STERILIZATION OF MEDICAL PRODUCTS

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Sterilization of Medical Products

Edited by

Eugene R. L. Gaughran
Johnson & Johnson
New Brunswick, New Jersey

and

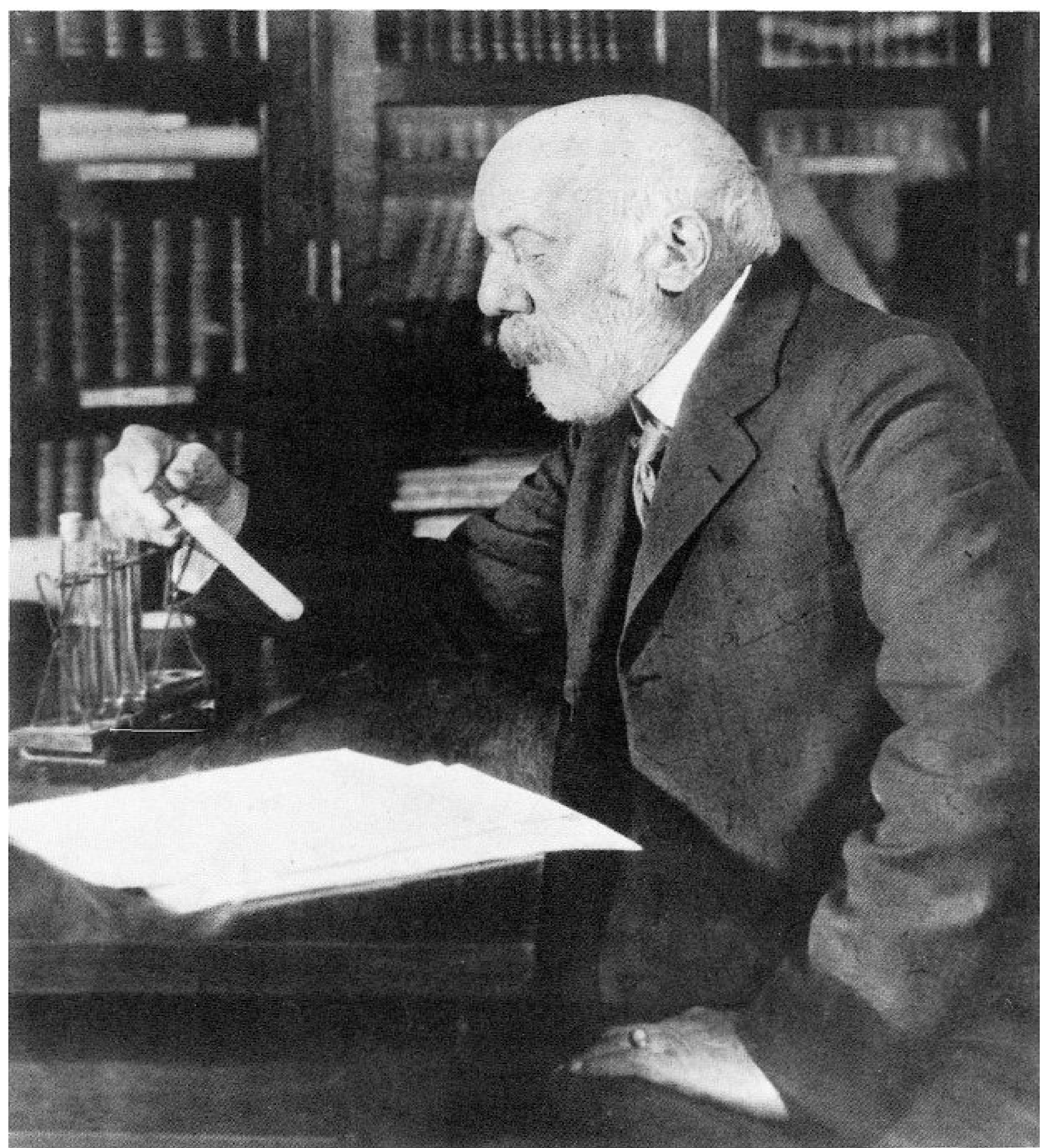
Karl Kereluk
Ethicon, Inc.
Somerville, New Jersey

Johnson & Johnson

1977

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.



Fred B Kilmer

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Modern Surgical Dressings.

By F. B. KILMER.

REPRINT FROM

AMERICAN JOURNAL OF PHARMACY.

PUBLISHED BY AUTHORITY OF THE

PHILADELPHIA COLLEGE OF PHARMACY.

HENRY TRIMBLE, Ph.M., Editor.

Publishing Committee:

HENRY N. RITTENHOUSE, SAMUEL P. SADTLER, WALLACE PROCTER, JOSEPH W. ENGLAND AND THE EDITOR.

Vol. 69 JANUARY, 1897. No. 1

CONTENTS.

The Murray Red Gum (Eucalyptus rostrata, Schlecht) and its Kino. By J. H. Maiden . . . 1
Gelsemium. By L. E. Sayre . . . 8
The Commercial Sources of Licorice Root. By H. N. Rittenhouse . . . 13
Second Pan-American Medical Congress. By Joseph P. Remington . . . 15
Commercial Ferruginous Pills—Blaud's Formula. By Wm. B. Thompson . . . 17
Adulterated Japan Wax. By Charles H. LaWall . . . 18
Petrolatum vs. Vaseline. By Louis Emanuel . . . 21
Modern Surgical Dressings. By F. B. Kilmer . . . 24
Chemical Analysis of the Bark of Honey Locust. Gleditschia triacanthos. By Louis P. Carstens, Ph.G. . . . 40
Alcohol as a Source of Error in the Titration of Alkaloids and Alkaloidal Residues. By Chas. Caspari, Jr. . . . 42
A Résumé of Recurrent Topics. By William B. Thompson . . . 44
Cinchona Cultivation in Bengal . . . 47
Recent Literature Relating to Pharmacy . . . 48
Editorial . . . 51
Reviews and Bibliographical Notices . . . 52
Minutes of the Pharmaceutical Meeting . . . 53
Classes of the Philadelphia College of Pharmacy . . . 55

Price, $3 per Annum, in advance. Issued in Monthly numbers of not less than 48 pages

SINGLE NUMBERS, 25 CENTS.

Address all papers for publication, etc., to the Editor.

All communications relating to subscriptions, advertisements, etc., to the Business Manager, F. YAPLE, 145 North Tenth Street, Philadelphia, Pa.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

MODERN SURGICAL DRESSINGS.

BY F. B. KILMER.

The surgical dressings in use at the present time by such practitioners as keep pace with the advancement of the surgical art are the products of the practical application of scientific knowledge. They are the outcome of the modifications and amplification of procedures that have been brought about in the evolution of surgical science.

Dr. Wm. Pepper states that "medicine and surgery have made more progress in the last twenty years than in the twenty centuries preceding." This statement may also be applied to the surgical dressing.

In the dawn of the present era of surgery, the teachings of Lister demanded that the dressings to be applied to a wound should be saturated with chemicals capable of killing germs "within the wound or coming from without." During this epoch antiseptics were empirically applied. A dressing that promised sure death to the microbe was in demand. In those days cloth was plastered with masses of pitch, paraffin fat and carbolic acid. The products were unclean—sticky, irritating and non-absorptive—directly the opposite to those in use at the present time. Crude as was this beginning, it contained the "living spark of truth that illuminated the mysterious darkness which for centuries hovered over wound infection." It brought blessings that "have soothed and removed untold suffering and misery—have saved millions of lives. For this gift to surgery we are indebted to Sir Joseph Lister."—*Gerster*.

During the decades that have followed the time of which we speak, the forward progress of the principles of antisepsis has been continuous.

The accurate scientific observations of bacteriology has determined the value of antiseptic substances, brought a knowledge of the nature of bacteria, their habits, their life, and shown their influence in the causation of wound infection. Such knowledge has given to the surgeon newer and better weapons than those first used in the combat against wound infection. The surgical dressing has always been to the front in the revolution and evolution of surgery. Caustic applications were early substituted for those which were mild, yet more potent. Many microbe-killers were found to be man-killers; others were shown to be valueless. Power to absorb wound secretion and exclude infection was made an essential requirement for wound-dressing material.

Prevention became both the watchword and the keystone of surgical technique. What is termed by Gerster "the conscientious practice of thorough-going cleanliness," was found possible of attainment by the use of antiseptics—"angels of cleanliness." Chemical sterilization has been combined with mechanical cleansing. Natural agents, as well as those instituted by the operator, have been called to the aid of the surgeon. In this transition, antisepsis has not been abandoned, but has developed into its higher form—asepsis. The antiseptic dressing has not been discarded, but has become aseptic. The terms antisepsis—asepsis, are not antagonistic; the one is not the antithesis of the other. "Asepsis is an exalted degree of cleanliness."

It is reached by the surgeon through the aid of antiseptics. The antiseptic agents employed to produce the condition of asepsis may be physical—heat, chemical—carbolic acid, etc., mechanical—washing. These may be supplemented by measures which exclude all bacteria. The aim sought is a condition of freedom of septic material or micro-organisms—asepsis.

Single user license provided by AAM. Further copying, networking, and distribution prohibited.

The Fundamental Law.—In the transition of surgical practice, which we have noted, the great

guiding principle first recognized by Lister has been strengthened, viz.: "that the presence of certain kinds of bacteria is an essential condition of wound infection." From this has been evolved the fundamental law that all materials which are to come in contact with the wound must be free from pathogenic organisms. To prepare a dressing which shall fulfil the requirements of this law would, at first glance, seem to be a simple undertaking. We find, however, that the task is not so easy of accomplishment when we note that over 150 species of bacteria are classed as pathogenic (6 pyogenic); in addition to this we have nearly 300 species of organisms classed as non-pathogenic for lack of information as to their disease producing power.[1]

These bacteria are widely distributed.

"There is no well-defined dividing line between pathogenic and non-pathogenic bacteria."—*Sternberg*.

It would be impossible in the manipulation of dressing material to separate or remove harmless bacteria from those which may be virulent. Therefore, in its practical application the fulfillment of the law demands that surgical dressings shall be free from all forms of bacteria.

All antiseptic agents do not possess the power to destroy or kill organisms. Therefore, dressings impregnated with antiseptics will not, of necessity, meet the demand. Hence, in the preparation of surgical dressings, the law must be construed to mean that, whatever may be the material and whatever may be the methods by which it may be prepared, in order to meet the requirements of surgery, the fundamental principle governing its production must provide that it shall be free from all micro-organisms.

The Infection of Dressings.—The materials which enter into surgical dressings, such as absorbent cotton, gauze, wool, are those which, in themselves, reach after, absorb and hold bacterial life. Every person and every object with which the dressing may come in contact in the course of its preparation, are liable to transfer to it infection. Infection through air is a possible factor.

Micro-organisms are readily disseminated through the air by the medium of dust. The air of a crowded room is always laden with bacterial life. In hospitals, the air is infected through the discharges of patients. The air of a physician's office cannot be kept free from infected dust. The dust on the drug-store counters, tables and shelves will always furnish a luxuriant bacterial garden.

Wherever people move about, they must, of necessity, transfer soil and create dust. If they move from infected centres, as do the inmates and attendants at hospitals, the visitors to the doctor's office or the patrons of a drug store, they spread infected dust.

Dressings may also become infected through the water used in their preparation. The water used upon the dressings should always be that which is boiling or which has been thoroughly boiled.

A greater source of infection arises from contact with the person who handles the dressing in the course of its preparation. Here the clothing of the operator is a possible germ carrier; his body is swarming with bacteria numerous in species, in uncountable numbers. Skin, hair and mucous membranes, even of persons who are healthy and of cleanly habits, furnish to bacteria a natural home for growth and multiplication.

In catarrhal conditions, skin disease, or wherever there is an increase of secretions, the bacteria of the body increase both in kind and in number. These sources of infection require more than ordinary attention.

Sterilization of the entire surface of the body is impossible. Yet we are confronted with the fact that the skin secretions, perspiration, dandruff from the hair, all mucous secretions, are a fruitful

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

source of infectious particles, fatal to asepsis if by any chance they should be transferred to the dressing. To even touch an aseptic dressing with hands not disinfected, to touch with prepared hands the eyes, nose, mouth or clothing, and then touch a dressing, would mean that infection would surely follow. Such a procedure would be an unpardonable violation of surgical cleanliness, a crime against asepsis. We must further take into account that the objects within the room where dressings may be prepared, including the air, the walls, furniture, floors, the tables upon which the dressings are laid every piece of apparatus, every object of any nature that may come in contact with the dressing, may be the means of transference of germ life. If such objects happen to be of the nature of organic material or those which hold moisture, the more readily do they become carriers of infection.

The maker of surgical dressings must have in mind, therefore, the materials of which the dressings are composed, that they are in their nature absorptive of infectious particles, that all objects connected with, all surrounding conditions, are sources through which infection may be carried to dressings during their handling and manipulation.

The Disinfection of Dressings.—Whatever the term disinfection has been made to mean elsewhere, when applied to surgical dressings it can only mean one thing—destruction of all micro-organisms in or upon the material. This process presents many varying problems. Bacteria show widely varying powers of resistance. Agents which destroy growing forms will not affect the vitality of their spores. The conditions of life and environment are all factors which must be taken into account in the disinfection of dressings. Thus, utensils and objects with smooth surfaces are readily disinfected, because any bacteria present will be found upon their outer surface; but when bacteria are enclosed in a rock-like mass, as they are in dried dust particles, where we find them surrounded by an almost impenetrable fortress, in dried pus, sweat, in dried secretions or flesh tissue, these organisms are protected by a varnish-like coating. Bacteria, within the fibre of cotton or wool, are enclosed within a cellulose structure. Therefore, in the disinfection of cotton, wool, silk, sponge and catgut, we find that there is presented a varying problem with each material. Chemical reaction is also a factor in disinfection that has been long overlooked. In the disinfection of dressings the nature of the materials and their behavior toward the disinfecting agent must be taken into account. Thus cotton may be disinfected in a solution of soda, but wool thus treated would be destroyed.

Wool may be disinfected in an acid solution, which, in turn, would destroy cotton. Catgut is affected by most chemicals; it is destroyed by moisture. Sponge tissue is affected by many chemicals; it is destroyed by moist heat. Oily substances are impenetrable by watery solutions.

The sole universal disinfectant is fire. It destroys the infection and the infected material. It is applicable to the disinfection of asbestos dressings, which have recently been recommended for surgical purposes. There is no one method or agent which, under all circumstances, will meet all conditions. Generally, more than one agent and several methods of procedure must be used together or in succession.

The writer has made a long series of investigations, having in view the possibility of disinfecting dressings with agents that would have no reaction with the material composing the dressing, that could be readily removed from the dressing, or, when allowed to remain within the dressing material, would have no effect upon wound tissue. In these experiments, such agents as electricity, gases, vapors, friction and pressure were employed.

The general method pursued was to infect fibres with a nutrient fluid containing bacteria, to then subject the infected fibres to the action of the disinfecting agent. The results may be briefly

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

summarized.

Electricity was not effective upon the organisms, except when electrolysis took place, as was the case when water or a solution of salts was the medium used in the transmission of electrical energy.

Oxygen gas when under pressure had a germicidal effect, especially so when the bacteria were in a moist state. Nascent oxygen was found to be a powerful germicide. Ozone gave similar results, as did oxygen. Carbon dioxide was found to be an inhibitant, but not a germicide. The gaseous oxides of nitrogen, except N_2O were found to be powerful in their action upon bacteria, but destructive to dressing material and productive of great irritation upon inhalation. Sulphur dioxide was found to be germicidal in the presence of moisture, but inapplicable to many classes of the materials used in surgical dressings. Chlorine gas is a disinfectant, especially in its reactions which takes place in the bleaching process, namely, union with hydrogen, and consequent liberation of oxygen.

The bleaching process, therefore, effectually destroys germ life. Iodine and bromine are energetic agents in the presence of moisture, but they react destructively with materials used in surgical dressings. Formaldehyde vapors possess a high power as a germicide. The vapors are highly irritating and destructive to flesh tissue. They are, however, applicable in the disinfection of some classes of material used in dressings, and are utilized in the processes hereinafter outlined.

During the mechanical process of carding cotton and other fibres, the fibres are subjected to prolonged friction, with consequent heat and electrical action. The results upon infected fibre passed through the process were interesting, and the process was found to be one of sterilization.

Experiments numbering many hundreds of series were made to ascertain the value of pressure as a sterilizing agent upon dressing materials. The results show that infected fibres may be sterilized by a pressure of 50 to 100 tons to the square inch. This process has been utilized in the sterilization of certain forms of surgical dressings.

With the discovery of a new species of bacteria there is said to be a new chemical born for its destruction.

But in the present day practice of surgery, only in a few instances, may we use chemical germicides for the disinfection of dressings and allow the chemical to remain in the finished product. The active chemical disinfectants are for the most part destructive to dressing fabrics as well as irritating to flesh tissue. Out of the many disinfectants lauded in days past for the impregnation of surgical dressings, but few remain. It has been found that dressings, even when impregnated with antiseptics, may still harbor germ life. In the presence of dry iodoform, dry corrosive sublimate, boric acid, germs will retain their vitality for a great length of time.

Though seemingly a contradiction of terms, it is, nevertheless, a truth born of experience to state that antiseptic dressings may be the means of conveying infection to a wound. Hence, the requirement that antiseptic dressings shall be free from micro-organisms.

In the list of agents applicable to the disinfection of dressing materials, heat ranks first in germ-destroying power. Heated air is precluded for use with cotton and some of the other substances used, for the reason that the temperature required for efficiency is destructive to the material. Heated air is quite inferior in disinfecting power to boiling water and steam. Boiling water almost instantly destroys most forms of germ-life; resistant forms succumb to its action in a few minutes.

Steam, then, holds the first place as a practical agent for the disinfection of surgical dressings. To be effective, it must be saturated (unmixed with air). Saturated or streaming steam circulating under moderate pressure reaches the efficiency and gives the results attained in boiling.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Practical Application.—Having passed in review some of the principles which underlie the preparation of surgical dressings, fitted to fulfil the requirements of surgery, we can best gain an impression as to their practical application by a brief review of the methods instituted by the author, which are now in working operation in the laboratories of Johnson & Johnson, at New Brunswick, N. J.

The buildings set apart for this work were built for this special purpose—made plain and tight to exclude dirt. They are admirably situated away from busy and dusty streets. For miles on either side stretches river and meadow-land, securing an almost dustless atmosphere. In fitting up the rooms in which the manipulations take place, the ideas kept in view were the exclusion of bacteria, easiness of keeping clean.

The walls and ceilings are glass-smooth. The floors are filled and polished. There are no closets or shelving, no cracks or crevices to harbor dust or dirt. The furniture consists of glass-topped tables with iron frame, allowing effectual and easy cleansing. The principal part of the work is done in the "aseptic room," so called because all things within it are at all times kept surgically clean.

The following is an extract from the rules governing this room:

> "Everything outside of this room, everybody and everything passing into this room from the outside are to be regarded as infected until subjected to special cleansing operations.
>
> "Everything required for use in this room, or being brought in, must be sterilized according to the prescribed rules.
>
> "All cleaning, sweeping and dusting must be done at the close of the day's work. Tools, apparatus, towels, aprons, aseptible clothing, etc., are to be sterilized in the sterilizing chambers. The floor must be well moistened before sweeping; dusting must be done with damp cloths. After sweeping and dusting, the covers upon the tables must remain for at least eight hours.
>
> "As often as may be necessary, the entire wood and iron work of the room must be washed with soap and water, then with antiseptic solutions; the room closed and fumigated with sulphur and steam."

Everything, whatsoever may be its nature or history outside of this room, is considered as infected (though, in fact, it may be free from germ life); it is, therefore, disinfected before being taken into the room. The entrance to this room is through an ante-room, which is a disinfecting station of the highest type. Through this quarantine all persons and things pass before entering the aseptic room. The persons who operate in this room are under charge of graduate surgical nurses.

The following extracts from the rules in force show the methods adopted for securing personal cleanliness:

> "Every person before entering the aseptic room must put on the prescribed washable garments (flowers, ornaments, jewelry, etc., must be removed). They must thoroughly wash and scrub their hands, forearms and face according to the prescribed rules.
>
> "*Hand Disinfection*.—(I) Scrub hands, face and forearms in a solution of ammonia and soap with a disinfected brush. By the aid of a knife or nail-cleaner, scrape all particles under the nails and on the margins.
>
> "(2) Wash again in ammonia and soap solution, then rinse in clean hot water and dry on a sterilized towel."

After this preliminary washing, operatives must pass at once into the aseptic room. Persons engaged in directly handling dressings must further put on sterilized over-dresses, caps, sleeves, etc., and again wash their hands with soap and ammonia, rinse them in clean water without drying, rinse in a solution of oxalic acid, finally in soda and alcohol without drying. After this washing, only such objects as have been cleansed and sterilized must be handled unless the hands are rewashed. If for any reason there is cause to leave the room, the sterilized garments must be taken off, and then, before re-entering, both the preliminary and final washing be again performed. Tracing the history of a yard of gauze on its way through these rooms, its course would be somewhat as follows: It is first rendered absorbent and bleached (in an adjoining department) and arrives at the ante-room to be made into

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

dressings. The jars in which it will be packed, with their tops, fastenings, etc., are brought to the same point from a bath in hot soda solution. If the gauze is to be impregnated with antiseptics, it is done in this outer or ante-room. The gauze, the containers, labels and all things pertaining thereto next pass into the sterilizing chamber. This chamber forms a part of the dividing wall between the ante-room and the aseptic room. The chamber is rectangular in form, large enough to hold a wagon-load of goods. It is constructed with thick walls made of metal, asbestos and other non-conducting material. The interior is lined with steam-pipe radiators for producing heated air within the chamber. Doors to the chamber open at both ends, one into the anteroom and the other into the aseptic room. These doors are steam-tight and held in place by ratchet screws.

The chambers are fitted with steam supply and escape connections, gauges for pressure and vacuum, safety valves, exhaust valves, etc. Cars of iron with trays carry the articles to be treated. Supply pipes controlled by valves admit live steam to the interior of the chamber. The actions involved in the operations within the chamber are:

(*a*) Preliminary warming of the materials to prevent condensation.

(*b*) Removal of air.

(*c*) Circulation of saturated steam unmixed with air under pressure through every fibre of the material, subjecting them to the highest possible action of this agent.

(*d*) Subsequent exhaustion of steam and substitution of heated air.

After the gauze passes into this chamber, the doors are closed and it then becomes a hot-air chamber. The air is then exhausted to a vacuum of 10 or 12 pounds; saturated streaming steam is then let in; the temperature soon rises to possibly 240° F., and the pressure gauge indicates 5 or 10 pounds. The steam pipes are now closed; the vacuum pump is again started until the proper vacuum is obtained.

Again steam is turned on, and so on, in turn, currents of saturated steam follow each other through the vacuum for from one to two hours. Every part of the chamber is penetrated, every fibre is subjected to the action of this highest of bactericides. The most resistant form of germ life must be reached and destroyed. From the sterilizing chamber the gauze passes directly into the aseptic room. In this room, all persons, tables and apparatus having been previously prepared, the dressings are cut, folded and packed in the jars, the covers laid on loosely.

(A large portion of this work is done by apparatus, to avoid touching with the hands.)

This work is rapidly performed, and the filled jars returned to the sterilizing chambers for a re-sterilization. This final sterilization effectually secures absolute safety against the remote possibility of infection by handling. After this final sterilization the jar seals are locked. For dressings packed in jars, this process is one of hermetic sealing, a partial vacuum having been formed within the jars during their heating and cooling. The finished dressings now pass on to be labelled, put in cartoons and made ready for shipment.

These same chambers are utilized for disinfection with formaldehyde vapors, the process being: first heating of the chambers, exhaustion of the air, filling the chamber with formaldehyde vapors, which penetrate every portion of the material; finally, exhaustion of the formaldehyde vapors, which are in turn replaced with heated air.

Sterilization Tests.—The effectiveness of sterilization procedures can be readily confirmed.

In the writer's laboratory the practice is substantially as follows: A portion of the dressing material (for example, a piece of gauze) is impregnated with an infected nutrient fluid. The thus

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

infected material is then dried in air, that the organisms may, as far as possible, be placed in a resistant condition. As a check experiment, a portion of this infected and dried material is placed in sterilized nutrient jelly in the culture chamber. This is done to ascertain whether the test material has surely been infected. The remaining portion of the infected material is then passed through the sterilization process, care being taken that it passes through like conditions as would the sterilized dressings.

In the case of gauze or cotton, the writer's practice is to wrap the test material in the centre of the package.

In testing catgut ligatures, the ligatures are moistened and untwisted; the infected material is then rolled up within the tissue and dried. After the infected material has passed through the sterilization processes, it is placed in nutrient media in a culture chamber. After a suitable time (at least three days) if a growth is found in the check experiment, we are certain that our test material was infected. If no growth has taken place in the infected material, that has passed through the sterilization processes, we are certain that sterilization has been complete in all the dressings. This conclusion needs no verification. The dressings have been prepared and sterilized by methods which exclude contamination. If a certain portion of material purposely infected, in passing through the sterilization process with them, is rendered sterile, it is conclusive proof that the whole of the dressings cannot fail to be sterile and aseptic.

The above method of procedure applies particularly to dressings containing no chemical antiseptic. Where the dressings are so impregnated, the process is varied as follows:

To avoid the restraining influence of the antiseptic upon the growth of the test organism, portions of the infected material, after passing through the sterilization processes, are placed in quite a large body of liquid nutrient media, which is shaken to dilute the antiseptic below its normal antiseptic potency; to carry this dilution still farther, a few drops from the first dilution are passed on to a second tube of culture media.

It has been found in the use of antiseptics that enough may adhere to the organism (especially to spores) to restrain development, though not destroying their vitality. This is obviated even in the use of strong solutions of an antiseptic by the dilution above mentioned.

In testing with antiseptics the test material is kept under a cultivation for at least a week. Development is often so retarded by the antiseptic tending to make hasty conclusions erroneous. In these tests with antiseptics, liquefied flesh—peptone—gelatine of Koch is usually employed.

Where no antiseptic has been employed, sterilized potatoes and other solid media have been found convenient.

The required test is the presence or absence of a growth which will liquefy solid media or produce form, color or odor characteristic of bacterial colonies.

This is verified when deemed necessary by a microscopical examination. In surgical bacteriology, the bacillus of anthrax is used as the standard test organism; whatever will destroy the vitality of this bacillus will destroy all the known organisms of wound infection.

Who Should Make Surgical Dressings.—In the past, dressing materials were largely the product of domestic industry and convict labor. We could not now tolerate supplies from such disease-breeding sources. In recent discussions by surgical authorities, the question has been raised as to the relative fitness of the surgeon, the pharmacist and the manufacturer as makers and purveyors of surgical materials.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

The apostle of modern surgery manufactured "Lister's Gauze" in his own kitchen. Sir Joseph's kitchen is doubtless a more fitting place for such work than is the office of many of his followers. Doctors' offices are not, as a rule, the most wholesome spots. Their upholstered furniture is in constant contact with the clothing and persons of patients carrying infections of every name and kind. Their tapestried carpets are filled with dust brought from pest-laden households. In the doctor's office we will find that tables, shelves, books and apparatus are spattered with debris from urinal examinations, pus from foul sores, dried excretions from diseased skin, pathological tissue, clotted blood and dried discharges from innumerable sources.

Streams of infectious matter continually pour into the rooms of the busy doctor and find a lodging-place in its paraphernalia. The unfitness of such surroundings for the production of surgically clean dressings is evident.

I claim for the American physician the highest of honors. I all but reverence the skill and genius of the American surgeon; but before I would attempt to prepare aseptic dressings in their offices, I should, in most cases, require that they be first cleansed and disinfected upon the lines adopted by health authorities for the purification of infected premises.

A certain hospital claims that its operating room is "the cleanest place in the world." All hospitals have not earned such a title. Many of them are attached to medical colleges where students and professors gather fresh from the dispensary clinic, from visits to infected houses, from dissecting rooms, from hundreds of sources of contagion.

Clinging to their persons and clothing may be found particles rich in pyogenic and pathogenic bacteria. In hospitals, the aggregation of infectious organisms cannot be avoided. Formerly, they were "hot-beds of infection." Now dangers are excluded only by the most rigorous procedures.

When dressings are prepared by the pharmacist, the work is generally performed in the drug store back room. This place comes far short of the conditions known as surgical cleanliness. The chemically clean graduate is still unclean in the eye of the surgeon. Counters covered with vegetable and animal drugs of all kinds are not suitable places upon which to lay absorbent gauze. Street and store dust, spatterings of syrups, extracts, oils, and all manner of decoctions, create a favorable lodging- and breeding-place for organic life. These are not wanted in surgical dressings. The pharmacist, though ordinarily clean in person and habits, familiar with soap and water in the pursuit of his calling, yet he is far from aseptic. Like the physician, he is constantly in contact with infection through the person of his patrons.

The hands that dispense beef tea at the soda counter, or that bring a jar from a mouldy cellar, should not touch sterilized material without cleansing. Thus there must be a radical change of environment before the pharmacist can attain success in aseptic technique, though he may, perhaps, rightfully claim conditions and facilities that are above those of the ordinary physician.

The facilities of the manufacturer, whose whole organization is adapted to the production of surgical dressings, are certainly more perfect than those of the surgeon, to whom such work is incidental. The environment of a room from which pathogenic organisms and septic matters are entirely excluded is superior to that obtained in the hospital or in the doctor's office. The room in which no work is undertaken except the handling of aseptic material will certainly be more nearly surgically clean than one to which infection has constant access. Persons whose only calling is that of preparing surgical material, who have been schooled in the principles underlying the infection and disinfection of dressings, are probably more competent to handle dressings than the doctor's student

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

or his attendants, to whom such work is of necessity relegated. In this work, as in many other instances, properly constructed apparatus is more efficient, more cleanly, more perfect, than hand work.

Further, an organization devoted exclusively to the manufacture of dressings, once having the details arranged to prepare a yard of dressing, can produce any number of yards more perfectly than if done as occasion may require, as is the rule in the hospital or in private practice.

To the manufacturer and dispensing pharmacist is due the credit of having made possible the universal application of the principles of modern surgery. They have supplied to the practitioner in the most remote regions appliances as perfect as those used in the great hospital centres. They have placed in the hands of the practitioner appliances that fulfil every requirement of the advanced art of surgery.

I hold that the preparation, selling and dispensing of medicinal and surgical supplies to the doctor, to the surgeon and to the public belong to pharmacy. Their application is the province of the practitioner of medicine and surgery, and I maintain that it will be to the betterment of surgery to receive all dressing materials from the hands of a competent pharmacist.

Training for the Work.—It is important that persons who are to handle surgical dressings in any capacity be familiar with the principles as well as the details of the work. They should also know why things are done as well as how to do them. The principles of surgical asepsis are applicable to the dispensing and sale of these materials. Therefore, the following epitome of a course in aseptic technique, devised for use in the writer's laboratory, may be found useful to many pharmacists.

In addition to the daily manual training under experienced persons, the operatives are required to attend stated instructions. These instructions are in the form of demonstrations of the processes, with an explanation of the principles involved. Those in attendance are given questions to be answered and experiments to perform. Text and reference books are furnished. The scheme is modeled upon the plan of a college extension course. Among the subjects are the following:

(1) The work of preparing surgical materials, its importance, its requirements.
(2) Definition and meaning of terms.
(3) Nature of the material used in dressings. (Fibres, cloth, ligatures, etc.)
(4) Preparation of materials, bleaching, rendering absorbent, etc.
(5) Kinds of dressings used in modern surgical practice.
(6) Uses to which dressings are put in surgery.
(7) Bacteria, their nature, conditions of growth, multiplication, products of their activity, with demonstrations of the means by which they may be transferred to and from persons and things.
(8) Wound infection.
(9) Infection of dressings.
(10) Disinfection—chemical agents and physical agents.
(11) Exclusion of bacteria.
(12) Sterilization.
(13) Disinfection of persons and things.
(14) Asepsis and aseptic technique in the preparation of dressings.

The entire course in my practice occupies several months—in fact, becomes a continuous course, as additional methods are constantly brought into practice.

Surgical Dressings in Commerce.—Dr. Gerster, in one of his addresses, condemned the use of ready-made products as sold in the drug store, on the ground that the gauge of success is purely commercial, only directed solely to profit.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Another writer affirms that the standard of such dressings is commercial in nature, the essential requisite being profit, and that they must be sold to meet competition. That in this the requirements of surgery are matters of indifference and generally matters of ignorance.

These statements were corroborated in a recent instance by a druggist in one of our large cities, who is commercially wise. He stated that to him quality, kind or make was no factor. Low prices were the sole criterion of value. Responsibility hovers over every field of the pharmacist's activity in dispensing dressings; we share the burden with the surgeon. Whoever has stood beside the surgeon in his operating room and realized how much depended on not only the hand, the training and the skill of the operator, but the absolute cleanliness in every movement, must realize that there are some things that cannot be expressed in a money ratio.

At such a time and in such a place the integrity of the dressing rises to supreme importance. Any neglect in its preparation, any misstep through the ignorance, cupidity and stupidity of any who have had to do in its history, is sure to be revealed. The issue of life or death in such a case should not be subject to the market rates per pound or yard. What results must follow the very common practice of dispensers who open packages of dressings, measure and weigh them over dusty counters with unclean hands, and send them on their mission? It would be more humane, perhaps, to send a lethal dose of strychnine. In the light of asepsis, to dispense morphine for quinine becomes a virtue when compared with the wilful contamination of a surgical dressing.

Poisons are put under lock and key, dispensed under rigid systems of precaution and checking.

The importance of the surgical dressing, the nature of its requirements, call for equal care. There is no article in the druggist's stock which should receive greater care and judgment. Upon every yard of gauze, sponge or ligature he dispenses hangs, perhaps, the life and death of a patient and the reputation of a surgeon. They should be guarded from every channel of direct or indirect infection.

A closet or a room, or a case should be provided for their reception that is cleanable; it should be cleaned often and kept clean. They should be sold within the containers in which they are packed in their preparation. They should never be broken open for sale or for any other purpose. They should be delivered to the surgeon so perfect that there can be no question as to their integrity, placing all the responsibility for their subsequent care in his hands. In dispensing to the public, every purchaser should be cautioned as to their nature and instructed in their handling and use. The price should meet the cost of the dressing plus a profit which will cover this service of advice, trouble and care.

Ninety-five per cent. of the 100,000 physicians in our land who apply these principles of surgery must look to the pharmacist for their dressing materials. In filling this demand, the pharmacist should supply such materials as will meet the highest surgical requirements. As far as the dressing is a factor, the surgeon at the country cross-roads, by the aid of the pharmacist, should be enabled to reach the advanced methods of the metropolitan clinic.

To attain this end in the making, in the buying, in the sale and in the dispensing, even to the most minute detail, there is required knowledge, skill, ability and finally a faithful application of the same.

---

[1] Buchner has shown that many of the common saprophytes classed as non-pathogenic, when injected under the skin, cause local abscess. I have recently witnessed serious results follow an experimental inoculation of a clean wound with mould spores supposed to be harmless.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

PREFACE

This volume represents the proceedings of a symposium held at the National Conference Center, East Windsor, New Jersey on April 30 and May 1 of 1976. The symposium was the first of a series to be organized as a tribute to Fred B. Kilmer, the first Director of Research of Johnson & Johnson and a pioneer in the sterilization of medical products as well as in the microbiological control of the environment. His classical paper of 1897 relating to this area is reprinted in this volume. Scientific curiosity was his driving force. So too was his desire to serve mankind, to achieve practical objectives. While his ability, skill, perserverance and strength were apparent in his diverse fields of activity, most striking was his capacity to look forward constantly. He was a student of history, but the future claimed his interest. It is entirely fitting, therefore, that a symposium on the sterilization of medical products be dedicated to this outstanding pioneer in this field.

On the occasion of the 125th anniversary of Kilmer's birth, the First Johnson & Johnson International Kilmer Memorial Conference on the Sterilization of Medical Products was held and the Kilmer Award established for outstanding contributions to this field.

The recipients of the First Kilmer Award were Dr. Charles R. Phillips and Dr. Saul Kaye for their work with ethylene oxide as a sterilizing agent. Their work was done at the laboratories of the Biological Department of the Chemical Corps at Camp (later Ft.) Detrick. In 1944 they were assigned to work on "biological decontamination". They concentrated upon gaseous fumigants. Ethylene oxide, the twelfth gas examined, proved to be very effective in their initial screening tests. While none of the standard text books or review papers on chemical disinfection mentioned this compound, Phillips and Kaye ferreted out some 30 obscure references, including several patents, which referred to the activity of ethylene oxide against microorganisms, as well as against insects. The parameters for its use as a "cold" sterilizing agent, however, were ill defined. The intensive work by Phillips and Kaye, reported in a series of papers in 1949, laid the foundation for the rapid adoption of ethylene oxide by the manufacturers and users of sterile medical devices.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

The awards were presented by Mr. Foster Whitlock, President of Johnson & Johnson International and Vice Chairman of the Corporate Board of Johnson & Johnson. Mr. Whitlock has been a staunch proponent of research within the Johnson & Johnson Family of Companies, as well as in the pharmaceutical and device industries generally. His sponsorship has made possible both the Kilmer Award and the conference.

Dr. Charles Artandi, Vice President of Research and Development of Ethicon, Inc., deserves special recognition for his continued and enthusiastic support of sterilization programs and for his guidance, from conception to conclusion, of the First Kilmer Conference.

The editors are grateful for the invaluable assistance and guidance given them by Dr. E. Martin in preparation of the proceedings of the conference and to Mrs. Carol Volpi for the superlative job in typing the manuscripts. Finally, the editors are indebted also to Dr. Robert Fredericks, Mr. Martin Koesterer, Mrs. Dorothy Collins, Mrs. Virginia Horvath and Mrs. Ruth Boyle for their assistance in the planning and the conduct of the conference.

New Brunswick, New Jersey	*Eugene R. L. Gaughran*
Somerville, New Jersey	*Karl Kereluk*
December, 1976	

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

CONTENTS

Fred B. Kilmer – Pioneer in Microbiological Control
Eugene R. L. Gaughran

The Effectiveness of Environmental Control in Manufacturing
G. Briggs Phillips

Importance of Bioburden in Sterilization Processing
William S. Miller

Microbiological Control of Sterilization Processes
Karl Kereluk

Quantitative Aspects of Microbiological Controls in Industrial Sterilization
Alan Tallentire

Discussion
Session Chairman – L. O. Kallings

Surgery, Sterilization and Sterility
Velvyl W. Greene

Ethylene Oxide Sterilization: A Study of Resistance Relationships
Keith L. West

Resistance Levels for Biological Indicators for Use in Sterilization by Ionizing Radiation
Robert Berube

The Use of Biological Indicators for Monitoring Wet Heat Sterilization Processes
Irving J. Pflug and Geraldine M. Smith

Discussion
Session Chairman – C. W. Bruch

Recent Developments in Pyrogen Testing
Stanley Marcus and Jerry R. Nelson

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Current Developments in the Toxicological Aspects of Ethylene

Oxide Sterilization
Robert J. Fredericks
Discussion
Session Chairman - J. E. Willson

Regulatory Review of Sterilization Control of Drugs in the United States
Mary K. Bruch
Regulatory Alternatives for Assuring Sterility in Medical Products
William C. Dierksheide
Compendial Standards for the Control of Sterilization and Sterility Testing - Their Development and Applications
Aubrey S. Outschoorn
Medical Device Regulation in Canada
Ronald W. Campbell
Control of Sterile Products in the United Kingdom
Alexander Bishop
Legislation Concerning the Sterilization of Drugs and Medical Products in the Federal Republic of Germany
Gerhard Schwenker
Regulation of Sterilization Control of Pharmaceutical and Surgical Products in Italy
Alfred D. Zampieri
Regulatory Review of Sterilization Control in Scandinavia
Lars Olof Kallings
Discussion
Session Chairman - A. Bishop

Index

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

FRED B. KILMER — PIONEER IN MICROBIOLOGICAL CONTROL

Eugene R. L. Gaughran
Exploratory Research Laboratories
Johnson & Johnson
New Brunswick, New Jersey

The man we are honoring by this Conference was born Frederick Barnett Kilmer on December 11, 1851 in Chapinville, Connecticut. Yet, through his adult life he was known as Fred B. Kilmer. He apparently did not accept Frederick for he is said always to have signed his name Fred B. Kilmer or F. B. Kilmer and did not consider "Fred" an abbreviation. His personal stationery and even his book plate bore the legend "Fred B. Kilmer".

The Kilmer family descended from the Palatinate emigrants who settled in Livingston Manor, New York, about 1711. Fred's father, Charles, was a preacher in the Methodist-Episcopal church and his mother, Mary Ann Langdon, was a descendent of the New England Langdon-Everetts. In accord with his religious background, he attended Wyoming Seminary at Kingston, Pennsylvania and later was graduated from the New York College of Pharmacy. He took advanced chemistry courses at Columbia, Yale and Rutgers Universities and received an honorary degree of Master of Pharmacy from the Philadelphia College of Pharmacy and Science in 1920. Kilmer was always referred to as Dr. Kilmer. No record has been found to indicate that he was the recipient of an honorary doctorate and it must be concluded that the title was a respectful form of address commonly accorded pharmacists years ago.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

FIGURE 1. The Kilmer Pharmacy - 1886.

Kilmer gained early practical pharmacy experience in Binghamton, New York, Plymouth, Pennsylvania, and Morristown, New Jersey before opening his own pharmacy in 1879 in New Brunswick, New Jersey, at the corner of Albany and Spring Streets. The pharmacy was located in the Opera House Building and appropriately called

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

The Opera House Pharmacy (Fig. 1). A frequent visitor was Thomas Edison, the great inventor of the 19th century. He spent considerable time behind the prescription department with Kilmer observing pharmaceutical operations – even going into the cellar to study the manufacture of soda water from marble dust and sulfuric acid. Edison exhibited a keen interest in drugs and chemicals and, at Kilmer's suggestion, purchased a U. S. Dispensatory. In addition to purchasing his prescription drugs "in quantity", Kilmer related in an article in the American Druggist that Edison purchased from him charcoal and other carbon substances used in his experiments with the incandescent lamp. Kilmer also related "the fact that when Mr. and Mrs. Edison were on their way to attend a performance at the Opera House, they would stop first in the drugstore… and Mrs. Edison would help to spruce up the unconventional inventor and remove traces of his hurriedly prepared toilet".

In 1878, Kilmer joined the New Jersey Pharmaceutical Association and took a deep interest in its activities. He served as vice president in 1884 and 1885, president in 1886 and secretary in 1887 and 1888. During this period and for the rest of his life he became interested in and championed professionalism in pharmacy.

In 1886, Kilmer became acquainted with Robert Wood Johnson, also a graduate of Wyoming Seminary, and there developed a close friendship which had a profound influence on the careers of both men. Robert Wood Johnson established, in 1886, the company of Johnson & Johnson in New Brunswick, New Jersey, a copartnership of his two brothers James Wood and Edward Mead Johnson. Operations began on the fourth floor of a building that was once a wallpaper factory. The factory was on Neilson Street, not far from the Kilmer Pharmacy. The first products were medicated plasters. It was not long, however, before a full line of surgical dressings, catgut and silk ligatures appeared. The company was incorporated in 1887.

Before coming to New Brunswick, Robert W. Johnson had organized the firm of Seabury & Johnson in 1874 in East Orange, New Jersey, to manufacture medicinal plasters. While exhibiting his plasters at the International Medical Congress in Philadelphia in 1876, he had the opportunity to attend an address by Joseph Lister, as well as to study the exhibit of the German firm of Paul Hartmann of Heidenheim showing "a complete set of all dressing materials as

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

they are in connection with Lister's method". Johnson sensed the importance of Lister's antiseptic method and undertook the manufacture of surgical dressings of the type advocated by Lister. Little progress was made with these dressings, and the Seabury and Johnson partnership was dissolved in 1885 on the condition that Johnson did not engage in business of like character for a period of ten years. "For a consideration", however, the covenant was "cancelled and annulled", leaving Johnson free to join his brothers, with manufacturing and laboratory entirely under his charge.

The Lister dressing of the time, as prepared in the Lister kitchen with a wash tub and clothes wringer, consisted of eight layers of cheese cloth saturated with carbolic acid, combined with resin and paraffin, cut into squares, with a piece of carbolated mackintosh cloth or a thin layer of gutta-percha tissue underneath the upper layer. The cheese cloth was actually a cheap unbleached nonabsorbent muslin. There is no doubt that this was a great improvement over oakum, but few hospitals in the world were in a position to make such dressings.

Out of the frequent discussions between Johnson and Kilmer came the realization that one of the greatest changes in the world of surgery was about to take place, and Johnson intensified his efforts to put Lister's discoveries to practical use. Their basic premise was that all antiseptic dressings should be as ready for surgery as the surgeon himself. Accordingly, such dressings should be assembled and packed for shipment to the remotest areas and still remain free from contamination. Furthermore, they envisioned antiseptic dressings constructed of their newly developed absorbent pure white cotton and gauze and designed in such manner that their easy application would be readily apparent.

Despite the magnitude of Lister's findings, only a handful of physicians in this country were aware of the discovery. To awaken medical interest in this surgical concept, Johnson & Johnson published a monograph entitled "Modern Methods of Antiseptic Wound Treatment". This was a compilation of recent notes and suggestions by eminent surgeons (D. Hayes Agnew, A. C. Bernays, John D. S. Davis, John B. Deaver, Hunter McGuire, Thomas G. Morton, N. Senn, Stephen Smith, Lewis A. Stimson and J. William White). The treatise, showing the talented hand of Kilmer, made its appearance in 1887, and went through five editions by 1893, with an

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

international distribution of one and one-half million copies. It incorporated the teachings of Lister, known as "Listerism", and was the only clear and accessible explanation of the new surgical concept. This monograph, along with the introduction of moist antiseptic dressings in 1887 by Johnson & Johnson, marked the real beginning of antiseptic surgery in the United States.

It is not surprising that Kilmer's close association with Johnson & Johnson would lead him to join the company. This he did in 1889 as Director of the Scientific Department. He did not, however, abandon his keen interest in and perceptive contributions to many aspects of pharmacy.

During his early years at Johnson & Johnson, he had the opportunity to put into practice his revolutionary ideas of production hygiene. Cleanliness quickly became a religion in Johnson & Johnson. The new buildings constructed for the manufacture of surgical dressings are described by Kilmer as follows:

> "The buildings set apart for this work were built for this special purpose—made plain and tight to exclude dirt. They are admirably situated away from busy and dusty streets. For miles on either side stretches river and meadow-land securing an almost dustless atmosphere."

Special attention was given to the grounds and the streets which were sprinkled and swept regularly. Lawns and shrubs were neatly clipped and areas around buildings painted white and kept free of litter. Inside the buildings even more scrupulous attention was given to cleanliness. In areas adjoining the manufacturing areas, corners were painted white; even in the stairways where the tread and riser met the wall, the corner was painted white. Kilmer described the dressings manufacturing areas in the following words:

> "In fitting up the rooms in which the manipulations take place, the ideas kept in view were the exclusion of bacteria, easiness of keeping clean. The walls and ceilings are glass-smooth. The floors are filled and polished. There are no closets or shelving, no cracks or crevices to harbor dirt. The furniture consists of glass-topped tables with iron frames, allowing effectual and easy cleaning. The principal part of the work is done in the 'aseptic room', so called because all

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

things within it are at all times kept surgically clean."

There was a set of elaborate rules governing this room. Kilmer goes on to say of the aseptic room:

> "Everything, whatsoever may be its nature of history outside of this room, is considered as infected (though, in fact, it may be free from germ life); it is therefore, disinfected before being taken into the room. The entrance to this room is through an anteroom, which is a disinfecting station of the highest type. Through this quarantine all persons and things must pass before entering the aseptic room. The persons who operate in this room are under charge of graduate surgical nurses."

In the anteroom, the operators donned prescribed washable garments and proceeded to scrub their hands, face and forearms in a solution of ammonia and soap with a disinfected brush, clean their nails, and wash again with ammonia and soap solution, rinse in clean hot water and dry on a sterilized towel. Kilmer goes on to say:

> "After this preliminary washing, operatives must pass at once into the aseptic room. Persons engaged in directly handling dressings must further put on sterilized over-dresses, caps, sleeves, etc., and again wash their hands with soap and ammonia, rinse them in clean water without drying, rinse in a solution of oxalic acid, finally in soda and alcohol without drying. After this washing, only such objects as have been cleansed and sterilized must be handled unless the hands are rewashed. If for any reason there is cause to leave the room, the sterilized garments must be taken off, and then, before re-entering, both the preliminary and final washing be again performed."

In speaking here of sterilized garments, it should be noted that the word sterilized had the same meaning then as it does today. The garments were actually autoclaved. It is a curious fact that in the 1890s the employees of Johnson & Johnson preparing and packaging surgical dressings in aseptic areas wore sterilized uniforms while many surgeons were operating in frock coats under conditions far short of surgical cleanliness.

In the dividing wall between the anteroom and the aseptic room was a sterilizer (Fig. 2). It was rectangular in shape with doors

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

at each end, one in the anteroom and the other in the aseptic room, and large enough to accomodate a wagon load of materials. The unit operated with saturated steam at pressures of up to ten pounds. The sterilizing temperature was said to be 240° F. This unit constitutes, to the best of our knowledge, the first industrial steam sterilizer in the United States, if not the world.

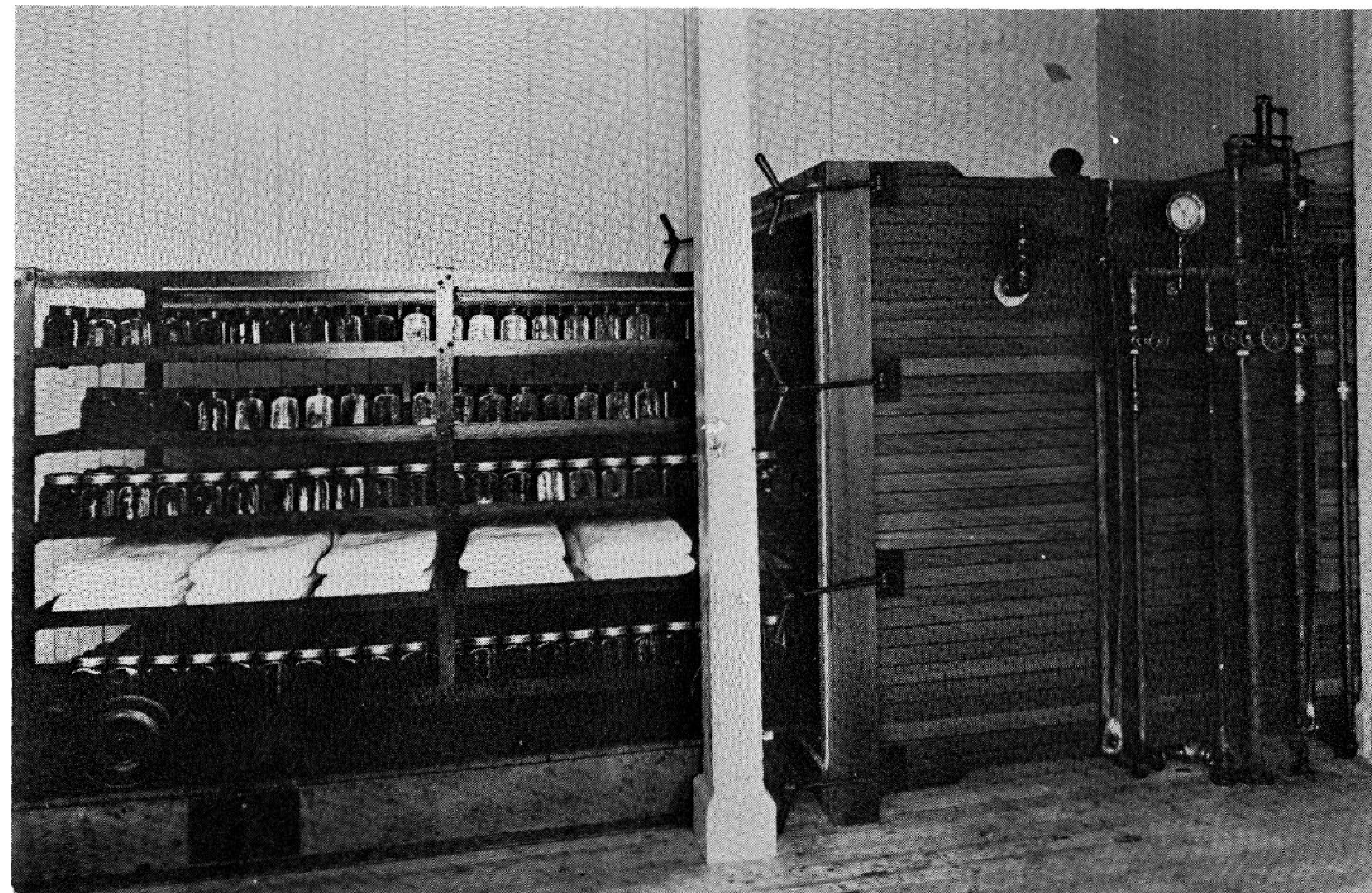


FIGURE 2. The steam sterilizer of the 1800s.

All materials employed in the manufacture of dressings were brought into the anteroom. This included the dressing components and all packaging components. If the dressing to be prepared was a medicated one, the gauze was impregnated in the anteroom. Then everything was placed in the sterilizer. After being subjected to the steam sterilization cycle, the materials were removed through the door in the aseptic room where the dressings were fabricated. This effectively reduced the bioburden on everything entering the aseptic area - except the operators. The operators were garbed, as noted earlier, in sterile uniforms. Kilmer recognized the need to
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.
avoid contamination by humans and to make things as automatic as

possible, for he noted that a large portion of the work in the aseptic room was “done by apparatus, to avoid touching with the hands”.

During the 1890s most of the dressings were moist and antiseptic, and were packed in glass jars. Johnson & Johnson had adopted glass jars in 1889. All components of the product and package received a preliminary sterililization before entering the aseptic room. After cutting, folding, etc., they were packed in the jars and sterilized with loose lids. After sterilization, the lids were clamped or tightened. It is assumed that Kilmer did not consider that the amount of water in the moist dressing was sufficient to achieve sterility if the jars were sealed before being subjected to the sterilization cycle. In 1894 an improved vacuum steam cycle and a method for automatically sealing the jars during the sterilization process were introduced.

In the “Linton Moist Dressing with 1:2000 corrosive sublimate, bi-sterilized, hermetically sealed” (Fig. 3), Johnson and Kilmer realized their two important goals: to provide the surgeon with “ready-to-use” Lister-type dressings packed to remain sterile even when shipped to the ends of the earth. A doctor could now have an antiseptic dressing in the time it took to open the package. Inherent, however, in this concept was an even more important element. A dressing must be more than antiseptic: it must also be free of microorganisms. This was the first step in the transition from antiseptic surgery to aseptic surgery.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

FIGURE 3. The sterile antiseptic dressing which played an important role in the transition from antiseptic to aseptic surgery.

In addition to merely marketing sterile antiseptic dressings, Johnson & Johnson published their formulas and rules, and illustrated their system. Others who followed put up a wet dressing in glass jars, similar in shape and style to those of Johnson & Johnson, even closely following the labels and cautionary seals, but without adopting any system of asepsis, and without sterilization. In one case, the maker openly stated that the sterilization of his products was done by the printer on the labels and that asepsis was a fad practiced by cranks.

In 1891 Kilmer established a bacteriological laboratory and repeated with great care the classical laboratory experiments of 1880 and 1881 conducted by Robert Koch and his associates in demonstrating the efficacy of hot air and steam in killing microorganisms. From these experiments Kilmer developed a procedure for verifying an industrial sterilization process. He adopted, as the reference organism, spores of Bacillus anthracis, an organism also favored by Koch. In the manner of Koch, Kilmer took spores directly from nutrient medium to inoculate a portion of the dressing material. He cautioned, however, that the test material be wrapped in the center of the package and care be taken that this package passed through the same conditions as would the other dressings in the sterilizer load. The ability of the

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

sterilization cycle to kill the test organisms under these

conditions provided Kilmer with assurance that the other dressings in the load were also sterile. Thus was born the biological indicator in industrial sterilization.

Kilmer noted, however, that this procedure was particularly applicable to dressings which did not contain antiseptics and cautioned against its indiscriminate use with antiseptic dressings. He explained the phenomenon of bacteriostasis and described how this could be overcome by dilution.

He recognized also that organisms subjected to inimical environments may be retarded in their development and suggested extended incubation to avoid erroneous conclusions. And it is acknowledged today that a marginal sterilization process which affords relatively low assurance of sterility may require prolonged incubation to detect growth of surviving organisms.

This concept of using reference microorganisms in assuring sterility was extended to all sterile products. Training manuals were prepared to cover all aspects of manufacture, and courses on the principles of asepsis were instituted for the employees.

Early in the 1890s Kilmer foresaw the trend to asepsis for he wrote:

> "Chemical sterilization and mechanical cleanliness are among the newer weapons that have been called to the aid of surgery. Antiseptic dressings have been made surgically clean. Antisepsis has not been abandoned, but has developed into its higher form: asepsis, and antiseptic processes have become aseptic."

In an effort to disseminate this concept he published, in 1897, his classical paper which we have reproduced for this Conference. He instituted, also in 1897, a company publication named Red Cross Notes. This periodical not only reported on developments in surgery, but contained articles by leading surgeons. It also contained descriptions of the Johnson & Johnson manufacturing processes and procedures for testing its products, with illustrations of its facilities and selected products. This publication, edited by Kilmer until 1928, did much to influence professional attitudes. A similar educational publication, Red Cross Messenger, was edited by Kilmer from 1908 to 1930. This was
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.
directed more to the pharmacist than to the physician. A third

publication, Notes and Abstracts, was started by Kilmer in 1921 for the purpose of presenting advances in operative techniques, new trends in medicine, and technical data.

Kilmer's work brought him into contact with many organizations. He held membership in the New Jersey Pharmaceutical Association (in which he held several offices), the American Drug Manufacturers Association (of which he was vice president), the American Public Health Association, the American Chemical Society, the American Institute of Chemical Engineers, the Chemists' Club of New York, the American Pharmaceutical Association, the New Jersey Sanitary Association, the New Jersey Microscopical Society (of which he was president), the Society of Chemical Industry of Great Britain, the Royal Society of Arts of London, the Society of Economic Biologists of England, the Council of North Britain Academy of Arts, the Institut für Arzneimittellehre of Braunschweig, the Sociedad Quí mica Argícola of Buenos Aires, the Institute of Jamaica, among others.

Kilmer, as a representative of Johnson & Johnson, played a very important role in the preparation of the various U. S. Pharmacopeia monographs and in drawing up the 1906 Pure Food and Drugs Act. He was also adviser to government in the framing of the statute known as Wiley's Law of Weights and Measures, providing for the proper labeling of all drug and food products.

He published widely in both professional and trade journals. His papers were said to run "the gamut from pure science to ironic whimsy". There were papers on the cultivation of medicinal plants, pharmacopoeial analysis, sanitation, pharmaceutical professionalism and education, practical pharmacy, and history of pharmacy. He was a pioneer in the First Aid Movement and authored the Johnson's First Aid Manual in 1901. Although this 100-page book was intended to accompany a case of first aid supplies, it gained worldwide popularity on its own and went through eleven editions by 1932, when Kilmer ceased editing it. As an additional move to further the First Aid Movement, he started the First Aid Bulletin in 1915, of which he said in the first edition: "So far as we know, the First Aid Bulletin is the first periodical devoted exclusively to first aid work published in this country and perhaps in the world. We believe there is a field for it, a very wide field… and it will not be very long before only the very ignorant will be at a loss as to what to do in an emergency".

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

While this Conference is concerned primarily with the area of Kilmer's contributions to the sterilization of medical products, there are many equally important facets to the talent of this remarkable man.

Shortly after joining Johnson & Johnson it was recognized that, in Kilmer, the company had not only a distinguished scientist, but also a talented writer. He soon became Manager of the Advertising Department and for 30 years held this job as well as that of Director of the Scientific Department. Until 1925 he was also in charge of all legal correspondence relating to patents, trademarks and litigations. In 1929 he organized a Museum of Surgical Products with the "main and most important object" being the "protection of trademarks, copyrights and patents".

As noted earlier, Kilmer was active in the field of sanitation and public health. For many years he was president of the New Brunswick Board of Health and adviser to the New Jersey State Board of Health. He wrote numerous public health bulletins and was able to put into practice a number of plans for the solution of water and milk supply problems, and the establishment of proper sewage disposal plants. It is interesting to note that as early as 1891, Johnson & Johnson had been operating its own large-scale water treatment plant for process water and as late as 1951, in the author's experience, met the U. S. Public Health standards for potable water.

Turning to another talent of Kilmer's, we find that he was not only an expert on medicinal plants, but transformed his garden into a botanical garden and also established a replica of an ancient monastery garden at St. Peter's Hospital in New Brunswick. His horticultural and chemical studies added materially to our knowledge of ginger, kola, pawpaw, belladonna, and other plants. The Philadelphia College of Pharmacy and Science, in recognition of Kilmer's contributions in this field, in 1935 named their botanical gardens the "Kilmer Gardens".

Strangely, the name of Kilmer has been perpetuated not because of the distinguished scientist and talented writer, Fred B. Kilmer, but because of his son, Alfred Joyce Kilmer. Alfred, better known as Joyce, was born in 1886 in New Brunswick, the year Fred came to New Brunswick. He was killed in action in the First World War and has since been remembered as a symbol of poetic

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

idealism, and especially for his poem "Trees".

Fred Kilmer's writings never reflected the tragic aspects of his family life. A son and daughter died in infancy. Another son died as a suicide at 26 and Joyce was killed before his 32nd birthday. With the loss of Joyce, Mrs. Kilmer devoted her life to the perpetuation of the memory of Joyce Kilmer. So there is a U. S. Army Camp Kilmer; there are dozens of American Legion Posts throughout the United States named Joyce Kilmer Posts; there was a Liberty Ship in World War II named Joyce Kilmer; there are Joyce Kilmer streets, parks, trees, forests, etc.

This is not to say that Fred B. Kilmer was not recognized. During his lifetime he was recognized as a respected writer on scientific and medical subjects, acknowledged for his wisdom and almost prophetic understanding of the future. Upon Dr. Kilmer's death in 1934, Time magazine described him as "the most revered pharmaceutical chemist in the country". The editor of the New Jersey Pharmaceutical Association's Journal of Pharmacy described him as "one of the most talented and discerning writers in the pharmaceutical field". The American Druggist called him "one of the most fascinating individuals American pharmacy has given to the world".

Today there are two Kilmer Awards, curiously enough, both bequeathed by Kilmer. In his will, he left the earnings from a $3,000 bequest to the American Pharmaceutical Association to award a prize in his name for "the most meritorious paper in pharmacognosy" and a bequest of $1,000 to the New Jersey Pharmaceutical Association, which gives an annual Kilmer Prize for "the most meritorious paper submitted to the Association by a graduate pharmacist".

With this Conference we pay homage to a man who possessed a rare and keen insight into all the facets of pharmacy. The Johnson & Johnson Kilmer Award has been established as a tribute to his pioneering work in microbiological control of the environment and in the field of sterilization, and as it is presented this year and in future years, it will recognize men who have made significant contributions in these fields.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

THE EFFECTIVENESS OF ENVIRONMENTAL CONTROL IN MANUFACTURING

G. Briggs Phillips

Health Industry Manufacturers Association
1030 - 15th Street, NW
Washington, D. C.

INTRODUCTION

This discussion is concerned with the control of the microbiological environment in areas used for the manufacture of medical devices. The discussion will attempt to outline the major elements of a control program and how they may be used in an overall scheme for the manufacture of medical products having a minimum level of microbiological contamination prior to the sterilization process. The importance of controlling the bioburden on such products to the sterilization cycles to be subsequently employed will be covered in other presentations.

From a historical perspective it would be interesting, at another time and place, to consider the development through the years of the technology presently available for environmental control. Environmental control of microbiological contamination, in reality, is little more than the application of techniques to provide barriers to isolate, contain, or prevent the transfer of microbial populations.

The heritage of barrier technology, of course, dates to antiquity. One recalls, for example, the mention by pre-Biblical writers such as Pliny and Pollux of the use of face masks made of animal membranes by miners to avoid the inhalation of dust. Actually, to trace the early development of techniques for microbiological control and isolation is much the same as a chronology of the early history of bacteriology. The chronology would start perhaps by identifying the glass flasks and other apparatus used in the early 19th century by Schulze and by Schwann to test the theory of spontaneous generation and heterogenesis. From this point one can proceed to the studies by the obstetrician

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Semmelweis in devising techniques for the control of sepsis in childbirth. This was followed by Lister's treatise on antiseptic techniques and by the biological barriers devised by Davaine, Koch, Petri, and others. Also contributing to the technology were the studies of Berthelot and Thierfelder, in the latter part of the 19th century, in their attempts to produce germ-free plants and animals. The list of contributors to the technology of environmental control is long and continues to the present. The point to be made, however, is that the technology of environmental control has, through the decades, developed hand-in-hand with the science of microbiology; and the ability to contain, control, sterilize, and manipulate microbial species has provided a basis for the development of pure culture techniques and for the continued development of the science of microbiology.

In the following, I shall discuss environmental control techniques used in the manufacture of medical products within the context of a total system generally found in medical device manufacturing environments.

ENVIRONMENTAL CONTROL – THE TOTAL SYSTEM

Before proceeding to specific methods of environmental control, it is well to emphasize three points:

1) In minimizing microbial contamination on medical products during manufacture, it is necessary to understand the process by which products become contaminated.

2) Although we will emphasize today the specific tools for environmental control, these should be considered as but a part of a total control system for medical product manufacturing.

3) In applying environmental control techniques, it is generally necessary to institute testing programs to evaluate the efficiency of the control procedures.

I would like to briefly discuss these three points. Figure 1 illustrates that the nature of the contamination we wish to control is generally that classified as particulate-biological-viable. With regard to the medical product itself, Figure 2 shows a simplified scheme of how products may become contaminated. Figure 3 identifies nine elements of a total program of

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

environmental control for minimizing microbial contamination on medical products. Here the important point to mention is that management's policy for environmental control and factors such as training, in-plant hygiene practices, and even product design all play a part in the effectiveness of an environmental control program.

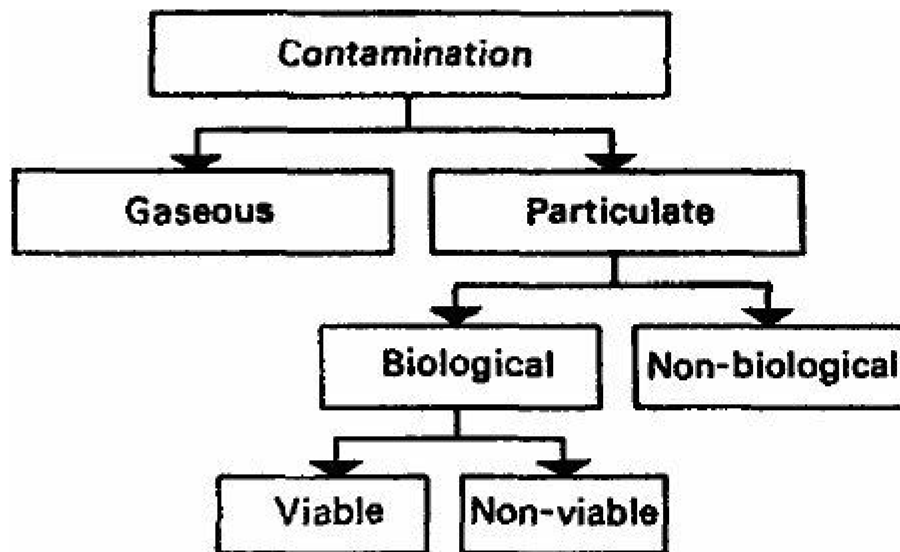


FIGURE 1. The nature of contamination.

People and Work Activity
Airborne Dust and Vapors
Work Surfaces, Tools and Fixtures
Materials and Water
Direct Contact
Direct Transfer
Fall Out
Self-Generated
PRODUCT

FIGURE 2. The process of product contamination.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

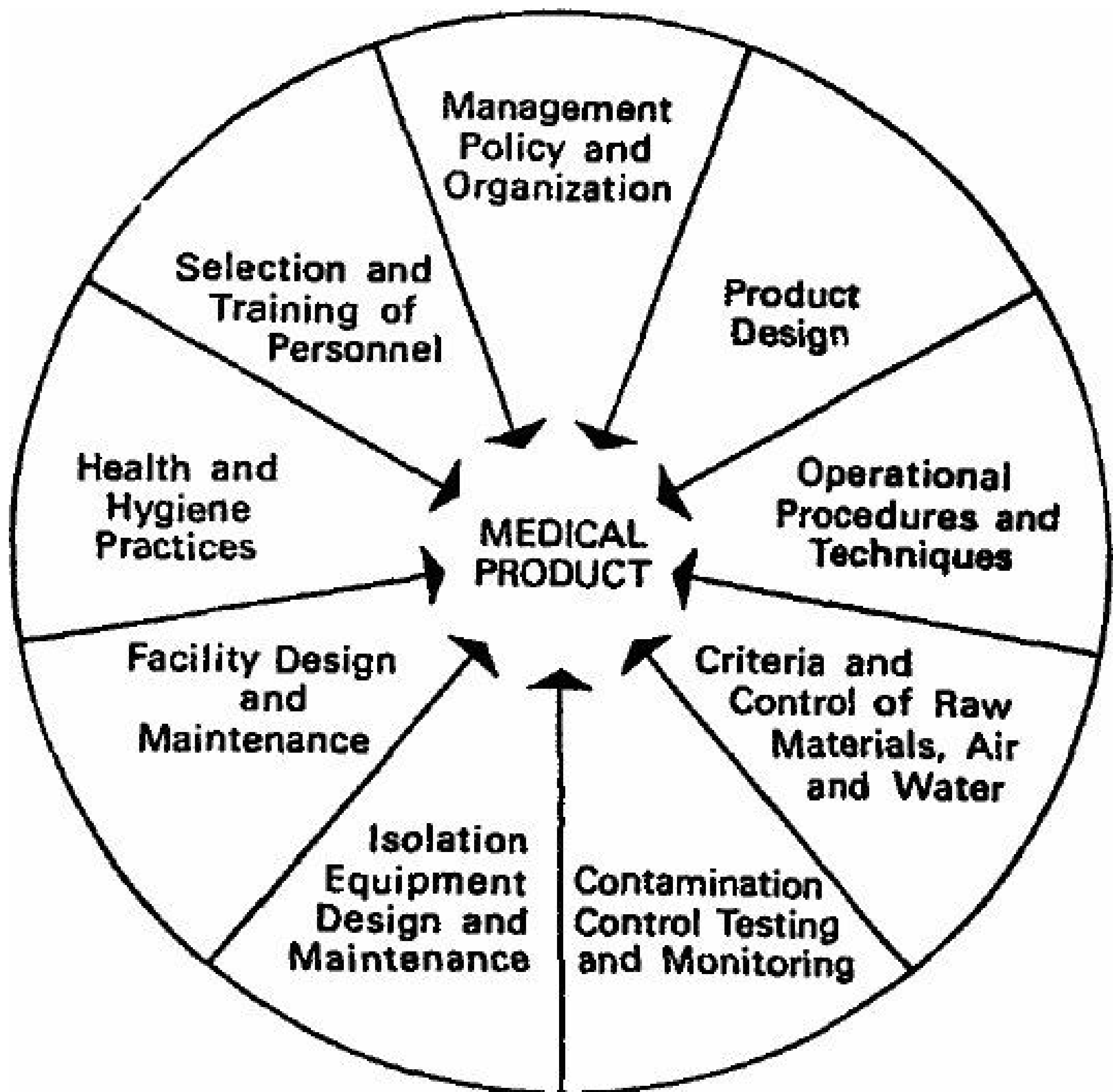


FIGURE 3. The elements of a medical product environmental control program.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

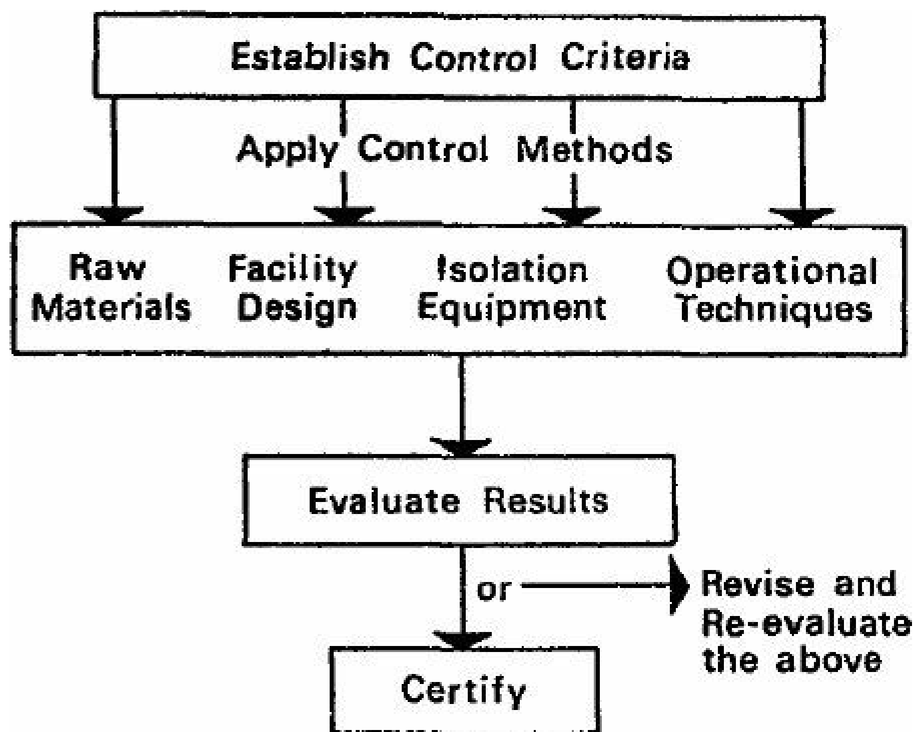


FIGURE 4. A scheme for environmental control.

With regard to Figure 3, it is significant to emphasize the importance in the United States of the generation and implementation of Good Manufacturing Practice (GMP) guidelines and regulations as they apply to environmental control whether required by regulatory agencies or developed and implemented on a voluntary basis by industrial firms. GMPs should take the form of written policies, manuals, directives, etc., and Standard Operating Procedures (SOPs) should specify the control measures relative to the nine elements in Figure 3. Most of you are undoubtedly aware of the active programs now under way in this country for the development of overall or umbrella-type GMPs as well as GMPs relating to the production of sterile products. I think that there can be no doubt that GMPs will, in the future, be considered the overall form of documentation by which the effectiveness of environmental control during the manufacture of medical products will be measured.

Figure 4 illustrates the requirement for a testing program to evaluate environmental control techniques being applied. Here the major point is that it is first necessary to establish criteria for the performance of control systems and to evaluate, by a suitable testing program, whether or not the control was achieved. In all probability the results of the evaluation of environmental control systems will be an important element for being in

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

compliance with GMP regulations of the future.

THE TOOLS OF ENVIRONMENTAL CONTROL

For the purpose of this presentation, it is convenient to classify the tools available for implementing environmental control in the following manner:

1) Design of the manufacturing facility.
2) Control of the quality and movement of air.
3) Control of process water and liquid effluents.
4) Isolation equipment for device manufacturing.
5) Control of manufacturing techniques.
6) Methods for sanitation and hygiene.

Design of the Manufacturing Facility

In considering the design of the manufacturing facility itself as one of the tools for environmental control, one must first realistically evaluate the degree of control needed for a particular medical product and then evaluate the extent to which the design features of the facility can effectively and economically provide a reasonable and practical degree of control. In other words, the planning stage in specifying facility environmental control systems is of utmost importance. Remember that environmental control systems within a facility can be expensive, not only from the initial investment point of view, but also because of the need for periodic repair, maintenance, and replacement. Good facility design following adequate planning can be valuable in containing and controlling environmental microorganisms, while indifferent or inconsistent arrangements can complicate or limit the effectiveness of microbial control. In most instances the function of the facility itself in providing a degree of control will center around the bioburden of the products being manufactured. However, in some instances when infectious or hazardous agents are involved in the manufacture, the facility features may also be expected to provide the control required to assure that hazardous materials do not escape from the facility.

In addition to systems that control air and liquids within the facility, some engineering features that may be considered in the design are as follows:

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

1) Construction of floors, walls, and ceilings to provide surfaces that can be readily and easily sanitized or decontaminated.

2) The provision, when indicated, of change rooms and shower rooms for personnel.

3) Physical separation of manufacturing spaces that require control from those not requiring it.

4) Efficient layout of the controlled manufacturing environment to prevent spread of contamination.

5) Space for controlled entrance of raw materials or other materials that might bring in excessive contamination.

6) Use of intercommunication systems and other techniques such as viewing windows and speaking diaphragms to limit personnel traffic to and from protected areas.

7) Design of case work, benches, and other equipment, to permit easy cleaning and minimizing harboring of contaminants.

In some facility design concepts, the building itself can be considered as the second barrier, with the first barrier being some type of isolation equipment used immediately with or around the items being manufactured. In any case, it is convenient to approach facility design utilizing a functional zone concept which allows different zones to have different degrees of environmental control sophistication.

Control of the Quality and Movement of Air

In some instances air control may be part of the facility, but in other instances it may be part of certain isolation equipment used to limit contamination of medical products. In either case, just how air control systems can operate most effectively in limiting the spread of micro-organisms should be carefully considered. Some of the cardinal factors to be considered in air control systems are as follows:

1) The use of differential air pressures within a facility to provide control over the movement of airborne particulates.

2) Appropriate microbial filtration of air.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

3) Appropriate designs to control the degree of turbulence or nonturbulence of ventilating air.

4) The use of air lock rooms to assist in the control of air flow patterns.

With regard to the above, it is recommended that expert consultation be used in designing and selecting appropriate control systems. Decisions on the cost-to-benefit ratio of air control systems should not be left to an amateur. Particularly important are those considerations involved in the selection of laminar airflow systems and those relating to when or when not to recirculate conditioned air supplies. One should realize, for example, that the use of laminar airflow, HEPA filters, and Class one hundred, ten thousand, and one hundred thousand air are not within themselves panaceas for environmental control. While it is often possible to construct entire laminar airflow rooms for manufacturing, it is usually more effective and cheaper to provide laminar airflow cabinets and similar equipment directly over or around the manufacturing process. Also it should be clear that in many instances air control systems for the building depend in part on the type of containment equipment that may be used immediately around the manufacturing process.

As an example, there would be little requirement for an elaborate air control system in a room where the manufacturing process is being conducted within an airtight cabinet system. These various considerations, therefore, result in the conclusion that no one set of air control systems can be recommended because of the variety of sterile medical products manufactured and the many options for alternate control systems. With regard to the removal of microbial particulates from air, one should also remember that filtration is not the only method available and that HEPA filters are not the only filters capable of removing biological particles from air.

Some additional comments on laminar airflow are probably in order. While there is no doubt of the value of laminar airflow for providing better control of the microbial contamination in many diverse areas, it is important that proper attention be paid to its manner of use. Careful planning for the placement of equipment and supplies and control of the movement of people and objects in the laminar air stream is necessary. It is important to realize

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

that equipment or objects closest to the supply filter wall have the greatest degree of biological protection, while objects further downstream may not be as well protected. Environmental spaces protected with laminar airflow should contain only a minimum of equipment and supplies because cluttering of the work area will disrupt laminar flow patterns.

An important point to stress is that laminar airflow devices provide control over airborne particulate contamination only and will not remove surface contamination. However, unless airborne contamination is controlled, exposed surfaces will become contaminated because surface particle collection always occurs when airborne particles are present. Thus, in operations such as sterility testing, it is still necessary to reduce the microbial load on the outside of materials to avoid false laboratory results. Laminar flow will do an excellent job of maintaining the sterility or cleanliness of an article bathed in the airflow. An awareness of the turbulent air patterns created by the operation is necessary to avoid critical operations in turbulent zones.

Control of Process Water and Liquid Effluents

The implication of these two control systems in minimizing microbial contamination within a manufacturing facility is obvious. If the manufacturing process produces contaminated effluents which can add to the bioburden on the final product, appropriate treatment of the liquid should be considered. When various types of process water are used in manufacturing or in washing the manufacturing machinery, care should be taken that the design and the maintenance of such systems do not contribute also to the bioburden of the product. Caution should be exercised in the use of raw water supplies unless they are frequently and adequately tested for microbial content. Particular attention in the water systems should be given to device or configuration arrangements that are conducive to the multiplication of microorganisms. Experience has shown, for example, that certain systems and filters used to produce deionized water are particularly troublesome and often contain high levels of microorganisms when they are not properly maintained and serviced.

Isolation Equipment for Device Manufacturing

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Although good facility design and good manufacturing procedures

and techniques are often adequate in providing an appropriate degree of environmental control, in some instances additional barrier equipment is desirable. For example, a traditional method is to provide some type of ventilated enclosure or cabinet around specific manufacturing procedures. These so-called primary barriers can obviously be used to protect products from uncontrolled exposure to environmental contaminants, and they can also operate in preventing uncontrolled escape of materials being handled. In rare instances both objectives may be present simultaneously. An absolute barrier is provided by a cabinet that completely encloses the operation with manipulations being done with arm-length attached gloves. Partial barrier cabinets are not completely closed but depend upon airflow and air pressure to provide a barrier condition.

Many variations of isolation equipment have been developed to meet particular needs, and in recent years laminar airflow benches have come into wide use for sterility testing and other critical operations during the manufacture of medical products. As indicated earlier, the type and extent of the use of isolation equipment also relates to the control systems provided by the facility. In large manufacturing operations, experience has shown that emphasis on special isolation equipment placed as close to the manufacturing operation as possible is often more effective and less costly than other methods. Another isolation technique rarely used, but one that nonetheless can be effective, is that of isolating the human occupants in a manufacturing area. Thus it is possible to conduct certain operations in well-constructed clean rooms with the operators in the room wearing ventilated clothing to separate them from the environment of the medical device.

Control of Manufacturing Techniques

The cardinal point to be made with regard to the techniques and manipulations performed by humans in the manufacturing of medical devices is that these techniques should be carefully thought out and designed with microbial control in mind, and they must be well-supervised and conscientiously carried out. Because of the variety of medical devices, it is impossible to prescribe specific rules that should be followed. However, a major contribution to environmental control is achieved when workers are taught the same type of basic procedural rules as are used by operating room scrub nurses in the aseptic handling of materials.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Methods for Sanitation and Hygiene

These are also of major importance in determining the effectiveness of environmental control. The personal hygiene habits of employees and the training and motivation they receive in carrying out hand washing practices and in the wearing of gloves and masks where indicated are most important. In certain situations special lint-free clothing and hair covers can be used as part of the environmental control program. Special shoes are often used along with methods of reducing the tracking of floor contamination. Obviously, it is important to have some method of controlling the involvement of persons who may have a communicable disease or may otherwise have a temporary or permanent health condition that would be undesirable in the manufacturing area.

Proper sanitation through well-designed cleaning, scrubbing, and decontamination procedures of all equipment capable of contaminating the medical product is also a must. With respect to the use of chemical solutions, experience has shown that it is very important to have adequate control over the preparation of the solutions. Moreover, care must be taken to assure that the solutions used have not had their antibacterial properties degraded in some manner.

Determining the Effectiveness of Environmental Control Systems

Effectiveness of environmental control systems can be determined both before and after the fact. Measurement, microbiologically, of the final bioburden of some products ready to be sterilized can and should be done in connection with the establishment of proper sterilization cycles. However, the employment of environmental tests during manufacture will do much to validate the continuous effectiveness of the systems employed. Moreover, for each type of environmental control, there should be established, where possible, quantitative standards or criteria against which to test. Such criteria, together with the use of appropriate testing and microbiological sampling, provide a method of before-the-fact evaluation of environmental control effectiveness.

A short shopping list of some of the types of environmental control tests that can be considered in an evaluation program include:

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

1) Testing of HEPA and other biological air filters.

2) Certification of laminar airflow units.
3) Air velocity and air pressure measurements.
4) Microbiological tests of water and liquid effluents.
5) Microbiological air sampling.
6) Fallout tests with agar plates.
7) Microbiological sampling of surfaces.
8) Testing of germicidal ultraviolet lamps.
9) Testing of chemical disinfectants.
10) Periodic decontamination of cabinets.

CONCLUSIONS

This discussion on the effectiveness of environmental control systems has attempted to emphasize that environmental control is properly considered as a total system that encompasses not only the methods of controlling air, water, raw materials, surface contamination, etc., but includes many other factors, such as management's policy and organization, training and selection of personnel, and health and hygiene practices. These, taken collectively, constitute an essential part of what might be regarded as GMP guidelines used during the manufacture of sterile medical products.

With regard to the specific tools for environmental control, these include elements such as facility design, air quality and movement control, control of water and liquids, use of isolation equipment, control of operational techniques, and methods for sanitation and hygiene.

It has been emphasized also that for the environmental control methods employed in a particular manufacturing situation, testing methodologies are generally available to evaluate the effectiveness of the control methods.

Finally, it should be stated that what has been presented are some techniques and technologies available for environmental control. This does not mean that all are needed to adequately manufacture a particular sterile medical device, nor does it imply that alternate control methods not covered here will not be adequate.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

IMPORTANCE OF BIOBURDEN IN STERILIZATION PROCESSING

William S. Miller

Corporate Quality Assurance
Becton, Dickinson and Company
Rutherford, New Jersey

The subject of bioburden which, in the context of this discussion, means the types and numbers of microorganisms on materials to be sterilized, has received greater emphasis recently. First, discussions of Good Manufacturing Practices (GMPs) for medical devices have necessarily considered sterilization and sterilization practices. GMPs for devices have been in draft stage in the United States for a matter of years in anticipation of medical device legislation. Industry, associations, and the federal government have produced drafts. All deal to a lesser or greater degree with adequate sterilization processes and with sterility testing. These efforts have necessarily followed detailed scrutiny and discussion of our common current practices. As a result, there has been increasing awareness of the inadequacies of certain test procedures advocated by the _United States Pharmacopeia_ (USP), and further, there has been criticism of some biological indicator systems available to us.

The use of product tests as advocated in USP XVIII to confirm sterility is open to serious debate. The subject will not be reviewed here other than to refer to excellent discussions by other authors [1]. The statistical inadequacy of testing as few as ten or twenty products to detect low levels of contamination in large sterilization lots is readily apparent.

There are inherent problems in the use of biological indicators which are not indicative of the natural microbial contamination of the product to be sterilized, but can render the sterilization process a poorly controlled one, or one of overkill. In either

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

case, the assay of biological indicators becomes academic and

there is no real basis for estimating the assurance of sterility following exposure to the sterilization cycle.

The bioburden on manufactured medical devices in most contexts can be estimated. In general, the higher the burden, the more severe the sterilization cycle must be. Conversely, the lower the burden, the lower the cost of sterilization processing. Regardless of level, a valid estimate of sterility assurance can only come from a knowledge of the level of that burden.

For some years we have advocated, where feasible, that sterilization cycles for medical devices be based on quantitative estimates of bioburden on those devices. This approach has a number of advantages to the manufacturer, the medical community and to patients. These advantages are quantitatively related to product cost and to a resulting ability to estimate probability of contamination on sterilized product. The following discussion relates to methods and to the value of bioburden estimates in establishing sterilization cycles.

There is one condition where the estimate of contamination levels would be of inconsequential value. Bioburden levels bear little, if any, relevance to sterilization processes if those processes are absolute, that is, if all living organisms were killed with certainty. However, absolute sterilization in the medical device industry is, to my knowledge, impossible. In terms of volumes, the vast majority of products are sterilized by ethylene oxide or irradiation. Neither process provides absolute assurance that all contaminants are killed. Because of this well recognized, and we should add and emphasize, technically acceptable fact, we deal with the issues of probabilities of contamination on our products which are labeled sterile.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

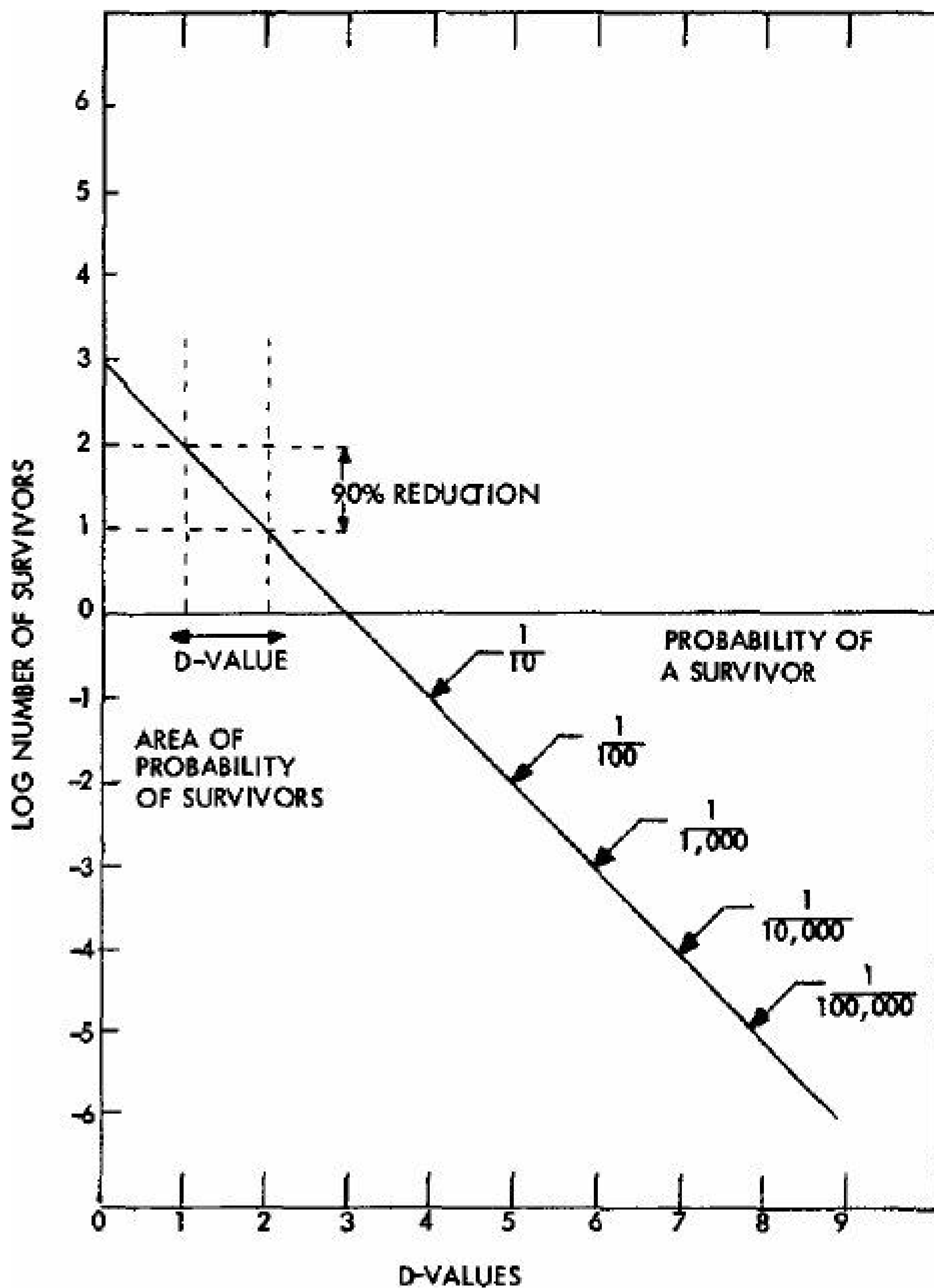


FIGURE 1. Microbial death rate curve and levels of probability of survivors.

Figure 1 illustrates one means of visualizing the effect of the sterilization process on microbial contaminants. The ordinate indicates simply the concentration of viable organisms in logarithms. The abscissa indicates exposure time to a sterilizing agent. Mathematically, the number of organisms decrease to the point of less than one survivor. Therefore, if we consider that the reduction is not absolute, but rather a probability function, then we have a reasonable way to express the effect of further exposure times. This expression is in terms of probability of a survivor. Thus, a D-value (that time of exposure to ethylene oxide gas or to an irradiation dose required to reduce the concentration of organisms by one logarithm) exposure of one survivor results in

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

one chance in ten that that organism will survive. A second D-value exposure will result in odds of one in one hundred that a survivor will occur, and so forth. It is easy to see in this context that one never reaches sterility in the absolute sense. There always remains a probability, no matter how small, that there will be a survivor.

A standard of probability has been widely discussed in the U. S. and in Europe for sterile medical devices. The value is not official in the U. S., and there is certainly justification for greater or lesser levels for certain types of devices or for certain applications in use of devices. For example, one could easily justify the need for a lesser chance of survivors in an implantable device than in a sterile petri dish. The medium for contaminant growth in the first is human tissue. The medium in the second is nonliving material used in vitro.

The commonly accepted value of probability for sterile medical devices is 1×10^{-6} or one chance in one million of contamination. This description, of course, is not a satisfactory one in practice. To the user, it would mean that only one medical device in one million labeled sterile will evidence viable contamination. To the manufacturer, faced with the usual problem of converting concepts into realities, there are difficulties in proving this product characteristic. Obviously, proof through product sampling is not possible.

Our own approach to assuring contamination of less than one in one million relies on the use of biological indicators. These are utilized for cycle establishment with both ethylene oxide and ^{60}Co irradiation processes.

The number of indicators used is based on product contamination levels. In one mode, a highly quantitative contamination estimate is employed to define a sterilization cycle of acceptable effectiveness with maximum efficiency. In the second mode, crude estimates are used to derive an effective but inefficient cycle. Both modes are discussed below.

Quantitative estimates of product contamination lead to well calibrated biological indicators which are acceptable simulators for measuring behavior of organisms on devices. The use of
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.
indicators rather than natural product organisms is based on the

limitations of microbiology and the cold facts of statistical sampling. When one deals, for example, with a sterilizer lot size of 100,000 and an average contamination load of ten organisms per unit, there are ten million contaminants. However, the sensitivity of microbiologic tests is such that assays of survivors on devices are insensitive at this level. Further, a large number of units would be sacrificed in assaying for any assurance of sterility of the lot. With indicators, ten million organisms may be placed on 100 carriers at 1×10^5 organisms per carrier. The carriers are exposed to the cycle with the product load. Their assays provide excellent simulation of the fate of product contaminants.

We have devised a program for such quantitative derivation of cycles. The methods have been described elsewhere [2, 3, 4, 5]. In general, they are suited to high volume, continuous production processes where uniform contamination potential exists.

TABLE I

COUNTS OF AEROBES ON SYRINGES AS FUNCTION OF MANUFACTURING PERIOD

Syringe	Total aerobic count for syringe	
	Hand packaging (1973)	Automatic packaging (1975)
1	8	0
2	5	1
3	184	1
4	2	0
5	1	5
6	1	1
7	1	3
8	2	0
9	15	0
10	8	0
11	8	0
12	1	0

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

13	1	1
14	6	0
15	TNTC*	0
16	1	0
17	3	1
18	0	2
19	0	0
20	0	0

*Too numerous to count.

TABLE II

EFFECT OF EXPOSURE OF SYRINGES TO AMBIENT AIR ON MICROBIAL COUNTS

Syringe treatment	Max count (CFU)+ per syringe	Avg count (CFU)+ per syringe	% Sterile
Control*	10	0.8	60
One week exposure	TNTC	>167	0

*Removed from manufacturing line and assayed immediately.
+Colony forming unit.

Investigations of factors affecting contamination control have indicated that product handling and air exposure are extremely important. With our types of products and processes, we find that consistently low organism numbers exist on products manufactured by automated processes. This is illustrated in Table I. Representative product counts are given for two manufacturing periods for nonsterile, plastic syringes. Time of manufacture, however, is not the critical variable in my opinion. The units manufactured in 1973 were assembled automatically, but were packaged by hand. Those manufactured in 1975 were packaged automatically, thus eliminating any chance of human contact. These results illustrate a cardinal rule for minimal contamination, i. e., avoid handling of product parts. A second rule for uniformly low contamination levels is to avoid prolonged exposure to factory air. Table II illustrates the effect on counts of exposing device

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

components to plant air for one week as opposed to normal manufacturing where the exposure time on uncovered lines or in open containers is less than eight hours. Marked changes occur in terms of maximum count per syringe, average count per syringe and the percentage of units which are sterile. The latter value ranges as high as 90%, because of the in-process treatments, including high temperature molding.

With well controlled manufacturing conditions, the numbers of organisms contaminating products can be reliably estimated. For example, it was found in one study involving over one year's work that the total number of organisms on loads of 225,000 syringes was 85,000 with the probability of exceeding this number being one in over 1,000,000. Estimates such as these provide the number standard for a biological indicator to be used to establish a cycle.

A second characteristic of the spore indicators is that they must exhibit a resistance to sterilization processes which exceeds that of all normal product contaminants. The referenced papers contain descriptions of the techniques and analyses used to confirm this property. Table III illustrates representative data from a trial comparing the resistance of spores to that of organisms on products. Non-sterile devices, and indicators located on paper strips are exposed simultaneously to sterilant. The results of sterility tests for graded exposure times provide fractional responses. From these it is simple to construct curves showing death rates and to compare them. From Table I it is apparent that the rate of sterilization for products is greater than that for indicators.

TABLE III

STERILITY TESTS OF PRODUCTS AND BIOLOGICAL INDICATORS FOR RESISTANCE COMPARISONS

EtO gas contact time (min)	FT Medium	Products (no. pos/no. tested)	SCD Medium	Biological indicators (no. pos/no. tested)
X	2/40		3/40	9/10
3X	1/40		0/40	5/10

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

4.5X	0/40	0/40	0/10
7.5X	0/40	0/40	0/10

FT = Fluid thioglycolate
SCD = Soy casein digest

The response of indicators is also used for another purpose. From the spore curve, it is possible to calculate an approximate D-value. This value, calculated for ethylene oxide processes in minutes, indicates the time interval required to reduce spore strip viability by a factor of ten. On the basis of this estimate, we can return to Figure 1 for computation of the time required to reduce strip viability to zero and then proceed to add six additional D-values for an assurance of sterility at the 1×10^{-6} level.

It is clear that with this approach, a cycle of minimum time with an estimate of probability of sterility is obtained. It should be emphasized that an extensive period of time is required to obtain adequate data for a program such as this. Further, a given set of data is of value for only a relatively narrow product range. Any significant difference in process, materials, or environment which can affect the microbial load appreciably requires a separate program for estimation of contamination level and resistance comparisons.

If the quantitative measurement of contamination levels is impractical, as may be true for very low volumes, irregular production or for new products, what is the valid approach to sterilization cycles? In these cases, we employ the traditional approach of overkill backed by our technical judgment and past experience. In terms of controls one would rely, for example, on a hygienic environment for manufacturing, clean product components, and processes which introduce minimum contamination. Obviously a sampling of products intermittently is desirable to detect and eliminate unreasonable microbial levels by suitable manufacturing modifications. Other controls for establishing a valid cycle and for monitoring cycles routinely include the use of well-calibrated biological indicator spores. In our own operations we use up to 100 spore strips at 1×10^5 spores per strip. Obviously, the significant physical parameters of a cycle are measured routinely as a matter of good practice.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

The cycle time, in the case of ethylene oxide, is selected subjectively, is regidly certified, and is beyond doubt a case of overkill. The cycles may vary from 6 to 18 hours, and are calibrated to assure kill of a high count of biological indicators. Further, products are sterility tested after the cycle to assure that indicators are not providing nonrepresentative results. The statistical limitations to tests of 20 products out of one-quarter million in the lot were referenced previously.

The total effect of a subjective approach to cycle establishment is, in my view, safety of products but to an undefined degree. Further, we must suspect inefficiency of the cycle with probable gross overkill and with unnecessary testing. However, both factors are irreducible. The risks of reducing cycles to a level which will just kill an arbitrary biological indicator are too great to undertake. The assurance of safety provided by hours of exposure to gas in a traditional cycle length is great. The only way to improve efficiency is by way of the quantitative approach described earlier.

In summary, the greatest efficiency for sterilization processes in manufacturing lies in the use of quantitative contamination data. Lacking this, intermittent estimates which form the basis of adequate cycles are acceptable. However, these in my view result in inefficiency in processes due to overkill.

REFERENCES

1. Pflug, I. J. (1973). Heat sterilization. In Industrial Sterilization. G. B. Phillips and W. S. Miller, Eds. Duke University Press, Durham, N. C.; pp. 272-276.

2. Miller, W. S. (1972). Establishment of ethylene oxide sterilization cycles. Bull. Parenter. Drug Assoc. 26:34-40.

3. Berube, R. (1974). Control and quantitation of the sterilization process. Presented at the 17th Biological Safety Conference, Raleigh, N. C., Oct. 15-17.

4. Berube, R. (1974). Certification of biological indicator controls for industrial sterilization. Presented at the 74th Annual Meeting of the American Society of Microbiology, Chicago, Ill., May 12-17.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

5. Miller, W. S. (1975). The role of production hygiene in sterilization control. Presented at the Contamination Control Symposium, Gothenburg, Sweden.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

MICROBIOLOGICAL CONTROL OF STERILIZATION PROCESSES

Karl Kereluk

Ethicon, Inc.
Somerville, New Jersey

INTRODUCTION

Microbiological control of sterilization processes for many years was exercised under the impression that sterility was absolute in concept, and by definition when the product passed a conventional sterility test, e.g., the test given in the United States Pharmacopeia (USP), it could be considered sterile. Inference of sterility through utilization of biological indicators has been a more accepted approach during the last two decades. Although the limitations of the sterility test are now being recognized, it has only recently been widely accepted that sterility is impossible to verify in the absolute sense. Kelsey [1], in a recent article entitled, "The Myth of Surgical Sterility" has concluded the following:

> "Although sterility is in theory an absolute term, in practice it may only be regarded as at best relative and at worst misleading. It is a philosophical concept that can never be unequivocally demonstrated in a real world. Experience has shown that it is virtually impossible, even if it is honest, to change the definition of a term that has been in use for many years; we may need a new term to indicate 'the state of having been sufficiently freed from microorganisms to be deemed safe for some special purpose by some competent body'. The abandonment of the term 'sterility' and the acceptance of some other term would remove confusion and enable the important matter of providing microbiologically safe medical products to be more rationally and realistically considered."

The proof or demonstration of sterility by industrial microbiologists for any sterilization process has been achieved by the use of a microbial spore challenge. This microbial challenge generally consisted of exposing a resistant species of

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

microorganism, usually a bacterial spore preparation, to the process. The level or number of spores deposited on a substrate is high and reflects an abnormal situation which would not be encountered in everyday use. It is assumed that when a microbial challenge has been made to some lethal agent and no viable growth of organisms is observed upon incubation in an appropriate sterile culture medium under optimum conditions, then the load may be considered to have been exposed to sterilizing conditions. Such microbial challenges are generally referred to as biological indicators (BI).

Thus, BIs indicate sterility or sterilization by inference. This is true if they are used as a microbiological process control to validate a sterilizer, or to develop an exposure time for a particular packaged medical device or product. When medical products and/or devices are sterilized with an adequate "margin of safety"*, and the BI tests are shown to be negative, it is assumed that the articles so processed are sterile.

The use of BIs and their acceptance as a sterilization process control are gaining recognition, and some authorities believe that the use of BIs should replace the USP sterility test.

HISTORY OF BIOLOGICAL INDICATORS

The first recorded use of BIs, as microbiologists know and use them today, was by Koch in 1881 [2]. Koch employed a variety of microorganisms including spore preparations on various carriers such as filter paper strips, glass plates, and silk thread. At first, all the BIs used by Koch were challenged to dry heat; later, he used steam.

Shortly thereafter, in 1891 [3], F. B. Kilmer, the first Research Director of Johnson & Johnson, used a mixed culture slurry (probably a pure culture of anthrax) on gauze patches to challenge a sterilization process. It is important to note that Kilmer was the first to use a BI in a manufacturing operation, and to release commercial products as sterile based upon bacteriological testing. The following is a direct quote from his article published in 1897 and reproduced elsewhere in these Proceedings:

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

"A portion of the dressing material (for example, a piece of

gauze) is impregnated with an infected nutrient fluid. The thus infected material is then dried in air, that the organisms may, as far as possible, be placed in a resistant condition. As a check experiment, a portion of this infected and dried material is placed in sterilized nutrient jelly in the culture chamber. This is done to ascertain whether the test material has surely been infected. The remaining portion of the infected material is then passed through the sterilization process, care being taken that it passes through like conditions as would the sterilized dressings.

"In the case of gauze or cotton, the writer's practice is to wrap the test material in the centre of the package.

"In testing catgut ligatures, the ligatures are moistened and untwisted; the infected material is then rolled up within the tissue and dried. After the infected material has passed through the sterilization processes, it is placed in nutrient media in a culture chamber. After a suitable time (at least three days) if a growth is found in the check experiment, we are certain that our test material was infected. If no growth has taken place in the infected material, that has passed through the sterilization processes, we are certain that sterilization has been complete in all the dressings. This conclusion needs no verification. The dressings have been prepared and sterilized by methods which exclude contamination. If a certain portion of material purposely infected, in passing through the sterilization process with them, is rendered sterile, it is conclusive proof that the whole of the dressings cannot fail to be sterile and aseptic."

A British physician at the end of the 19th century reported using eggs as a convenient "biological" indicator. Raw eggs were placed within the load of the sterilizer and if, upon completion of the exposure, the eggs were "hard-boiled", the physician considered his load sterile. Although the method was crude, to say the least, and quite doubtful as a "true indicator of microbiological sterility", it should be noted that the physician even at that time appreciated the need for a method of determining the efficacy of his sterilizer.

There have been many variations in the form and use of biological indicators, ranging from the use of the garden soil approach of many German microbiologists, to the inoculated carrier

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

concepts employed by many of today's manufacturers of sterile disposable devices. For example, Ecker in 1937 [4], proposed using a BI consisting of air-dried and powdered garden soil (one gram) in a paper package inserted into the center of a test pack or surgical drum. Many other European microbiologists have used Ecker's method or modified it [5, 6, 7] for determining the sterilization capability of their sterilizers. It is their argument for this type of soil BI that soil samples as test materials supposedly simulate practical conditions under which surgical and medical supplies become contaminated. However, it is the opinion and concensus of modern microbiologists that "clean" spore preparations of a single bacterial species deposited upon paper strips are best suited for BIs because they are more reproducible than the garden soil BI, which may have several spore-forming species with varied populations within any given soil sample. An extension of the inoculated paper strip idea is the actual inoculation of product with a predetermined level of a specific microbial species. During the last decade, the use of inoculated product has been encouraged by USP XVIII and XIX. Incentives for the use of inoculated product BIs are reduced sample size and early release of products under documented optimal conditions.

A summary of the recognized minimum requirements of various sterilization agents and the appropriate BIs with suggested decimal reduction expression is given in Table I. The table also presents the terminology used later in this report.

The use of Bacillus stearothermophilus as the microorganism of choice in the BI for moist heat (steam) sterilization was recognized by the National Institutes of Health (NIH) in 1955, who recommended it as a sterilizer control or monitor in licensed establishments preparing biologicals. This organism lacks pathogenicity, pyrogenicity and toxicity. It was also recognized by NIH that, as a thermophilic microorganism, it would not grow at the official NIH and USP sterility test incubation temperature of 30-35°C [8].

TABLE I

MINIMAL REQUIREMENTS FOR STERILIZATION AGENTS AND BIOLOGICAL INDICATOR ORGANISMS

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Sterilization agent	Minimum requirement			Organism and level	D-value expression $D_{(\quad)} =$	Interception point of spore destruction curve
	Temperature °F	Concentration	Time			
Steam	250	NA	12 min	B. stearo-thermophilus 10^5	$D_{250°F} =$	TDT
	270	NA	2 min		$D_{270°F} =$	
Dry heat	320	NA	1-2 hr	B. subtilis var. niger 10^6	$D_{320°F} =$	TDT
	356	NA	½ hr		$D_{356°F} =$	
Ethylene oxide	Ambient to 130	Variable (0-1200 mg/l)	1-24 hr	B. subtilis var. niger 10^6	$D_{(Temp)}$ used (Conc) used =	TCDT
Radiation	NA	NA	Dose (Mrad) variable with time	B. pumilus 2×10^4	$D_{Mrad} =$	MDD

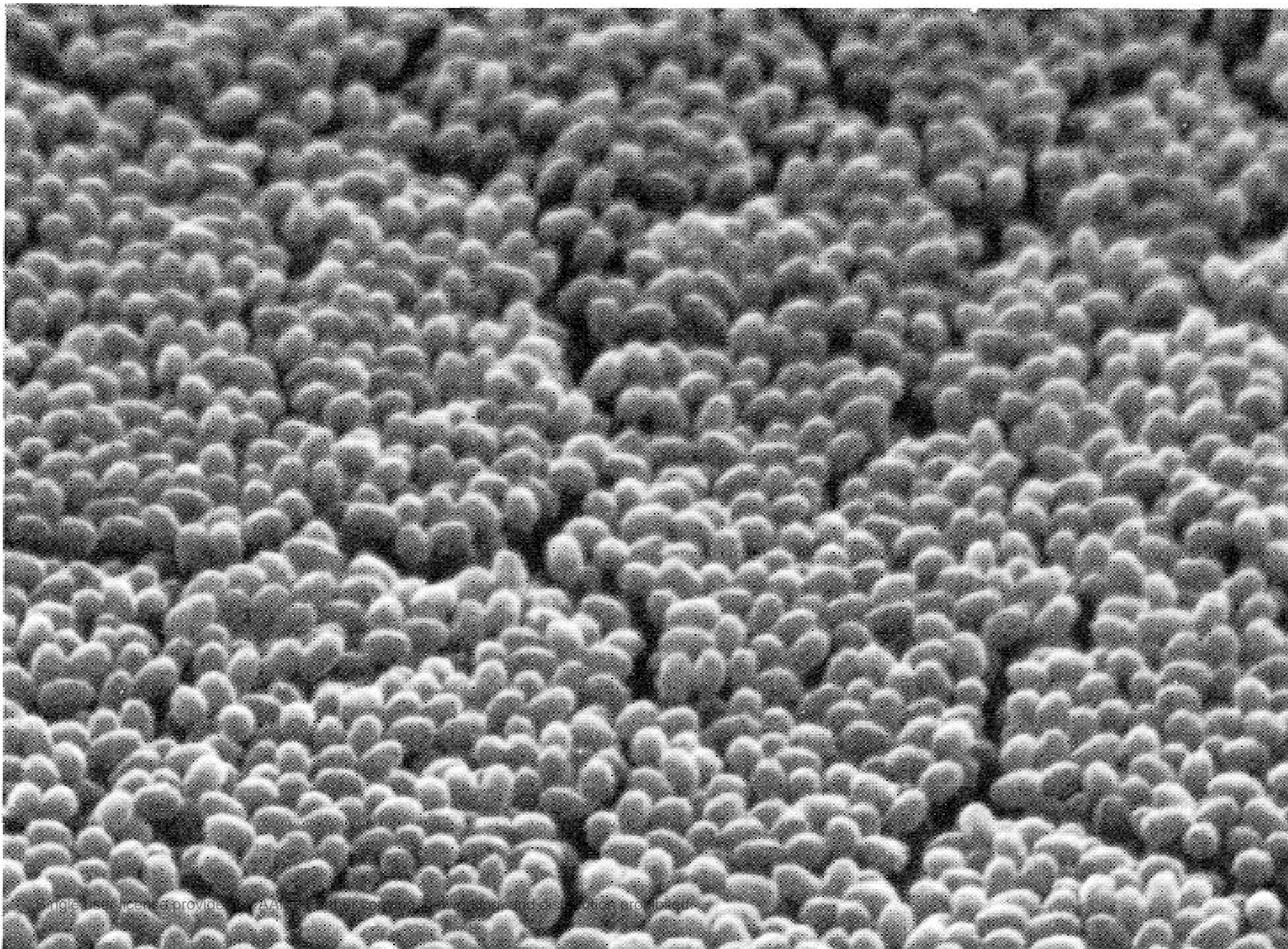
NA = Not applicable
TDT = Thermal death time
TCDT = Thermochemical death time
MDD = Mrad death dose

Since the early days of cobalt-60 sterilization, microbiologists at Ethicon, Inc. chose to work with spores of Bacillus pumilus for their biological process control. The particular organism, B. pumilus E601, was characterized and deposited with the American Type Culture Collection (ATCC) and has been given the number 27142. This organism has become accepted as the biological reference for radiation sterilization in the United States and Canada, as well as in many other countries in Europe and the Far East. An inoculum of 2×10^4 spores of B. pumilus, directly pipetted onto a suture, has been our process challenge for radiation sterilization, both electron beam which commenced in 1956, and cobalt-60 since 1964. Figure 1 shows a clean B. pumilus preparation deposited on a cotton suture, magnified 10,500 times.

Biological indicators, as we know and use them today (spore preparations on paper strips), were first introduced as a commercial product in the fall of 1957, at the American Hospital Association meeting in Chicago, by the American Sterilizer Company (AMSCO) of Erie, Pennsylvania. The Castle Company, two years later, also introduced commercial BIs very similar to those of AMSCO. Other manufacturers such as Baltimore Biological Labs,

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Colab, and 3M Company followed later. Today there are as many as eight manufacturers of BIs, and they are as varied as the number of manufacturers producing them. The performance of the different BIs also varies [9, 10]. Prior to 1958, AMSCO had been constantly besieged with questions from the Central Service Departments of hospitals concerning methods of assuring the sterility of exposed materials or the efficiency of their sterilizers. Prior to 1958, Brewer, et al., at Hynson, Westcott and Dunning [11], had been equally concerned about the cost and limitations of sterility tests and described a control for sterilizing procedures. The control used a thermophilic spore forming organism which was neither “pathogenic nor a strong producer of pyrogens”. The organism would not grow at ordinary storage temperatures of biologicals or hospital solutions. A complete description of the preparation of BIs was eventually presented in the Journal of Pharmaceutical Sciences [12]. A standardized population of 1.9×10^7 on paper strips survived for 12.4 minutes at 250°F in a steam autoclave, but was killed in 14 minutes at the same temperature.



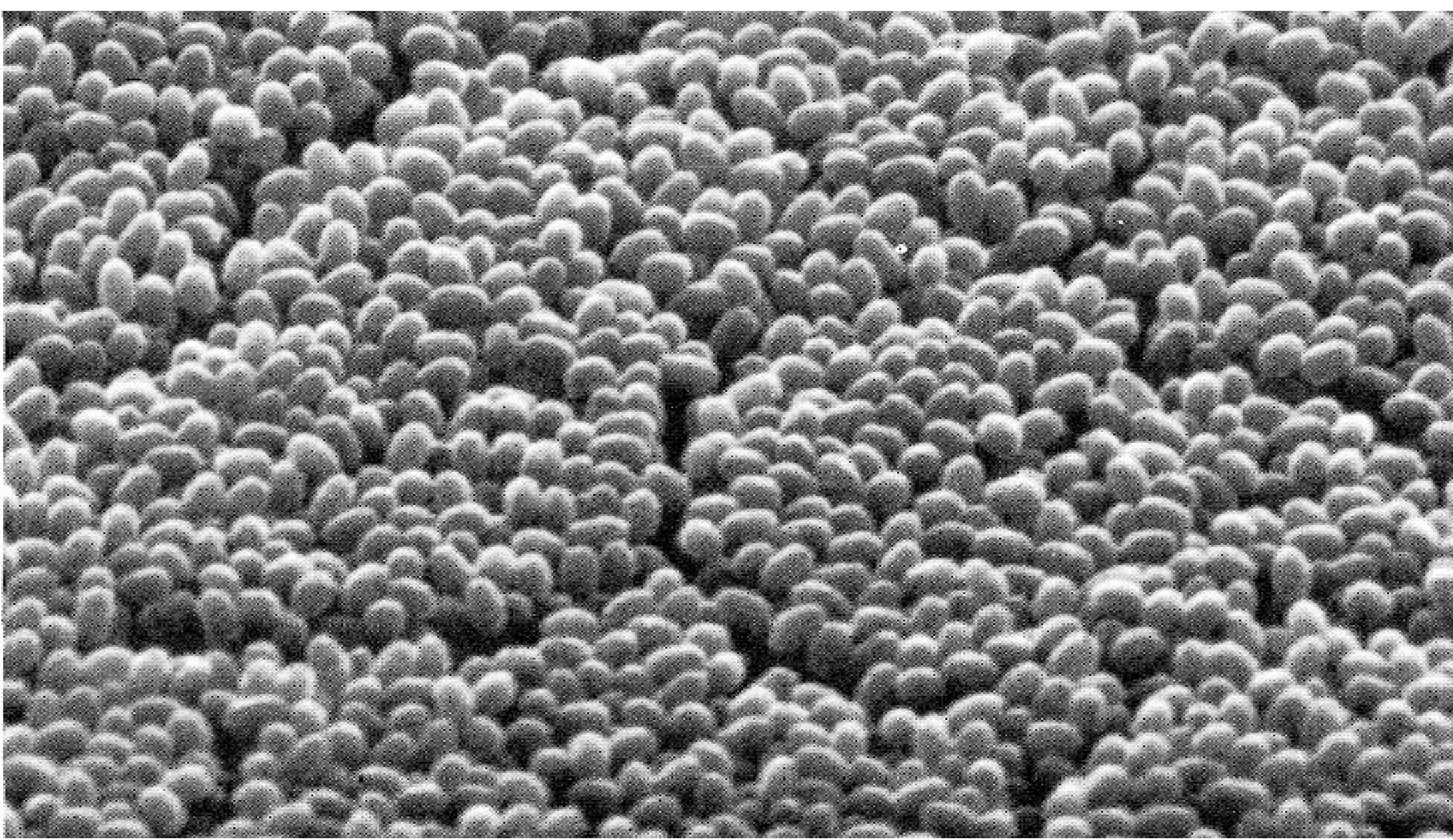


FIGURE 1. A clean preparation of Bacillus pumilus spores. Magnification: × 10,500.

All in all, however, the basic concepts of Koch and Kilmer have not changed. The use of anthrax spores on a thread, (Dr. Koch's method), in my opinion, is no different in concept than that of the current AOAC sporicidal test employing Clostridium sporogenes on four silk suture loops. The intentional inoculation of gauze and gut sutures with "infected nutrient material" conceived by Kilmer in the late 1800s as a microbiological challenge test for sterility is similar to what we are currently doing at Ethicon, Inc. today, with the intentional contamination of sutures with B. pumilus as BI challenge.

There have been no further changes, discoveries, or technological breakthroughs in microbiological process control for sterilization of medical products since Kilmer's work. However, strides have been made in food processing, where the importance of microbial destruction of the botulism organism led microbiologists to develop the kinetics of microbial destruction by heat as we know them today. We have really borrowed the food microbiologists' approaches for microbiological process control, and adapted them for use in the manufacture of sterile medical products treated with other sterilants. Yet, there still remains a need for

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

improvement in our microbiological process controls and greater insights into our current methods of using BIs.

PERFORMANCE OF BIOLOGICAL INDICATORS

There are recent publications citing the performance of BIs for steam and ethylene oxide sterilization [9, 10]. These investigations on BIs, as they are manufactured today, generally deal with spore populations impregnated on paper strips designed to meet the performance requirement of surviving a certain period of time, but being killed in an additional time increment. The results of these investigations indicate a wide variation in resistance among the commercially prepared BIs used in steam and ethylene oxide sterilizers. Thus, at 250°F in steam, only two out of seven of the commercial BIs performed according to their label specifications. Similar results were obtained for ethylene oxide exposures where only three out of six met their labeled specifications. To date there has been no published survey of performance data (survival and kill) for BIs used in radiation sterilization such as exist for steam and ethylene oxide sterilization. This is probably due to the fact that most users of cobalt-60 sterilization produce their own BIs and perform only the in-house quality assurance tests they deem necessary.

Performance of *Bacillus pumilus* as Biological Indicator for Radiation Sterilization

Ethicon, Inc. has employed *B. pumilus* E601 for over 12 years as BI for cobalt-60 sterilization. The Ethicon, Inc. strain E601 of *B. pumilus* was characterized and deposited with the ATCC and was assigned the number 27142. As mentioned earlier, this organism has become the biological reference standard for radiation sterilization in the United States and Canada, as well as in many other countries in Europe and the Far East.

One of the advantages of the cobalt-60 sterilization process over steam and ethylene oxide is its simplicity and reproducibility. The absorbed dose in megarads (Mrads) can easily and reliably be measured by any of several physical-chemical dosimetric methods, whereas in all other sterilization processes, microbiological variability in resistance to various processes still remains to be adequately controlled. Optimal conditions of

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

recovery have been developed with the use of B. pumilus as microbiological control for irradiation sterilization. Zuk et al. [13] have indicated the following optimal conditions for B. pumilus:

Temperature of incubation	- 32°C
Incubation time	- 7 days
Recovery medium	- Columbia Broth
pH	- 7.0-8.0

It is under these optimum conditions that microbiological variation and resistance may be minimized. Biological indicators for the suture sterilization program at Ethicon, Inc. consist of inoculated product (sutures), as they are convenient to make and also employ actual production material rather than paper strips or other carriers. Standard Size 1 Sutupak* sterile sutures containing 17-18 inch strands (e. g., cotton) in an aluminum foil packet are inoculated with 0.2 ml of a washed spore suspension of 10^5 B. pumilus E601 suspended in 80% isopropyl alcohol - 20% water.

Figure 2 depicts a clean spore preparation of B. pumilus deposited upon an unglazed cotton suture, at a magnification of 10,500 times; in Figure 3, the same preparation is magnified 70,000 times, showing the topographical characteristics of B. pumilus spores.

Using the above optimal conditions for the B. pumilus BI, Figure 4 presents a typical destruction curve for spores contained in a glass vial (wet preparation) after exposure to incremental doses of cobalt-60 irradiation. A decimal reduction value (D_{Mrad}) can be determined from such a spore destruction curve. D_{Mrad} is defined as the amount of absorbed dose that will reduce a microbial population by 90%, but this is only applicable to pure culture work. There are several references which point out the difficulty in obtaining reproducible values using heterogeneous microbial populations [14, 15, 16]. In our case, a D_{Mrad} of 0.30 was obtained.

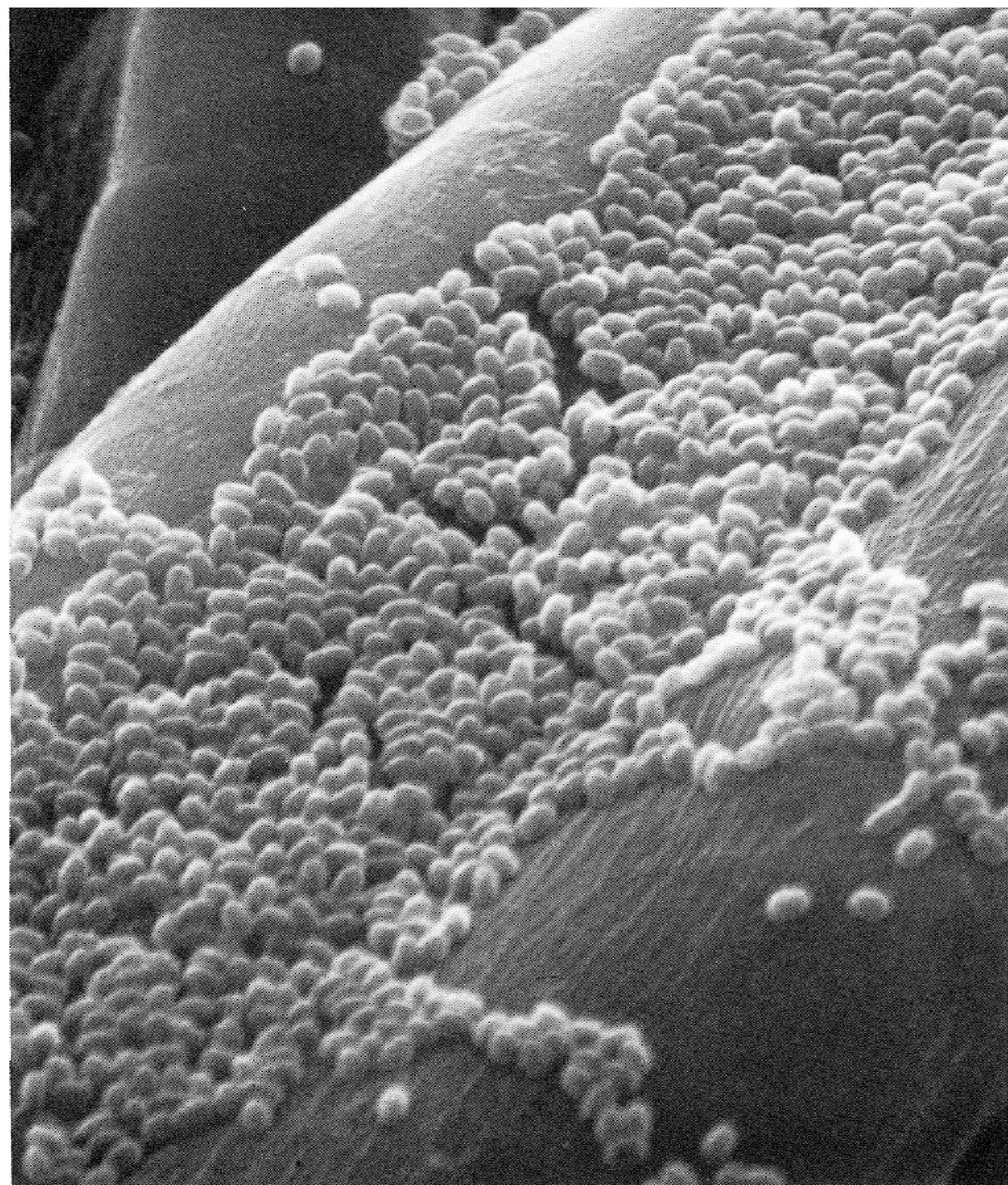


FIGURE 2. A clean preparation of _Bacillus_ _pumilus_ spores deposited on unglazed cotton suture. Magnification: × 10,500.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

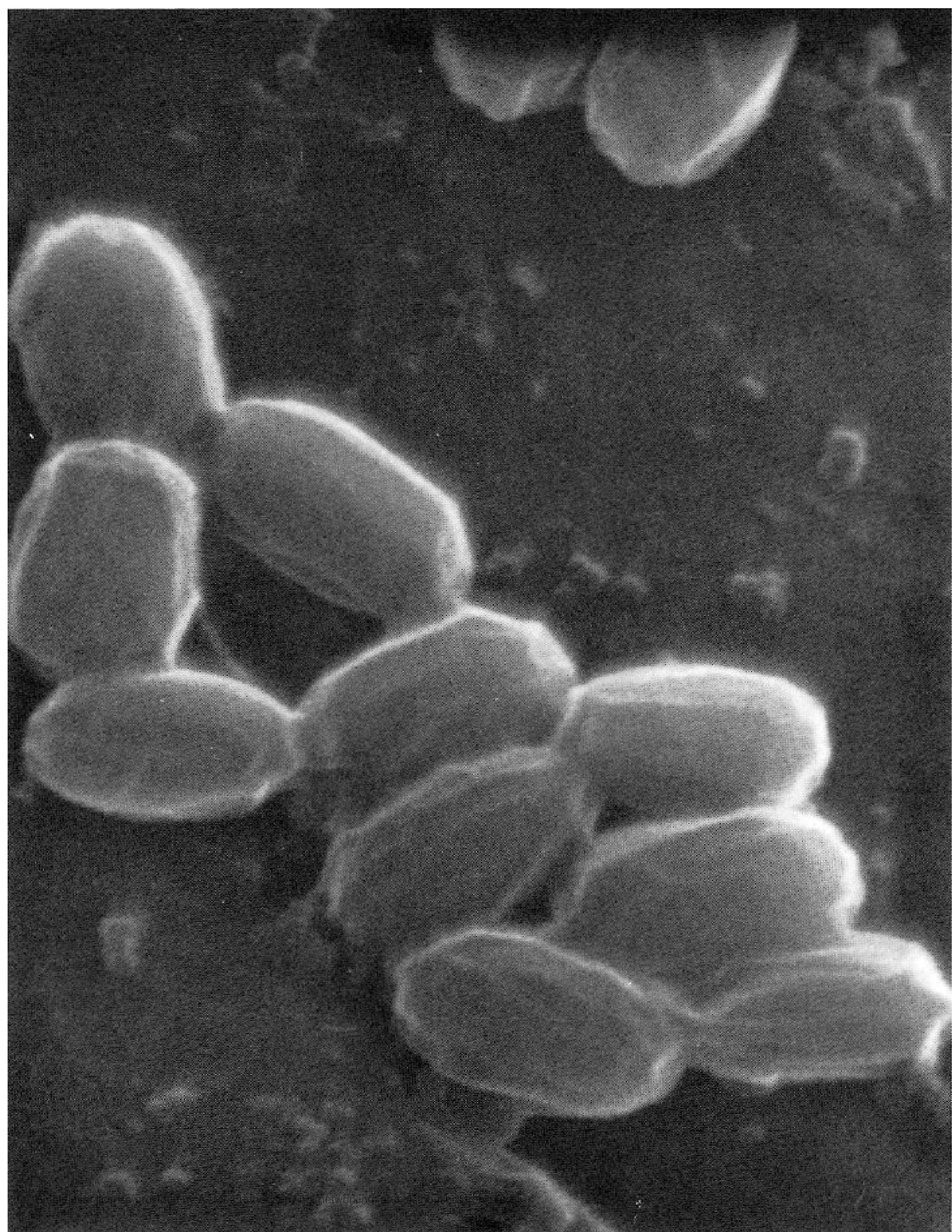


FIGURE 3. Spore preparation of Bacillus pumilus. Magnification: × 70,000.

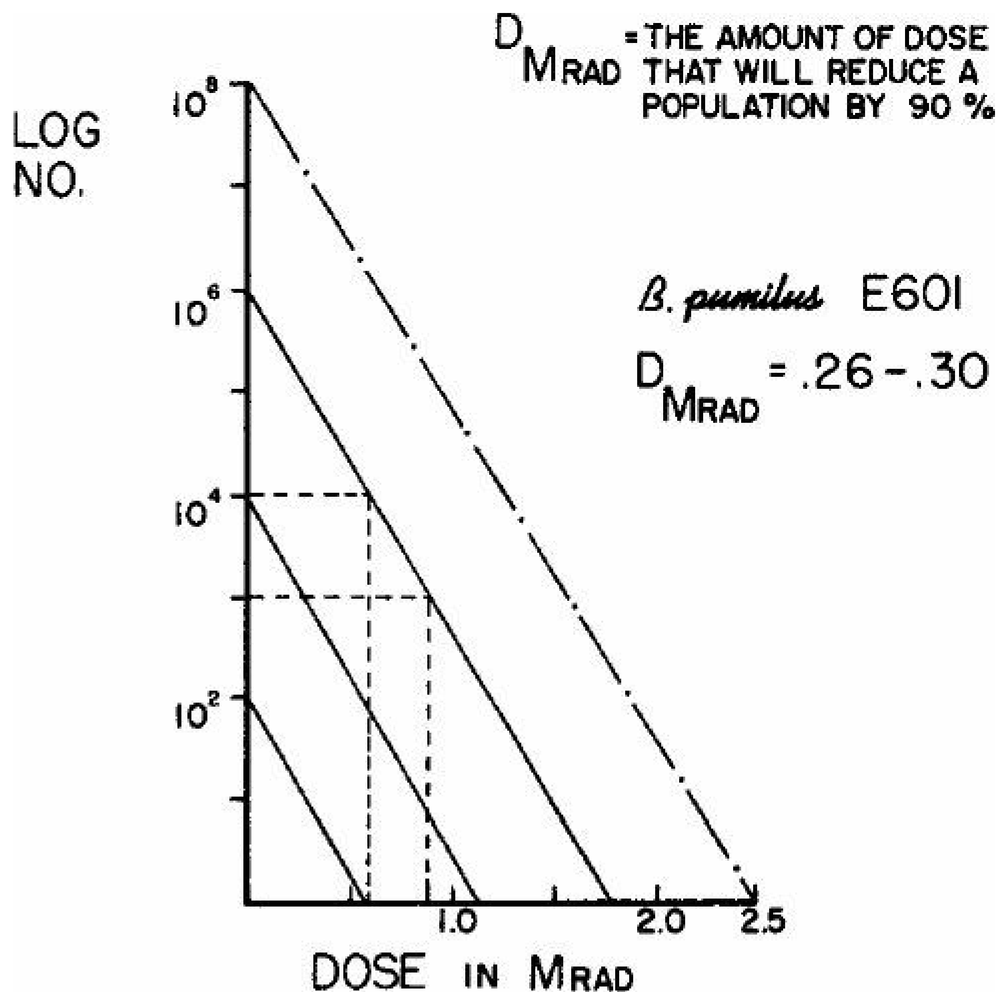


FIGURE 4. Determination of D_{Mrad}-value for a pure culture of Bacillus pumilus spores to obtain a decimal reduction dose.

The kinetics of microbial destruction employing the most widely accepted sterilization agents have been amply covered in many other publications [14, 15, 17]. Historically, it has been generally accepted that sterility is an absolute phenomenon with any of these agents, but the advent of a "probabilistic" approach recognizes that positives can occur in microbiological process controls (use of BIs) even under the most rigidly controlled conditions. The presence of a positive has been best interpreted as a probability function, i. e., a probability of having a nonsterile product or BI. Figure 5 depicts the spore destruction curve of B. pumilus E601 having a D_{Mrad} of 0.3 (wet preparation in glass vial) extrapolated to a probability of having a survivor. The probability extrapolation function is depicted as the probability of a BI being positive. It can be seen from the figure

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

that a wet preparation of 2×10^4 B. pumilus spores exposed to incremental doses of radiation extrapolates to give a probability of one positive in 10,500 BIs. Table II summarizes the history, on an annual basis, of the number of positive BIs recovered from irradiated products at Ethicon, Inc. since 1964. Of some million inoculated sutures using 2×10^4 B. pumilus spores, to date there have been only 44 positives. If one were to determine the actual or "under use conditions" probability of a positive, the number should be close to 1:23,000. If one were to note this probability on Figure 6 and draw a straight line back up to the starting point of 2×10^4 spores of B. pumilus, the intercept would be just under 1.2 Mrads. A "back" calculation would reveal a decimal reduction value (D_{Mrad}) for the "in use condition" of the inoculated sutures to be 0.29.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

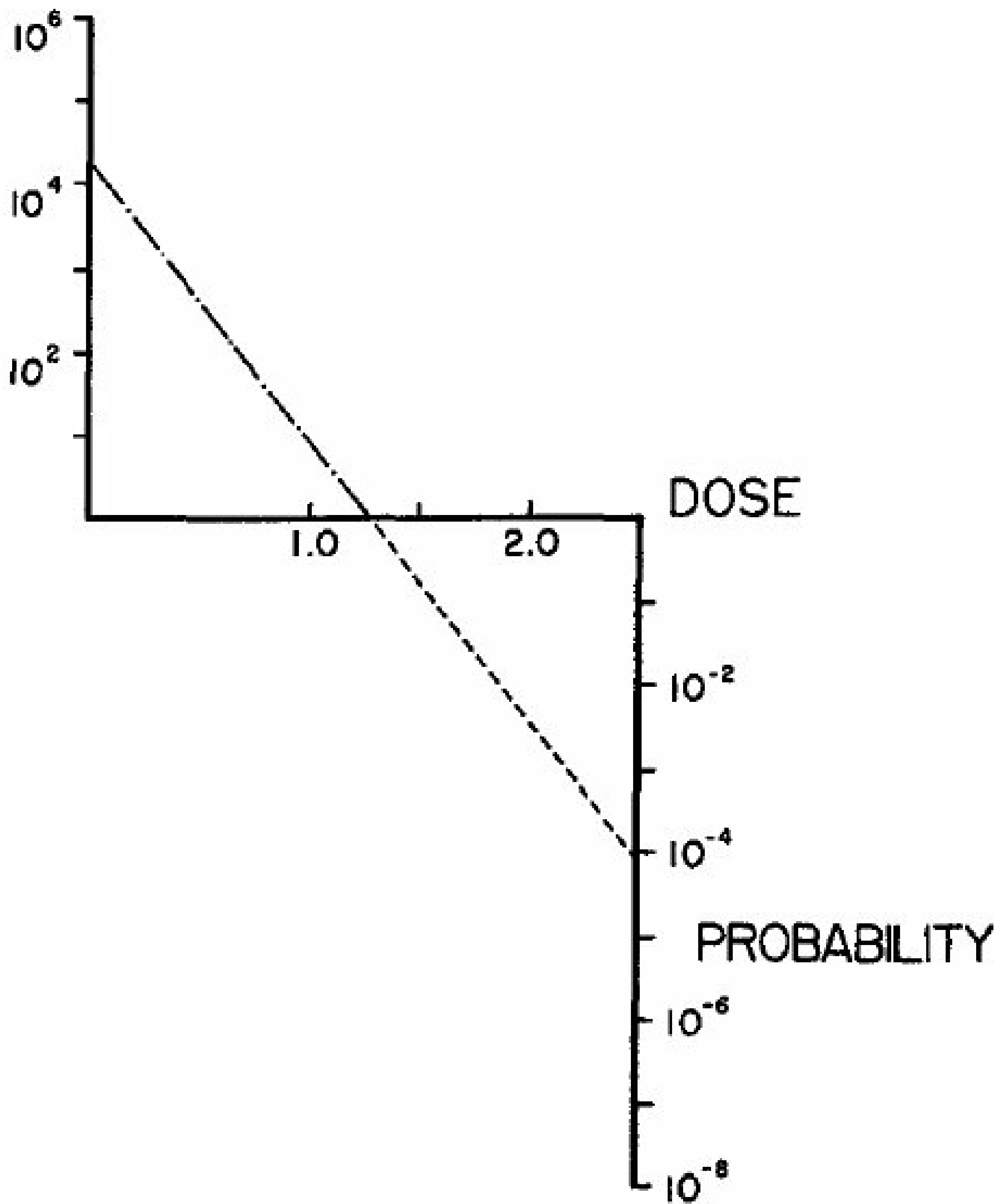


FIGURE 5. A typical spore destruction curve for *Bacillus pumilus* E601, and extrapolation to a probability of a positive BI.

TABLE II

PERFORMANCE OF *BACILLUS PUMILUS* BIs (2×10^4)

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

1964 - 1975

Year	No. of BIs tested	No. of BI positives/year
1964	53,092	7
1965	161,466	15
1966	163,427	3
1967	138,302	7
1968	83,986	2
1969	73,171	2
1970	55,171	3
1971	61,780	2
1972	62,428	0
1973	85,747	1
1974	45,910	2
1975	31,589	0
Totals	1,016,069	44

In the light of the above, the continued use of BIs of *B. pumilus* spores for routine, conventional sterility testing seems to be a futile exercise in reestablishing the probabilities of a positive. With the historically proven reliability and reproducibility of the irradiation process, its inherent simplicity, and highly predictable effect on spore preparations of *B. pumilus,* and the increased recognition that physical-chemical dosimeters are more accurate than biological preparations, continuation of the routine use of BIs or product sterility testing becomes open to question in irradiation processing.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

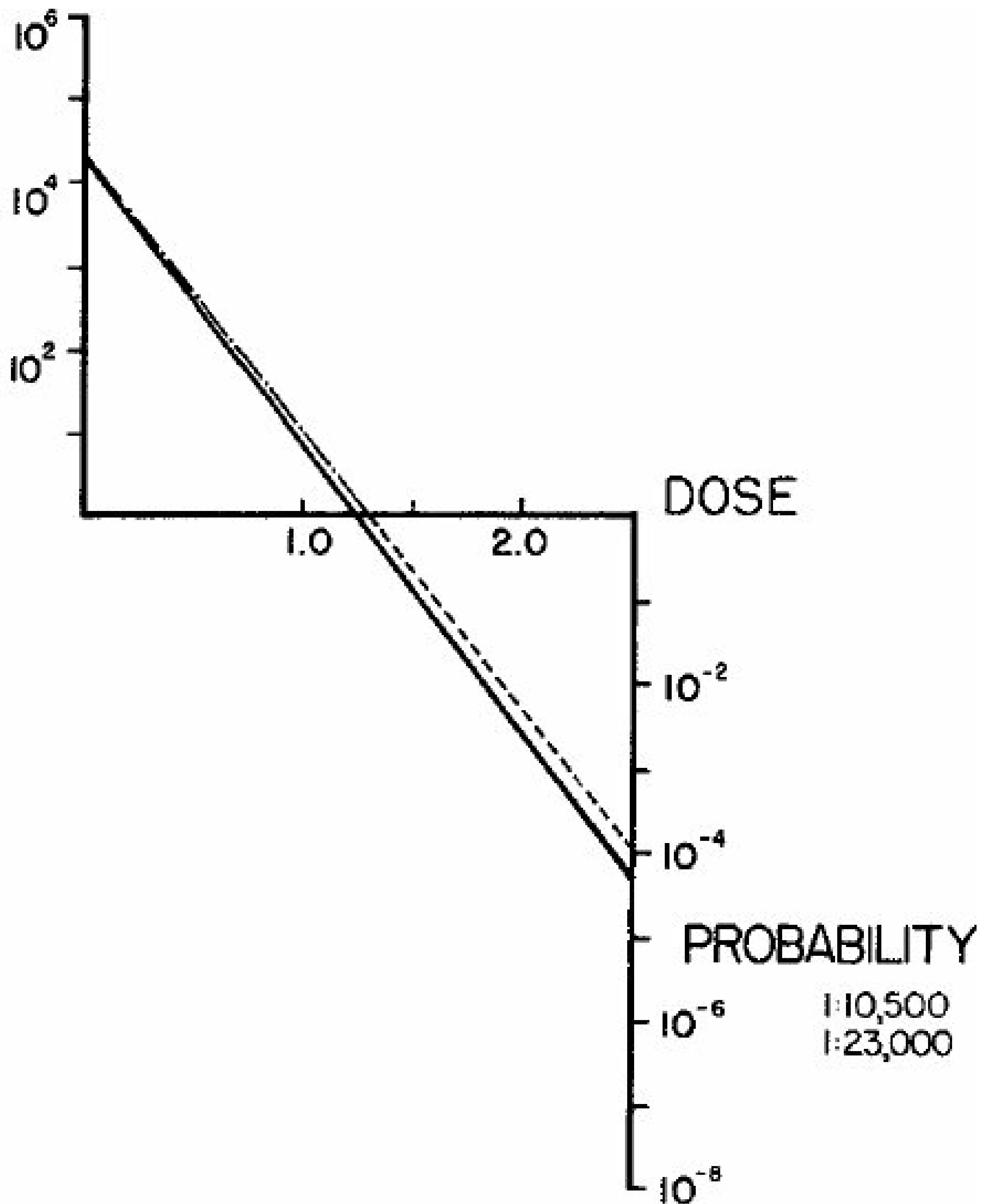


FIGURE 6. The probability of obtaining a nonsterile biological indicator (Bacillus pumilus) by a spore destruction curve and probability of a nonsterile biological indicator, from 12 years' experience.

One must consider the use of dosimetric methods for the routine control of a radiation sterilization process, and shift

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

microbiological efforts from routine use of BIs and product sterility testing to periodic monitoring of the actual microbiological parameters which are pertinent. In addition to the use of dosimetry, microbiological profiles should be provided by auditing the following:

1) Determination of the presterilization microbiological levels on products (bioburden) according to types and resistance of the organisms.

2) Enumeration of viable and nonviable particulates of selected environmental air samples and the enumeration of selected surfaces for microbial content.

3) Determination of the Mrad death dose for finished products with its indigenous microbial flora.

The above parameters could be considered as a "dosimetry release program" or plan which will give assurance that the highest sterility "level" or the greatest probability of not having a nonsterile product is obtained for radiation sterilization.

GRADED BIOLOGICAL INDICATORS

A single level microbiological preparation has been used for approximately the last 20 years to monitor a sterilizing process or agent and to develop an exposure time for sterilizing different packs, items, etc. During this time, microbial kill has been measured in terms of growth and no growth of the single level BI. Furthermore, a 14-day incubation period was required to give sufficient recovery time for process-damaged spores. The use of a single level BI was far from efficient because it required considerable repetition of incremental exposures before a selected exposure time could be arrived at.

It became evident to one manufacturer of biological indicators [18] that if bacteriological death is essentially logarithmic and the death rate (K) is constant under reproducible sterilizing conditions, then the initial number of spores used on a BI would influence the exposure time required to bring the count down to zero (or to unity on a log scale). This manufacturer pursued the idea of producing a graded biological indicator (GBI) using separate strips of paper impregnated with 10^2 to 10^8 spores,

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

respectively, i. e., each strip contained one level of spores. This was the beginning of a GBI, referred to as the Spordex Scale*.

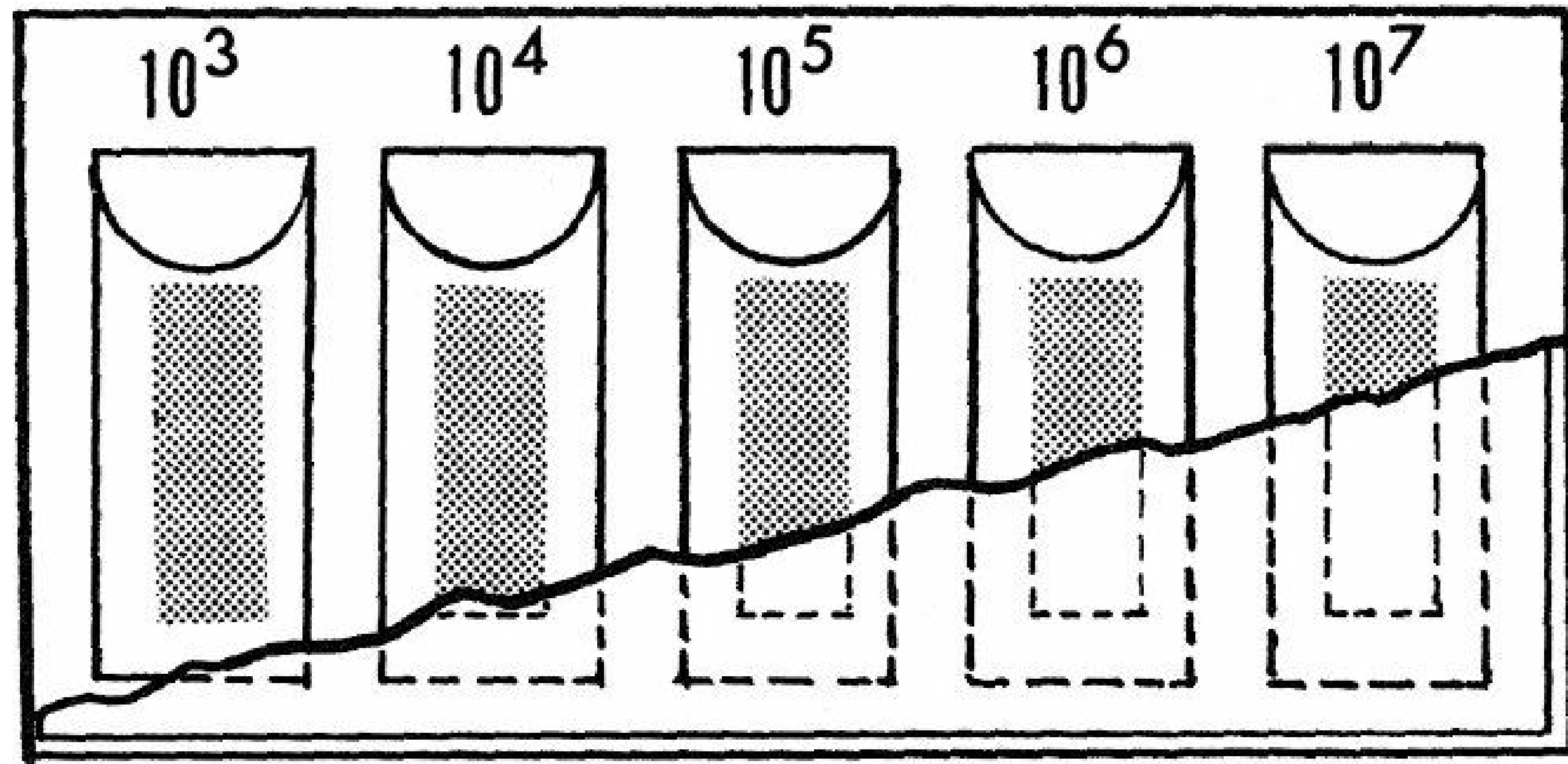


FIGURE 7. A graded biological indicator set.

The GBI, an extension of current BI technology, will enable an investigator to determine quickly a decimal reduction value after only two exposures, determine a “margin of safety”, as well as extrapolate an approximation of the probability of a spore survivor. In addition, kill or no kill (growth or no growth) levels can be established in 24 to 48 hours, in place of the 14- or 7-day incubation times associated with single level BI preparations.

Graded Biological Indicators and Ethylene Oxide Sterilization

The value of the GBI approach was recognized at Ethicon, Inc. several years ago, and the graded system has been employed to gather microbiological data in its application in ethylene oxide (EtO) sterilization programs.

The GBI employed in generating the microbiological data consisted of a series of levels of a clean spore preparation of B. pumilus E601 deposited on individual sutures or paper strips to give counts of from 10^4 to 10^7 per sample. An example of the GBI is

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

presented in Figure 7. Since Ethicon, Inc. has had a long and successful history using 2 × 10^4 *B*. *pumilus* as BI for irradiation sterilization, it was only logical to use the same organism for ethylene oxide sterilization after determining that its resistance to EtO was similar to that of *B*. *subtilis* var *niger*.

In use, the GBI is placed in five locations in an ethylene oxide sterilizer: 1) front-top; 2) front-bottom; 3) middle; 4) back-top; and 5) back bottom. With GBIs distributed in these five positions of a sterilizer, a "grid" is formed from which sterilizer performance can be determined under load conditions. The pattern of the grid is depicted in Figure 8 and has been referred to in our investigation as the "Graded Grid".

Sterilizer position	Graded inoculated levels				
	10^3	10^4	10^5	10^6	10^7
1) Front-top					
2) Front-bottom					
3) Middle					
4) Back-top					
5) Back-bottom					

FIGURE 8. A graded biological indicator reporting form to record the results in growth (+) and no growth (–).

The Graded Grid concept, in theory, can have "forecast" potential for any parameter which might decrease the efficacy of overall EtO sterilization processing. For example, if the 10^4 BI has been chosen as the decision point for passing or rejecting a sterilized load of items, and if past experience has shown that the EtO equipment and process have been successfully killing the

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

10^7 BI regularly, then any 10^7 positives and perhaps a few 10^6 positives will indicate that the process is declining in effectiveness. As more 10^7, 10^6, and some 10^5 become positive, the microbiologist should indeed realize that something is happening in the cycle or exposure, and the process is approaching the rejection criteria of 10^4. Of course, the assumption is made that the BIs are of uniform batch-to-batch resistance, and that the quality assurance program would confirm that they are above suspicion.

Anomalies may occur in the Graded Grid approach: it has been observed on occasion that a single 10^4 BI preparation is positive and all the higher BI challenges of 10^5 to 10^7 are negative. Although data is being compiled and currently undergoing review by our statisticians, one of the early observations indicated that the infrequent number of 10^4 and other single level positives indeed fit a classical random selection. This random selection could be explained by Ernst [19] who has suggested the phenomenon of "spore occlusion", which may account for the random positives occasionally encountered in EtO sterilization.

The Graded Grid system has been employed in the routine sterilization of products at our facility for more than one year. These results too, are currently being compiled and evaluated on a production-to-production run basis for several EtO cycles.

A low temperature EtO cycle was evaluated using the expanded (ten positions instead of five) Graded Grid concept and five sets of eight BIs per position. The exposure times were two, four, and six hours. The results for the three exposure times are presented in Table III.

TABLE III

SUMMARY OF FRACTION-POSITIVE* RESULTS OF GBIs EXPOSED TO LOW TEMPERATURE COMMERCIAL EtO CYCLE USING GRADED GRID CONCEPT

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Position	2 hours					4 hours					6 hours				
	10^4	10^5	10^6	10^7	10^8	10^4	10^5	10^6	10^7	10^8	10^4	10^5	10^6	10^7	10^8
1	0/5	4/5	5/5	5/5	5/5	0/5	0/5	0/5	1/5	1/5	0/5	0/5	0/5	1/5	0/5
2	1/5	4/5	5/5	5/5	5/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5
3	0/5	1/5	5/5	5/5	5/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5
4	2/5	4/5	5/5	5/5	5/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5
5	1/5	2/5	5/5	5/5	5/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
6	1/5	2/5	4/5	5/5	5/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
7	1/5	5/5	5/5	5/5	5/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5	0/5	0/5
8	2/5	3/5	4/5	5/5	5/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	1/5
9	2/5	3/5	5/5	5/5	5/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
10	0/5	3/5	5/5	5/5	5/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Totals	10/50	31/50	48/50	50/50	50/50	0/50	0/50	0/50	3/50	7/50	0/50	0/50	0/50	1/50	1/50

*Fraction represents the number of positive BIs (those showing survival of the indicator organism) over the total number exposed.

The effect of a two-hour exposure time on the 10^4 preparations was a kill of 40 BIs from a total of 50; in a four-hour exposure period, all BIs up to 10^7 were killed. The The six-hour exposure resulted in only two positives, one at 10^7 and one at 10^8. The criteria for releasing the load at the 10^4 level indicate that a six-hour exposure would be adequate. The above example of an actual interrupted cycle of incremental exposure times of EtO on a Graded Grid biological system could aid any manufacturer in EtO cycle development as well as determination of an adequate exposure time. The Graded Grid system takes the same time to run as an equivalent number of single level BIs, but the information gained through a graded system is by far the most useful and meaningful.

It is anticipated that the various responses obtained from the Graded Grid system can be correlated with a prediction of the probability of sterility acceptance of a given load, when such predictions may not be possible using other microbiological methods.

SUMMARY

Biological indicators have been employed as microbiological sterilization process controls for approximately one hundred years. Since the time of Koch and Kilmer, biological indicators

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

have not changed in basic concept or use. It has only been in the last five to ten years that sterilization microbiologists have begun to recognize the futility of efforts to run single level BIs as a microbiological routine control measure in radiation sterilization. They now also recognize the need for environmental and microbiological control of products prior to sterilization in order to provide a microbiologically safe product.

The use of multiple level or graded biological indicators is just being recognized as an improvement in aiding sterilization microbiologists to obtain meaningful data, and the graded indicators will undoubtedly become routinely employed in the near future.

This report commenced with a direct quote from Kelsey's publication on the myth of sterility. It is fitting to conclude this summary on the use and interpretation of microbiological controls (biological indicators) as sterilization monitors with another Kelsey quote [1].

> "Sterility testing (product control) thus involves a compromise choice of conditions for a limited objective… It is a very crude technique indeed for assuring the sterility of medical products. The alternative to product control is to control the process. The problem is to decide what sterilization process can be relied upon to produce sterility in all circumstances."

The use of biological controls for process controls can be defined by paraphrasing Kelsey. Biological indicators can be used to demonstrate that a sterilization process has sufficiently "freed" an article of microorganisms to be deemed safe for some special purpose by some competent group in order to provide a microbiologically safe product.

REFERENCES

1. Kelsey, J. C. (1972). The myth of surgical sterility. Lancet 2:1301-1303.

2. Koch, R. and Wolffhugel, G. (1881). Untersuchungen über die Desinfection mit heisser Luft. Mitt. Kaiserl. Gesund. 1:301-321.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

3. Kilmer, F. B. (1897). Modern surgical dressings. Amer. J. Pharm. 69:24-39.

4. Ecker, E. E. (1937). Sterilization based upon temperature attained and time ratio. Mod. Hosp. 48, 86-90.

5. Von Stutz, L. (1960). Die Bedeutung der Höchst-resistenten Thermophilen Sporenbildner bei Sterilisation. Med. Klin. 39:1722-1723.

6. Flury, F. (1962). Beitrag zur Sterilisationstechnik and Sterilisationskontrolle in einer Spitalapotheke. Thesis (No. 3202), Swiss Federal Institute of Technology, Zurich.

7. Kurzweil, H. (1957). Neue Methoden der bakteriologischen Testung von Dampfsterilisationsapparaten, Schweiz. Z. Pathol. Bakteriol. 20:505-510.

8. Memorandum. (1955). National Institutes of Health, Division of Biological Standards, August 23; cited in Reference 12.

9. Kereluk, K. and Gammon, R. A. (1974). A comparative study of biological indicators for steam sterilization. Dev. Ind. Microbiol. 15:411-416.

10. Gillis, J. R., Woodrow, J. R., Powell, K. A., and Halleck, F. E. (1975). Evaluation and standards of biological indicators for steam and ethylene oxide sterilization. Dev. Ind. Microbiol. 17:417-430.

11. Brewer, J. H., Heer, A. A., and McLaughlin, C. B. (1956). The control of sterilization procedures with thermophilic spore-formers. Bacteriol. Proc. 56:61.

12. Brewer, J. H. and McLaughlin, C. B. (1961). Dehydrated sterilizer controls containing bacterial spores and culture media. J. Pharm. Sci. 50:171-172.

13. Zuk, I. T., Parisi, A. N., and Borick, P. M. (1972). Optimization of biological indicator outgrowth and recovery conditions. Presented at the 71st Meeting of the American Society for Microbiology, Philadelphia, Pa.

14. Stumbo, C. R. (1973). Thermobacteriology in Food Processing. 2nd ed. Academic Press, New York.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

15. Pflug, I. J. and Schmidt, C. F. (1968). Heat destruction of

microorganisms. In Disinfection, Sterilization, and Preservation. C. A. Lawrence and S. S. Block (Eds.). Lea anf Febiger, Philadelphia, Pa; pp. 63-105.

16. Tallentire, A. (1972). Aspects of microbiological control of radiation sterilization. In Proceedings of the U. S. P. Conference on Radiation Sterilization. Washington, D. C., Oct. 9-10. Jacob Slonim, Ltd., Tel-Aviv; pp. 85-103.

17. Rubo, S. D. and Gardner, J. F. (1965). A Review of Sterilization and Disinfection, Year Book, Chicago. Lloyd-Luke Ltd., London.

18. Kereluk, K. (1975). Quality control in sterilization procedures: biological indicators. In Quality Control in Microbiology. J. E. Prier, J. Bartola, and H. Friedman (Eds.). University Park Press, Baltimore, Md.; pp. 25-39.

19. Ernst, R. R. and Doyle, J. E. (1968). Sterilization with gaseous ethylene oxide: a review of chemical and physical factors. Biotechnol. Bioeng. 10:1-31.

---

*Margin of safety - This term, as used here, is defined as follows: The additional exposure time beyond that which has been demonstrated to kill a predetermined level of a resistant microorganism under reproducible sterilizing conditions.

*Trademark of Ethicon, Inc., for precut, multiple suture strands in a hermetically sealed package.

*Trademark of the American Sterilizer Company, Erie, Pennsylvania.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

QUANTITATIVE ASPECTS OF MICROBIOLOGICAL CONTROLS IN INDUSTRIAL STERILIZATION

Alan Tallentire

Department of Pharmacy
University of Manchester
Manchester, England

The cornerstone of a modern sterilization process involving inactivation of microorganisms and of certain of the controls exerted on it, is the time/survival curve for heat or gas treatment, and the dose/survival curve for treatment with radiation. Although survival curve analysis has been exhaustive and detailed and has reached a high level of sophistication [1], a cursory examination of the form taken by these curves will serve to reveal broad underlying principles. Familiarity dictates that examples be drawn from the field of radiation processing, but it is doubtless appreciated that just the same principles operate in processing with heat or gas.

SURVIVAL CURVES

Figure 1 depicts typical shapes of dose/survival curves obtained when populations of pure cultures of microorganisms are exposed to increasing graded doses of high energy radiation. Three features are evident:

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

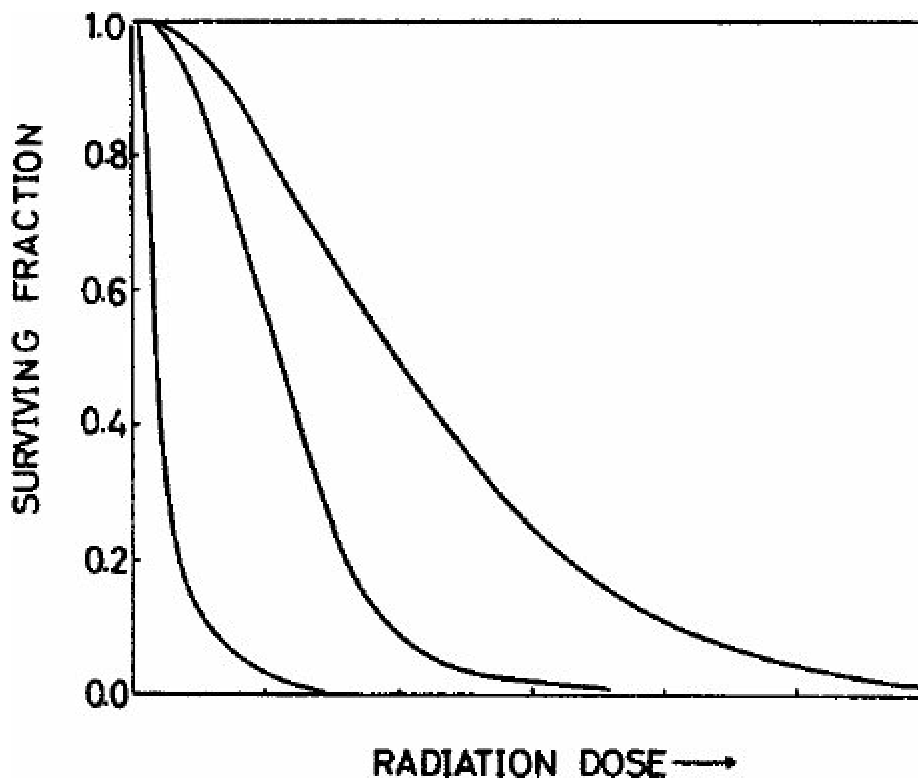


FIGURE 1. Shapes of curves drawn from typical dose-survival data plotted on arithmetic scales.

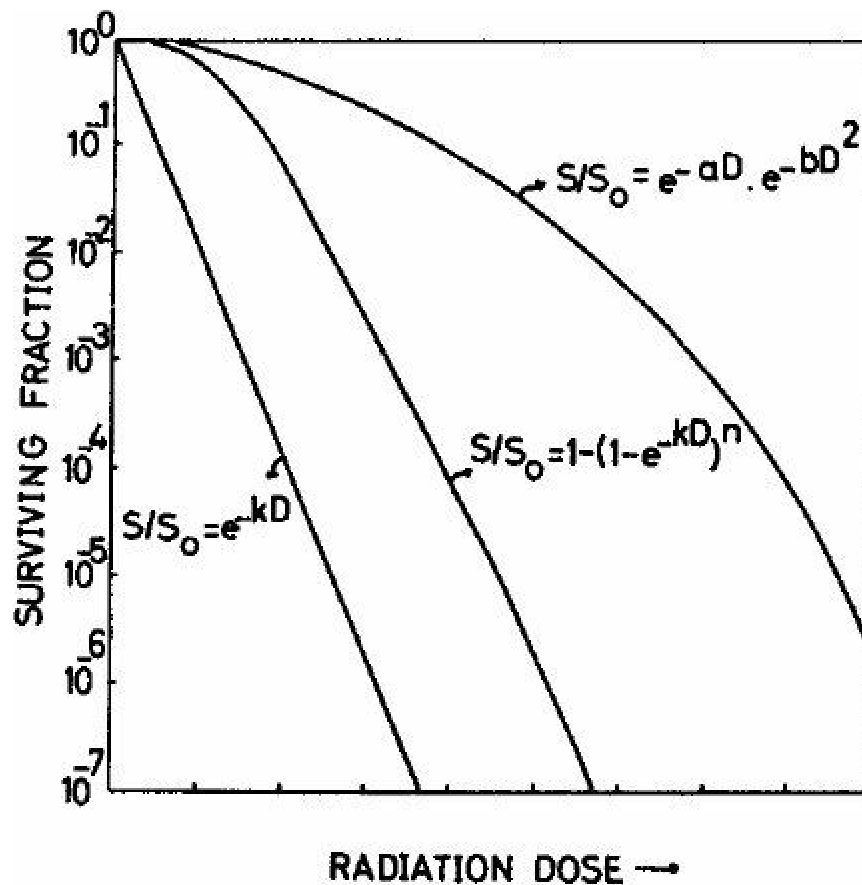


FIGURE 2. Curve shapes derived from replotting curves given in Figure 1 on a semilogarithmic plot.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

a) With increasing radiation treatment, the fraction of viable cells progressively decreases.

b) The rate of decrease can vary over the dose range.

c) At relatively high radiation doses, the fraction of cells surviving approaches zero.

Such curves are reminiscent of those generated from the simple negative exponential function

$$y = \underline{A}e^{-\underline{k}x} \tag{1}$$

in which $\underline{A}$ and $\underline{k}$ are constants. On taking logarithms, (1) transforms to the linear expression

$$\log_e y = \log_e \underline{A} - \underline{k}x \tag{2}$$

Replotting the curves given in Figure 1 on a semilogarithmic scale produces curves with the different general shapes shown in Figure 2. These shapes are wholly described by simple exponential expressions. It is common practice in examining principles underlying sterilization processing to consider the strictly linear dose or time/log survival curves. The idealized curves shown in Figure 3, taken from Bruch [2], embody these principles.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

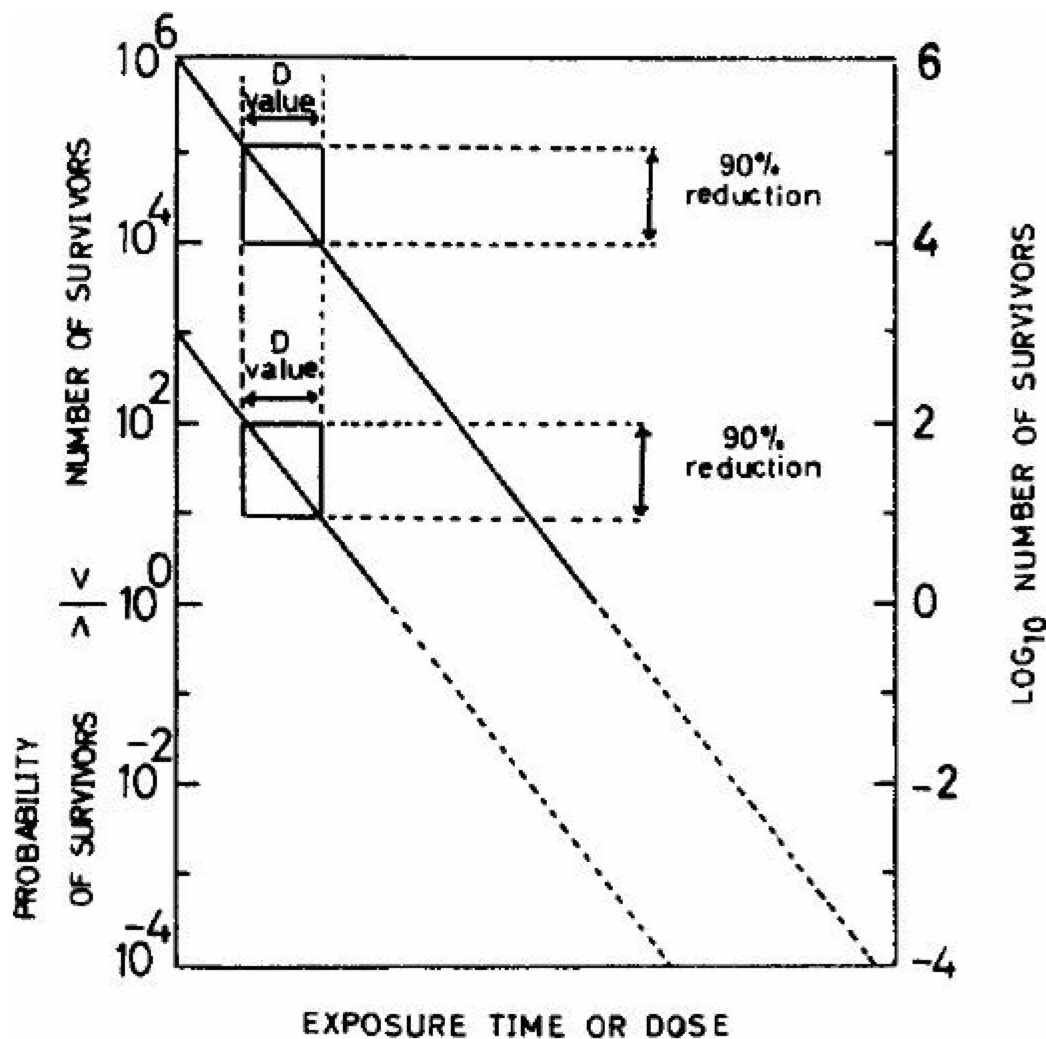


FIGURE 3. Idealized microbial inactivation curves depicting concept of probability of survivors and importance of initial numbers of organisms.

The upper curve describes the behavior of the microbial population on an item initially possessing 10^6 organisms of a given type. Over the measurable range of survivors (down to one viable organism), the slope is constant, its numerical value being a measure of the response of the organisms to the treatment. Below one viable organism, where estimates of numbers of survivors are impracticable, the curve is extrapolated, giving levels of survival that are represented as fractional numbers of viable organisms. Obviously these fractional numbers do not exist, but they can be translated into probabilities of the existence of survivors. For example, the application of a treatment giving a survival level of 1/10 of a viable organism really means that there is a 1 in 10 chance of the existence of a survivor on the particular item given that particular treatment. Similarly, a somewhat greater treatment gives theoretically 1/100 of a viable organism and this is interpreted to mean that there is a 1 in 100

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

chance of the existence of a survivor on an item with this particular microbial load given this particular treatment. The inference is then that, irrespective of the extent of the sterilizing treatment, sterility in absolute terms cannot be guaranteed; the condition achieved with increasing treatment is one of decreasing the probability of the presence of a surviving organism.

The lower curve describes the behavior of the same population of microorganisms present on an item at an initial level of 10^3. It is seen that the two curves are parallel, a fact (provided that the extrapolation is valid) leading directly to a second inference; namely, for a given treatment, the lower the initial microbial load, the less chance there is of the existence of a survivor.

These two principles, deduced simply from a consideration of survival curve shape and form, are basic to all modes of sterilization processing.

BIOLOGICAL INDICATORS

The survival curve is also the basis of the two types of biological indicator in current use. To draw attention to their inherent limitations, consideration is given to the description of biological indicators appearing in the recently published Addition to the 9th French Pharmacopoeia and entitled "Sterilization of Disposable Medical and Surgical Equipment and of Wound Dressings and Sutures by means of Ionizing Radiations" [3]. With the publication of this Addition, the French Pharmacopoeia requires that a microbiological check of effectiveness be carried out on each sterilization batch using biological indicators placed inside or on the surfaces of the articles to be sterilized. The indicators are either deliberately contaminated articles or supports made of material resembling as closely as possible that of the article to be sterilized or its packaging, contaminated with 10^8 spores of *Bacillus pumilus* E601 or *Bacillus sphaericus* C_1A.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

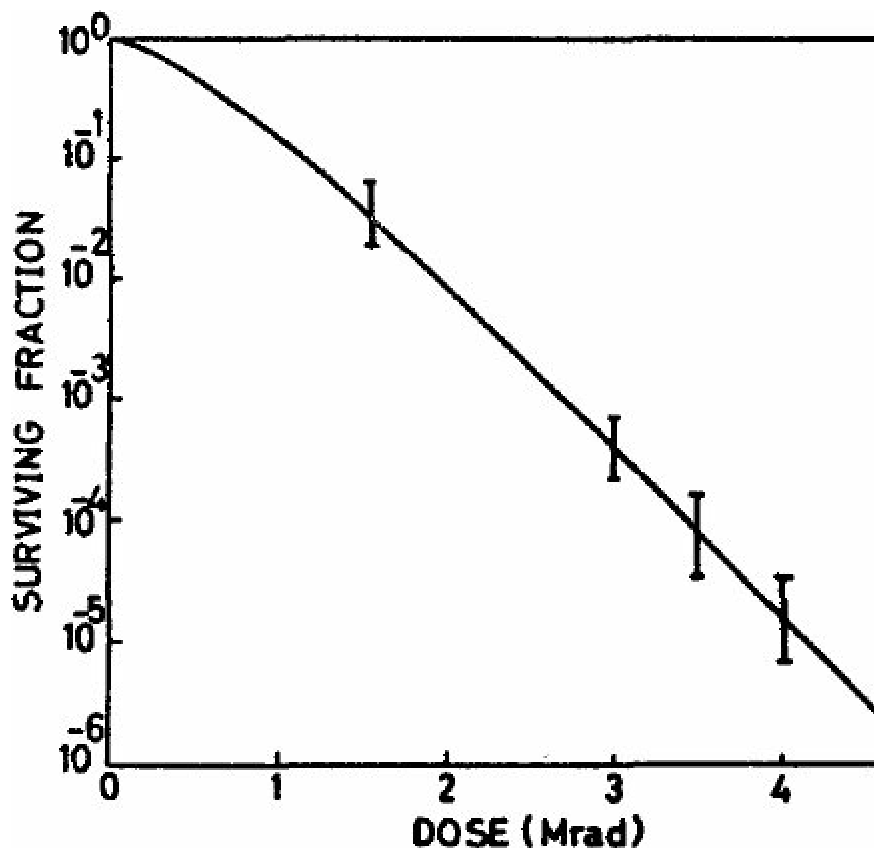


FIGURE 4. A typical "reference inactivation curve" supplied with test pieces of B. sphaericus spores employed for monitoring a radiation plant [5].

The indicator employing B. sphaericus spores is typical of those devised by Dr. E. A. Christensen and his colleagues for the monitoring of a radiation plant [4]. It comprises cells of a radiation-resistant microorganism dried in a complex medium so that their resistance to radiation is sufficiently high that measurable numbers of survivors are present following exposure to the process radiation dose. This is evident from an examination of the so-called "reference inactivation curve" which is an integral part of this type of biological indicator (Fig. 4). In determining the relative efficiencies of different radiation sterilization facilities, the imprecision of this biological indicator, as evidenced by the appreciable error bars on the curve, is recognized and replicate determinations of numbers of survivors at each of three different radiation doses are required [5]. This design allows a reasonable comparison of reference and test responses. In contrast, the French Pharmacopoeial requirement is that indicators (no specified number) are exposed to a single dose of radiation (the process dose) and then scored to check whether the level of inactivation achieved corresponds to that expected from the reference inactivation curve. Thus, at a minimum dose of

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

2.5 Mrads, an inactivation factor of "about 10^3" should be achieved and at a process dose higher than 2.5 Mrads, an appropriate higher factor is required. The critical question in this context is "what degree of confidence can be placed on determinations of inactivation factors using the B. sphaericus indicator in this way?"

In our laboratory we have carried out a substantial number of replicate determinations of inactivation factors using single indicators exposed to the same nominal dose of γ-radiation in the range commonly used in processing. From the variance associated with these determinations, we calculate that the 95% confidence limits about a single measurement of an inactivation factor of 10^3 derived from a single irradiated indicator are 3.3×10^2 and 3.1×10^3, values corresponding to doses of radiation of around 2.2 and 2.9 Mrads respectively on the reference curve. Similarly, if ten indicators are used with each sterilization batch for estimation of an inactivation of 10^3, the confidence limits are 7×10^2 and 1.4×10^3, corresponding to around 2.4 and 2.7 Mrads. The latter values are certainly acceptable; however it is questionable whether the time and effort expended on collecting such biological data are worthwhile when more precise physical and chemical methods for routine measurement of radiation dose are available.

The biological indicator employing B. pumilus spores is of a quite different type; it is a growth/no growth indicator (termed by the French Pharmacopoeia, "all or nothing test") and is restricted to testing the effectiveness of a minimum radiation dose of 2.5 Mrads. The reason for this restriction becomes evident on examination of certain dose/survival data for B. pumilus E601 spores generated in our laboratory in an attempt to rationalize the French Pharmacopoeial description. Specifically we were asking "Why a challenge of 10^8 spores?" and "What form should the indicator take to fulfill its role effectively?"

Initially we sought the response of B. pumilus spores to γ-rays when present in a variety of conditions during irradiation. For example, spores were irradiated in water suspension, or in a dried condition in the absence or presence of dried serum broth mounted on a variety of different supports. The radiation was least effective with spores dried from serum broth onto a polyethylene support enclosed in a sealed polyethylene envelope, conditions

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

similar to those devised by Christensen and his colleagues for indicators of _S_. _faecium_ cells and _B_. _sphaericus_ spores [6]. Figure 5 shows the response under this condition of _B_. _pumilus_ spores present originally at the stipulated number of 10^8 viable spores. The solid line is fitted to estimates of survivors derived over the practicable range of measurement, and the dashed line is an extrapolation into levels of radiation dose used typically in processing. Cursory examination of this curve suggests that a biological indicator modeled on such a response could be effective in a growth/no growth mode. Reading from the extrapolated part of the curve, it is seen that one survivor is expected at 2.0 Mrads, whereas at 2.5 Mrads, the probability of a survivor is around 1 in 100. In other words, apparently growth would occur at a level somewhat below the process dose, while there would be a good chance of no growth at the process dose itself. Closer analysis suggests, however, that such an indicator may not be able to do the job for which it is recommended.

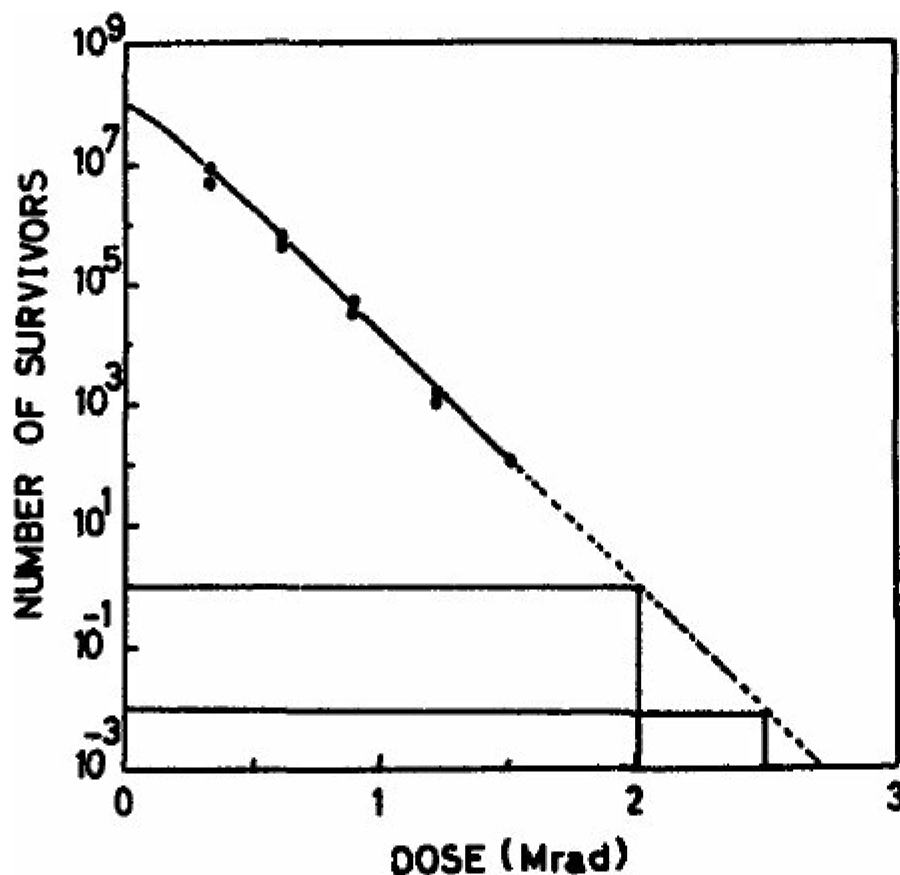


FIGURE 5. Duplicate measurements of the response of _B_. _pumilus_ E601 spores to γ-radiation when dried from serum broth onto polyethylene supports. The solid curve is described by the multihit expression $S/S_0 = 1-(1-e^{-9.4D})^{1.77}$ and the dashed line is an extrapolation of the linear part of this curve.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Suppose we have a population of indicators possessing, on average, 10^8 spores responding in the manner depicted in Figure 5 to irradiation. In reality these indicators will have, before irradiation, numbers of spores which are normally distributed about the average 10^8. Suppose each spore has an equal chance of inactivation by radiation and the population of indicators is given a dose sufficient to inactivate the 10^8 spores to a level, on the average, of one viable spore per indicator. The numbers of survivors on indicators will be distributed around this average of one according to the Poisson distribution, and those indicators that possess no viable spores will show no growth when scored, whereas those with one or more viable spores will yield growth on scoring. For an average of one viable spore per indicator, 37% of the population of indicators will exhibit no growth, even though they have received a dose of only 2.0 Mrads. In other words, using such an indicator and reading no growth as a measure of the effectiveness of the process dose (2.5 Mrads), there is an appreciable chance that one is reading the effectiveness of a radiation dose lower than the minimum that should have been given. Clearly a growth/no growth indicator possessing one level only of microbial challenge is not a sufficiently sensitive measure of the effectiveness of a stipulated sterilization treatment.

TESTS FOR STERILITY

Up to around the early 1960s, the burden of proof of attainment of a sterile condition rested almost exclusively on microbiological quality checks performed upon the sterilized product. These took the form of conventional tests for sterility, consisting of withdrawal of a particular number or proportion of random samples from a lot or batch of production items after application of a sterilization treatment, and testing these samples individually for the presence of viable microorganisms by incubation in appropriate growth media. The absence of growth in all samples was taken to mean that the population of items (lot or batch) was sterile.

On purely statistical grounds, the conventional test for sterility is now known to be inadequate as a primary method of assessment of microbiological quality (see for example, References 7 and 8). A second defect of this test is perhaps not as widely recognized; it concerns the sensitivity of the test. Under the

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

best known conditions of sterility testing, the frequency of spurious results originating from adventitious contamination, sometimes called "false positives", is about 1 in 10^3 items tested, a situation implying that this method of testing can only yield an estimate of the proportion of contaminated items ($\underline{P}$) in a population in the region where $\underline{P} > 10^{-3}$. Acceptable levels of $\underline{P}$ are generally much less than 10^{-3}, and detection of deviations from these are precluded by the very insensitivity of the test method.

Despite the objections to the conventional test for sterility on statistical and conceptual grounds, the form of the test itself possesses the following appealing features:

a) The test is effectively one of achievement; it is relevant to the sterilization treatment in that it measures inactivation of microorganisms present in their natural environment.

b) The test is subject to precise definition and therefore standards based on it can be stated in unequivocal numerical terms.

c) Generally the test is done with relative ease.

In view of these features, we have been wondering about using this form of test in a way other than in the conventional manner (i. e., other than in end product testing). One such way is to apply the test for sterility on production items after deliberate exposure to a treatment that is a fraction only of the sterilization treatment. Proper choice of the substerilization treatment should give a proportion of items contaminated, read in the test for sterility as the proportion of cultures showing growth, which is greater than the false positive level. In this way, the objection to the test for sterility on grounds of insensitivity is overcome. Such tests, we speculated at first, could be useful in two ways:

a) they might indicate the margin of safety achieved with a given sterilization treatment, and

b) they might reveal the frequency of radiation resistant microorganisms present on production items, and if desired, the types of these organisms.

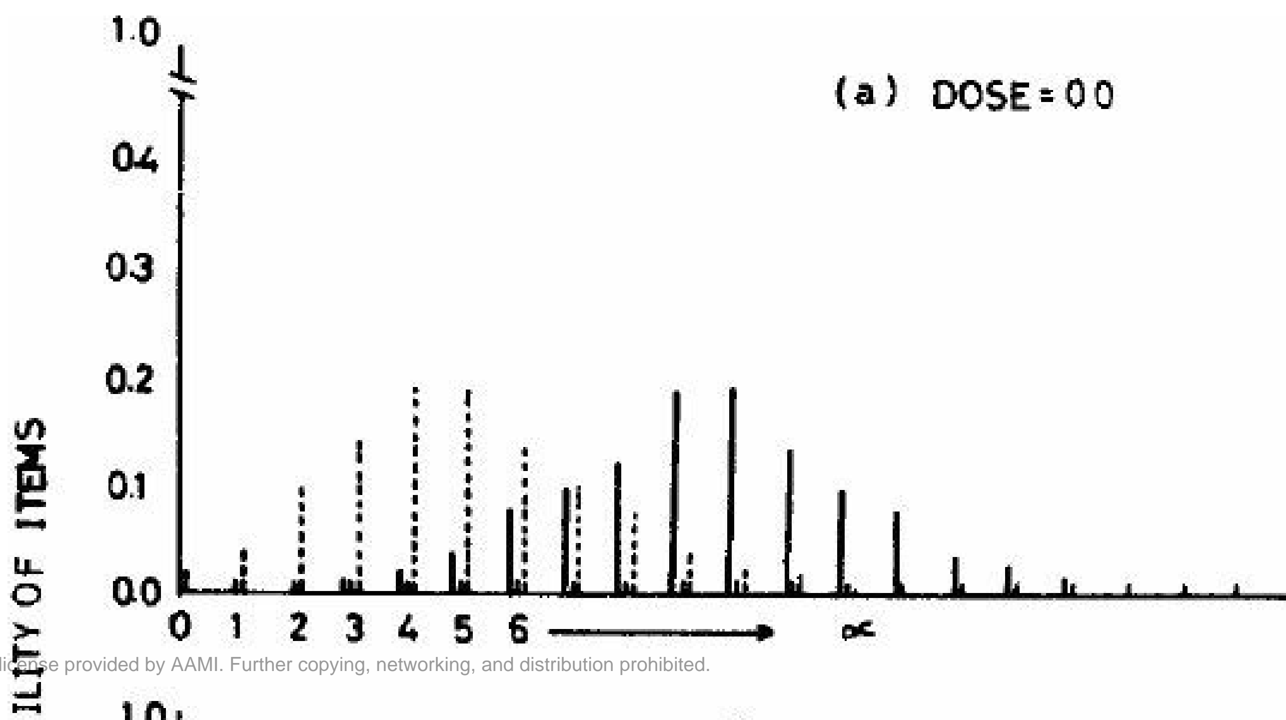
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

In the first instance, we explored the value of these speculations

by means of a wholly theoretical exercise [9].

MODERN RELATED FREQUENCY OF CONTAMINATED ITEMS AND RADIATION TREATMENT AND ITS VALIDITY

We assumed that prior to being given a sterilization treatment, items have distributed on them many types of microorganisms, and that the distributions of individual types of microorganisms take a particular form. For the sake of progress, we further assumed that initially the numbers of each type of microorganism on items are normally distributed (Fig. 6). On applying a dose of radiation to these items, microorganisms are inactivated according to particular functions, and the mean number of viable organisms/item decreases, with a corresponding relocation of the distribution about a lower mean value. With further irradiation, the proportion of items with low numbers of viable microbial contaminants increases and the distribution shifts further to the left. The resemblance of these distributions, shifting with increasing radiation dose, to Poisson distributions for decreasing mean values, is striking so we selected the Poisson distribution to describe the microbial status of items undergoing irradiation. Employing this distribution, we have been able to devise a model which, on evaluation, provides a quantitative description of events occurring on items possessing populations of microorganisms of different types as a function of increasing radiation dose. The model and associated assumptions are fully described elsewhere [9].



Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

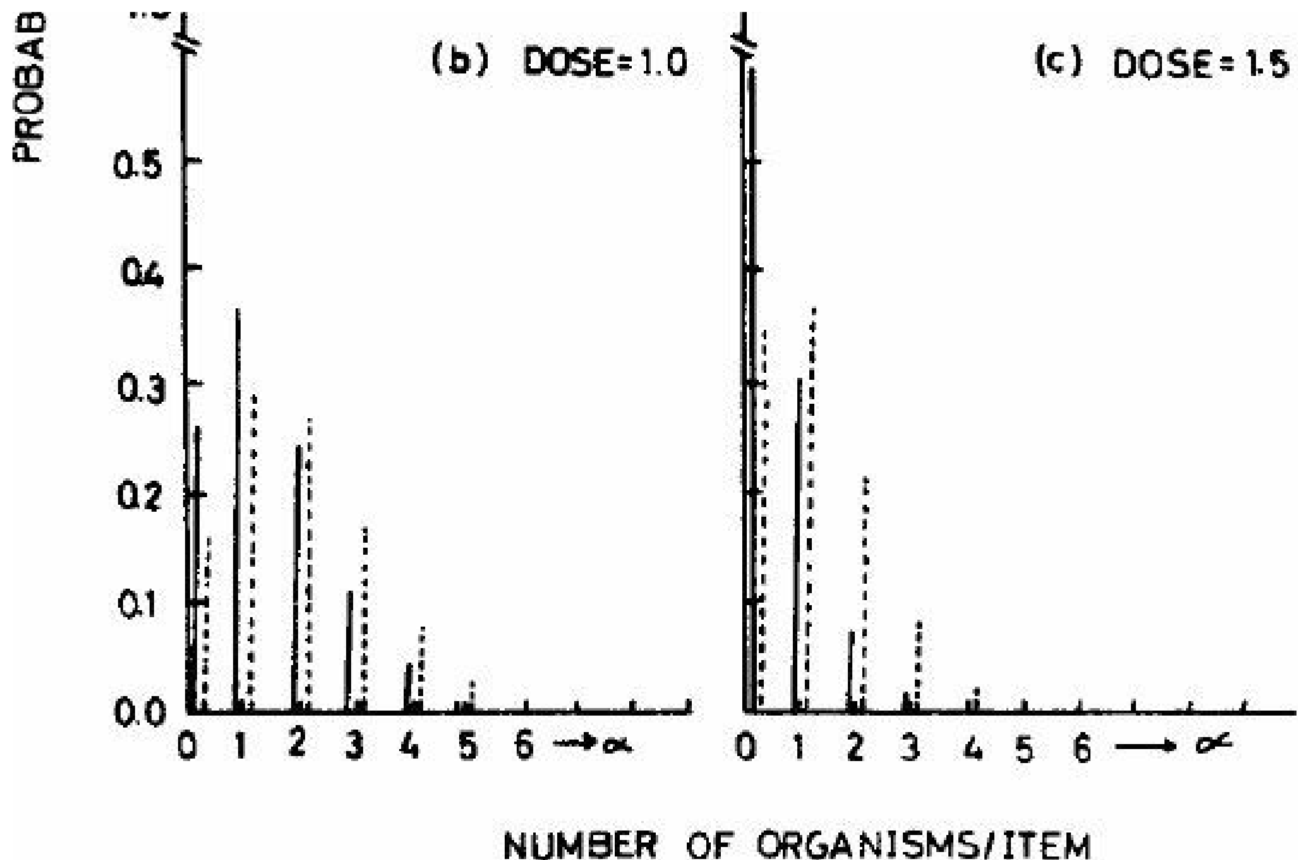


FIGURE 6. The change in the distributions of numbers of viable organisms of two types on items upon irradiation. The dashed lines indicate the distributions of Type R organisms and the solid lines those of Type S. Type R organisms possess a 2 × greater resistance to radiation than Type S. a) At dose = 0, the average number of viable organisms of Type R per item (μ^R) = 5.0 and the average number of viable organisms of Type S per item (μ^S) = 10.0; b) at dose = 1.0, μ^R = 1.80 and μ^S = 1.35; c) at dose = 1.5, μ^R = 1.10 and μ^S = 0.49.

Evaluations of the model appear as curves relating the probability of contaminated items and increasing radiation dose. Figure 7 shows diagrammatically the behavior of these curves for populations of items possessing different microbial loads prior to irradiation. The trend in all instances is for the curves to be displaced upwards and to the right when conditions on items prior to irradiation are worst (i. e., when items possess large numbers of contaminants or contaminants of high resistance). We should note, however, that in situations where there are rather low average numbers of resistant microorganisms/item in a mixed population of organisms (Fig. 7c), the curve can clearly depart form a linear from at high doses. This precludes the unrestricted extrapolation of the curve constructed from proportions of

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

contaminated items at doses giving levels somewhat greater than 10^{-3}, the false positive level. Thus, the direct estimation of the degree of safety achieved with an actual sterilization process from measurements of proportions of contaminated items obtained at less than process doses cannot be legitimately made if the model is valid.

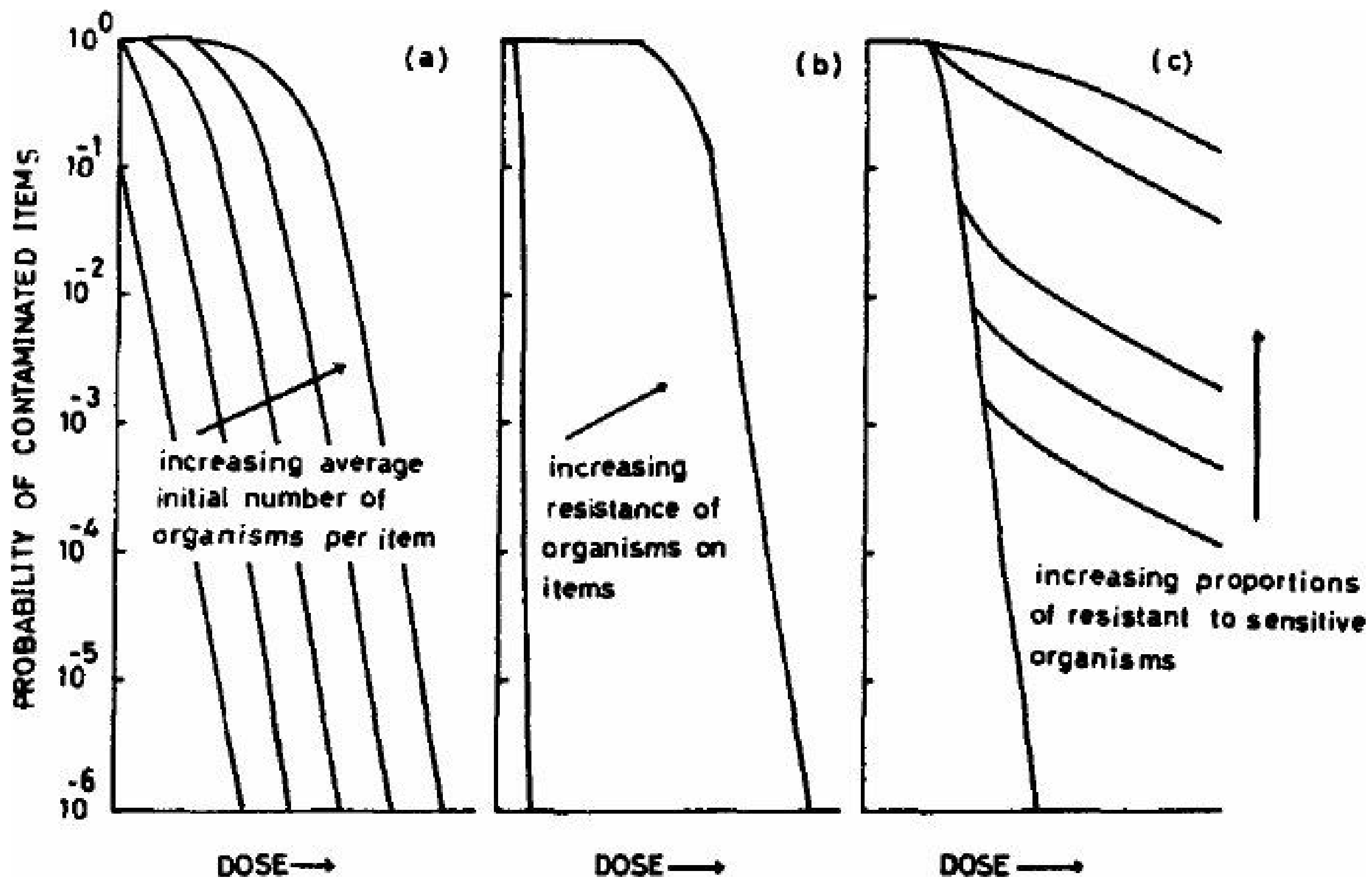


FIGURE 7. The influence of changing the numbers and resistance of viable organisms on items on the shapes of curves relating log probability of contaminated items and radiation dose.

The next obvious step has been to verify the model in the laboratory.

We have devised a test system in which it is possible to distribute, at a chosen frequency, particular species of microorganisms that respond in highly predictable and precise fashions to irradiation [10]. The test system is used to simulate the microbial load of items prior to treating them with known precise radiation doses. After irradiation over a given dose range

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

below the process dose, the presence of viable cells is recognized by a simple growth/no growth culture technique, an operation that essentially mimicks the sterility testing procedure. Measured proportions of contaminated test items have been obtained for varying initial average numbers of contaminants of a given species, for a given initial number of contaminants of different species, and for mixed populations of contaminants. From a knowledge of the initial average number of cells in the test system, of their distribution and their response to radiation, we have calculated probabilities of contaminated items over the same dose range, using the model. Figures 8 and 9 show typical comparisons of measured and predicted values. Clearly, with this simple test system, the model holds.

Examination of the curves reveal that the best achievement of a test employing irradiation of items, taken randomly, with subprocess doses is to detect moderate levels of contamination with resistant microorganisms. Presumably the same would hold for other methods of sterilization if subprocess treatments were given to random samples of items. We believe this feature may be utilized in designing tests to reveal the frequency of microorganisms present on manufactured items resistant to a particular form of treatment. It may also be used as a basis for developing a routine control procedure for assessing the microbiological quality of the overall production of items. Let us suppose that for a given manufacturing process of items destined for terminal sterilization, an accepted proportion of contaminated items has been obtained for a given subprocess treatment. Normally such a measurement would be made when the overall process of production is microbiologically under control, i. e., when the level of contamination on items during fabrication, assembly and packaging, prior to terminal sterilization, is acceptable. Alternatively, with a refined mathematical model it is possible that an accepted level might be generated from appropriate computations. According to our evaluations and results, departure from this controlled situation will be reflected in shifts from the accepted level. For example, large initial numbers of the same contaminants as those normally present cause an increase in the proportion of contaminated items, while a change to greater average resistance of contaminants does likewise. In principle, then, it is possible to monitor continuously the microbiological quality of the overall manufacturing process simply by measuring the proportions of contaminated items in random samples of actual

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

production items given subprocess treatments. The possibility of utilizing this principle in a production situation is presently under investigation.

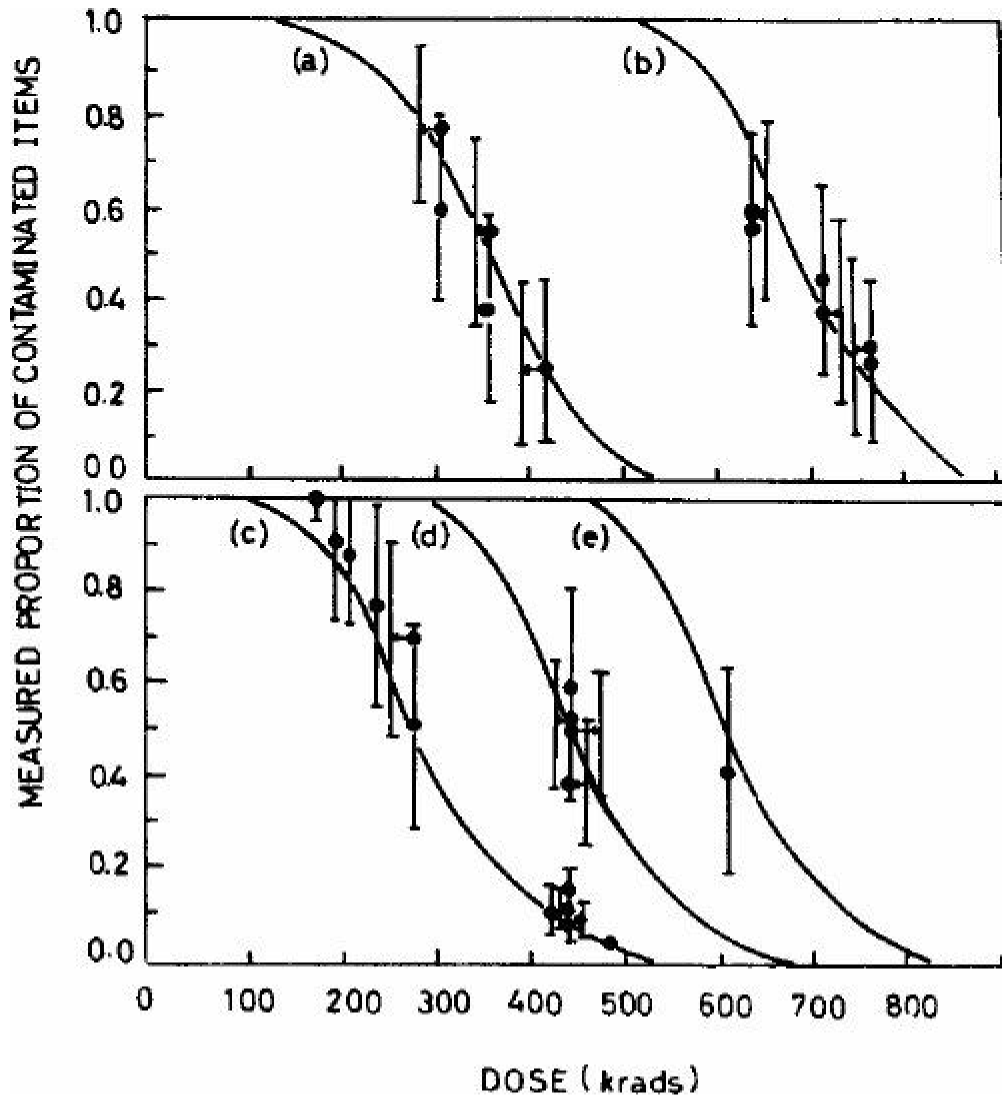


FIGURE 8. A comparison of measured and predicted levels of contamination for irradiated test items possessing initially different average numbers of viable B. pumilus spores per item. Curves are those predicted from evaluation of the model [9], and solid points are measured values. The error bars about the points are 95% confidence limits. Average number of viable spores per item: a) 60, b) 6000, c) 20, d) 200 and e) 2000.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

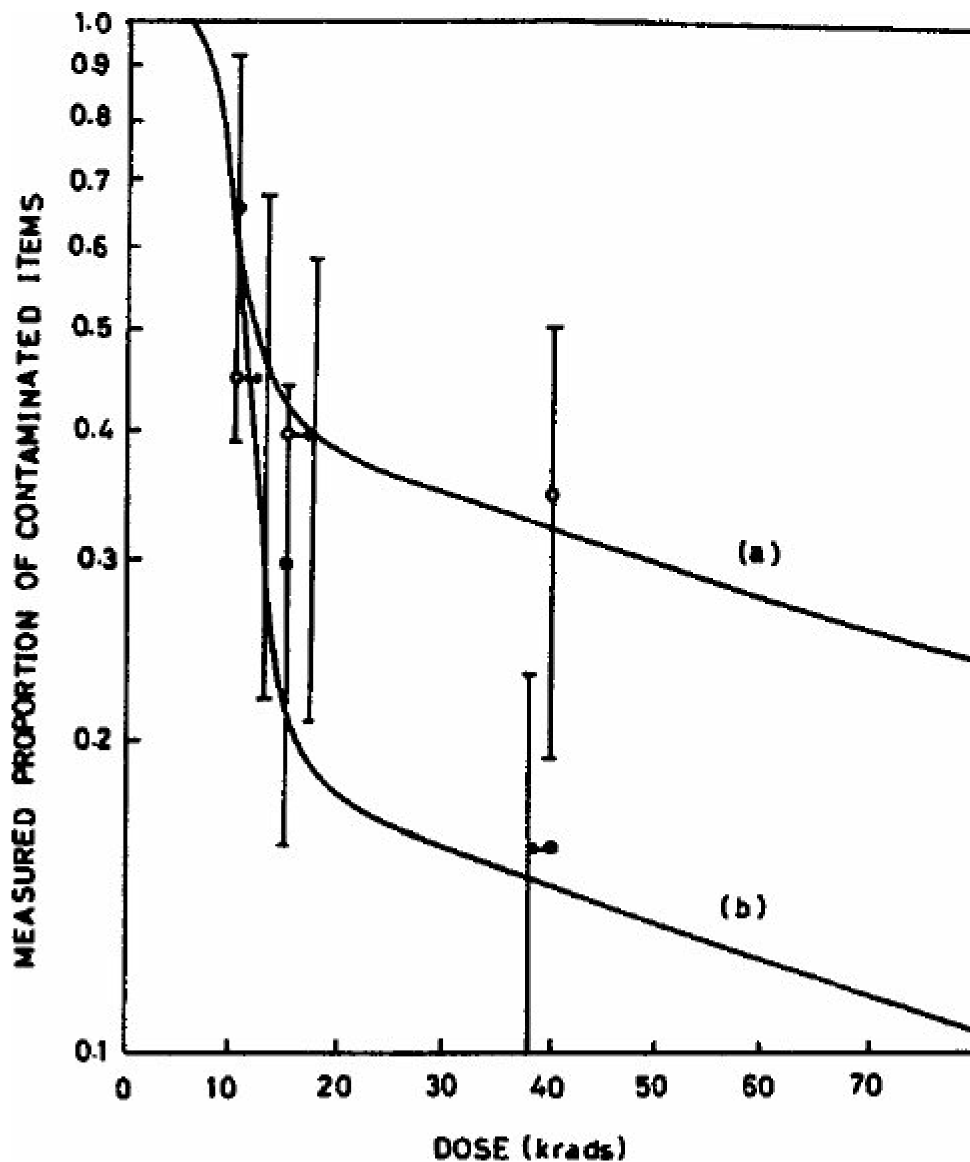


FIGURE 9. A comparison of measured and predicted levels of contamination for irradiated test items possessing viable organisms of markedly different resistance to radiation. Curves are those predicted from evaluation of the model [9], and points are measured values. The error bars about the points are 95% confidence limits. Curve (a) and open points: on average initially 0.5 B. pumilus spores and 100 Ser. marcescens cells. Curve (b) and solid points: on average initially 0.2 B. pumilus spores and 100 Ser. marcescens cells.

REFERENCES

1. Alper, T., Ed. (1975). Cell Survival after Low Doses of Radiation. John Wiley and Sons Ltd., London.

2. Bruch, C. W. (1973). Sterility assurance: product testing versus biological indicators. Aust. J. Pharm. Sci. 2:1-8.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

3. Additif no. 4 à la Pharmacopée française (9e édition). Bull. Ordre 170, Juillet 1974.

4. Emborg, C., Christensen, E. A., Kallings, L. O., Eriksen, W. H., Bjergbakke, E., and Holm, N. W. (1974). Control of the microbiological efficiency of radiation plants by means of B. sphaericus, Strain C_IA, Str. faecium, Strain $A_2$1 and Coli Phage T_I. In Experiences in Radiation Sterilization of Medical Products. International Atomic Energy Agency. Working Group Meeting, Risö, Denmark, June 5-9, 1972. IAEA, Vienna; pp. 53-66.

5. Recommended Code of Practice for Radiosterilization of Medical Products. (1967). In Radiosterilization of Medical Products. International Atomic Energy Agency Symposium, Budapest, Hungary, June 5-9, 1967. IAEA, Vienna; pp. 423-430.

6. Christensen, E. A. and Holm, N. W. (1964). Inactivation of dried bacteria and bacterial spores by means of ionizing radiation. Acta Pathol. Microbiol. Scand. 60:253-264.

7. Tattersall, K. (1961). Control of sterility in a manufacturing process. In Sterilization of Surgical Materials. The Pharmaceutical Press, London, pp. 198-203.

8. Artandi, C. (1974). Microbiological control before and after sterilization: Its effect on sterility assurance. In Experiences in Radiation Sterilization of Medical Products. International Atomic Energy Agency. Working Group Meeting, Risö, Denmark, June 5-9, 1972. IAEA, Vienna; pp. 3-14.

9. Tallentire, A., Dwyer, J., and Ley, F. J. (1971). Microbiological quality control of sterilized products: evaluation of a model relating frequency of contaminated items with increasing radiation treatment. J. Appl. Bacteriol. 34:521-534.

10. Tallentire, A. and Khan, A. A. (1975). Tests of the validity of a model relating frequency of contaminated items and increasing radiation dose. In Radiosterilization of Medical Products. International Atomic Energy Agency Symposium, Bombay, India, Dec. 9-13, 1974. IAEA, Vienna; pp. 3-14.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

DISCUSSION

Comment by L. Kallings:

I am very happy to be here and to meet old friends, and I appreciate the opportunity to keep in contact with industry. I am also very happy to serve as chairman of this session and have listened with interest to the scientific presentations that continued in the spirit of Dr. Kilmer. What you said, Dr. Gaughran, about Dr. Kilmer's abilities, is really amazing and remarkable. I think it is almost unbelievable that a person could be that much ahead of his time, not only in his mind and his ideas, but as well to have the ability to put those ideas into practical use and to demonstrate their soundness. Let us go on to the discussion.

Q. by S. Marcus:

Dr. Tallentire said that the false positive frequency, if I understood him correctly, is about one in a thousand. I had the impression that by using the kinetic approach which Dr. Kereluk described, it might be lower than that. And, if that technique were available, we could have even more confidence in sterility determination by microbiological culturing. The false positive frequency would be even less. But let me ask Dr. Kereluk and Dr. Tallentire to comment on the false positive frequency in microbiological testing for sterility.

A. by K. Kereluk:

When you get down to a frequency of one in a thousand, even with the best of your technicians, it is difficult to go beyond. As far as I am concerned, the break point is 10^{-3} in sterility testing and I believe Dr. Tallentire would agree.

A. by A. Tallentire:

In sterility testing, 10^{-3} false positives is the level seen by us for measurements taken over a number of years using a highly sophisticated technique.

A. by K. Kereluk:

We have had twelve years' experience and have come up with 44 positives out of a million samples. This speaks for itself. I am
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.
not looking forward to having my people work another twelve years

to reach 88. We should be looking for better ways to assure sterility by paying attention to microbiological control on the front end, instead of the back end of the processing.

Comment by C. Bruch:

I have enjoyed the discussion this morning. All four speakers complemented each other very nicely. I was intrigued particularly with the mathematical relationships which Dr. Tallentire presented.

I see two serious stumbling blocks in the U. S. relative to implementing what all four speakers were aiming toward. I have been with the FDA for ten years and I am concerned that ten years from now we will still be doing sterility testing. What I am saying is that if this symposium is going to have any influence in changing what is now being done in this country, we have to address ourselves to some really hard issues here. The first issue is the U. S. Pharmacopeia finished product sterility test. I started working with the USP in 1967. I tried to introduce D-values in USP XVIII, the 1970 revision. It was considered, in a sense, too radical and, I was told, we do not want to go with D-values in the sterilization chapter. Then, in 1975 for USP XIX, there were essentially no changes made in the sterilization chapter, other than the removal of 2.5 Mrad for radiation sterilization. There should be a sizeable updating of the sterilization chapter. I am not trying to be critical; I am just being frank. The USP sterilization chapter is out of date; it is just not up with modern technology.

The next agency I want to talk about is the agency that employs me. It relates to its inspectors. I am concerned how I, sitting in the regulatory agency, can bring to the FDA inspectors, the kind of thinking that was evidenced by our four speakers this morning. The FDA inspectors are most likely going into plants in the U. S. right now and the first thing they are going to be looking for is finished product sterility tests. That is what I face as a regulatory man.

Scientifically, I have no problem with what these four men have said and if it were my decision, and people came to me and asked if I would release a lot as being sterile on the basis of a dosimetry release or on the basis of sterilization kinetics, I
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.
would say "yes" if the sterility assurance is 1 in 10^6. Pflug,

referring to parenteral solutions, prefers 1 in 10^8. We have our differences there, but if someone gave me a D-value and I knew the initial contamination on the product, I would say no sterility testing is required.

This is the dilemma we are faced with. How do we take this knowledge and relay it to the FDA inspector? This is where I need help. How do we bridge the gap? How do we translate this kind of thinking to a practical basis?

Dr. Kereluk's slide on the graded series showed a positive at 10^7. A field inspector will find this hard to understand. I don't want to box in our field inspectors – they have a difficult job. But based on the contacts I have had with them and knowing their thinking, as soon as they have seen your records and found a positive, they will want to know if the lot was rejected. This is the kind of thinking I am concerned about. I like the graded series approach. But how can you translate this to a field inspector so that he won't get all excited if you have released a lot with a positive in it? Yet the positive was expected because you knew that was where you wanted or expected a positive.

Comment by L. Kallings:

In Scandinavia we use reference or official biological indicators and your inspectors could distribute such official spore preparations to a company to check their sterilization procedures. This is perhaps not being done here, but this is the way we are trying to do it.

Comment by A. Bishop:

In my country there is an august institution called the Anglican Church. The story is told of two army chaplains who were being demobilized together. One was an Anglican and the other a free churchman. As they parted at the railway station, the free churchman said to the Anglican, "Well, goodbye. It has been nice working with you and, after all, we are both doing the Lord's work." To which the Anglican replied, "Yes, we are both doing the Lord's work – you in your way and I in His."

I rather smiled when I listened to Dr. Bruch. We should not ask, "Is an article sterile?" We should ask, "Who sterilized it?" We should not expect to obtain any information from examining the

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

products. We have to satisfy ourselves that the man who is making the product knows what he is about.

Comment by S. Kaye:

Perhaps we will hear more about this problem in future sessions, but I did want to mention at this time something that bothers me a bit when one says that the bioburden can indicate to you what kind of overkill or overexposure you need to give, or should give. I think an important aspect of bioburden is the fact that you can now make your treatment, particularly gas treatment, precise. The difficulty is that if you overexpose any of the items, you may end up with a problem of residual ethylene oxide. So, the precise determination of bioburden should then be accompanied by a precise exposure commensurate with that particular bioburden. A 1000-fold or 10,000-fold excessive treatment will result in material trouble.

Comment by C. Artandi:

I am almost as impatient as Carl Bruch about the slowness of change. Two USP's ago we assembled for a major revision and made biological indicators respectable - about seventy years too late. About twelve or more years ago we decided that we were not going to rely on product sterility testing in terms of controlling our process. We reported that to the FDA and nothing happened. We then had an inspection and the inspector wanted to see our sterility records. We showed him that we were testing biological indicators. He immediately called his boss and our microbiologist had a long discussion with the boss, who was also a microbiologist. They finally agreed that it made sense, and that has been our criterion for many years. At this point we realize that biological indicators are tremendously useful in learning about the sterilization process, but to say that it is the ultimate solution in sterility control would be naive. I believe Karl Kereluk's paper proved that you can do a great deal of work without learning any more. The key thing is that you have to control the process where it is most effective. Looking at the new concepts, we are talking about statistical quality control in terms of sterilization. You want to look at your process from the very beginning. I would like to look at the raw materials; I would like to look at the various stages of the process; and then I would look for the weakest link in the process in order to have a way of affecting the bioburden. One must avoid becoming a purist. If the average count on a product is one, it would be wasteful to make a

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

tremendous effort to reduce it to one-half. If you plot the exponential curve of the "price of perfection", with cost as the abscissa and the percentage perfection as the ordinate, you will find that beyond a point it costs you an enormous amount to gain a little more perfection. What we have to teach and understand is that one should direct the efforts to where they are most effective. You should know your process. And, as Alex Bishop said, you should know the manufacturer – but I would also know the process. The process is the key. If you control the process, you can produce products which do not have to be tested for sterility.

Comment by W. Miller:

I agree completely in the case in which you have a product that might have an average count of one or less organisms; it is a complete waste of your resources to pursue that one. I think the effort has to go into those products which, because they are new and because of process variation, are producing higher counts which are really inhibiting a good sound evaluation of bioburden. I agree completely on that point. I want to make a comment on the issue of overkill. If it is any encouragement, where we do use the D-value computation method and, in fact, we could call reaching 10^{-6} an overkill situation, it is considerably less overkill than our old traditional subjective cycles yield. In my experience where we really applied this method across the board, we have gotten considerably less exposure to ethylene oxide.

Comment by C. Bruch:

I was in England the first part of April and took part in a postgraduate school on microbiological quality assurance given by the Society of Pharmaceutical Sciences at Chelsea College, Department of Pharmacy. I was quite impressed with the kind of dialogue that took place in this particular classroom. There were eight of us there dialoguing on sterilization as well as microbial limits for nonsterile products. By the way, I want to share with Dr. Marcus the fact that a representative from Organon, Holland, presented a paper in which he mentioned a false positive rate of 1 in 200 for some sterility testing. I questioned that, and he said they were quite satisfied to have a false positive rate of 1 in 200. Getting back to Alex Bishop, one of his associates gave a beautiful presentation about control of sterilization processes, but when he began his remarks, he said that, concerning those people who believe in biological indicators or finished product

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

sterility tests, we would like to invite them over to Russell Square and we would then proceed to take them down to the basement and shoot them. At least that is the message I got from his remarks and I refuted his comments. He was a very forceful fellow, a Ph.D. in Pharmacy. I thought, "Good, I hope the British can get that established over here; it would give me some leverage to use back in the United States". We then went into a discussion period and a gentleman from the Medicines Inspectorate spoke up. We really had a dogfight going thereafter. And, Alex, I wish you had been there, because the Medicines Inspectorate said that they are not going to give up finished product sterility testing. They brought up the Evans incident in 1972, and they pulled the same game that is pulled on me when I interact with the USP. The USP now keeps saying you must have a legal test, and this is the nub of this situation. The gentleman from the Medicines Inspectorate said to the representative from Alex Bishop's group, "What are you fellows going to say when the judge asks you from the stand, 'Did you run a sterility test?', and if you say, 'No', you have lost the case. You are guilty." Part of the nub here is this idea of legalities, as opposed to what is correct scientifically. I wish we had an answer for that.

Q. by J. Whitby:

Dr. Kereluk, did you do any radiation resistance testing on the survivors at the lower points of your curve? I also want to ask if Dr. Tallentire would just reinforce his comment about his combined curves. Some people always make the argument that each individual contribution can be taken as a separate item. Surely it is true that one has to take the whole population and the radiation resistance pattern of that population into account.

A. by K. Kereluk:

You are referring to the fraction negative curve. Yes, we are currently isolating organisms from a particular group of sutures. We are collecting those organisms and we are, unfortunately, at this time not able to run our kill curves. This is future work that we are lining up currently and I hope to be able to report that information at some future time.

A. by A. Tallentire:

On this point, we have done rather similar exercises involving examination of survivors for radiation resistance. We have devised

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

a rather neat little screening radiation test which is very readily done. I won't go into the details, but it will be appreciated that one can use a very simple screening method to find out whether the organism is a radiation resistant one or not, when cultured from an item which has been given a subprocess dose.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

SURGERY, STERILIZATION AND STERILITY

Velvyl W. Greene

School of Public Health
University of Minnesota
Minneapolis, Minnesota

Contrary to popular opinion, neither the practice nor the principles of sterilization originated in the surgical amphitheater or even in the hospital. The image, now almost universally held, about the hospital as a temple of health, salubrity, and sanitation, is actually quite recent – not more than 80 or 90 years old. The use of the term "surgical sterility" would have been derogatory a century ago! Yet today the words are practically an inseparable couplet and the image they are intended to convey is one of ideal cleanliness, the most perfect state of freedom from microbes we can hope to achieve in this imperfect world.

The very convening of this conference, however, suggests that this image does not really impress the professionals who deal with the realities of contaminants and infections. Notwithstanding the layman's ideal, the words "surgery", "sterilization", and "sterility" still require a footnote or two of explanation.

Perhaps the problem is focused by the very sequence of words in the title: Surgery, Sterilization, and Sterility!

First came surgery. When and how are themselves fascinating excursions into history. It is not my function to review the history of this awesome and noble profession, but it should be noted that surgeons had been operating on human beings for 600 and more years before the elementary principles of asepsis and anesthesia were even gingerly introduced or suggested about the middle of the 1800s. And it should also be noted that most of these operations were gory, bloody horrors which were mostly followed by death from infections. Until the latter half of the 19th century, most surgery was fairly well doomed to failure. We

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

are talking about mortality rates considerably higher than 50%; we are talking about operations in which the mark of a successful surgeon was not how many people he cured, but how fast he could amputate a limb, or get in and cut the stone out. There were some faltering attempts, even before Lister, of reducing the sepsis rate by introducing a variety of chemicals ("antiseptics") into wounds. But the progenitors of today's surgeons were more concerned with therapy than prevention; more impressed by dramatic breakthroughs than tedious attention to boring detail; and were remarkably reluctant to listen to advice from nonsurgeons. (At least, this is the impression I gained by reading the historical reviews written by the Wangensteens.)

While much of this was going on, the beginnings of thermal sterilization were being developed independently and concurrently in the food industry. In 1809, Nicholas Appert won a 12 thousand franc prize offered by the French government for developing a method to preserve foods for the armed forces. Appert published directions for the preservation of a wide variety of foods in glass bottles which he heated for hours in boiling water. Although nothing was really known at the time about the relationship of microorganisms and food spoilage, the empirical methods developed in the very early years of the 19th century were imitated successfully by home and commercial canners. By 1860, a sterilization process of a half hour was being used to can foods for soldiers in the American Civil War. And in 1874, a patent was issued in Philadelphia for the manufacture of a closed and controlled retort that is remarkably similar to the food sterilizers used commercially today.

When were sterilization and surgery actually united? The answer, of course, depends on one's definition of sterilization. And this polemic quite naturally introduces the controversy of Lord Joseph Lister. His work is too well known to this audience to merit review. There is some legitimate argument about whether he achieved his lifesaving successes independently - through thought and trial and reason - or whether he stole his ideas from Semmelweis who published his revolutionary thesis twenty years earlier. No matter. The basic principles of antisepsis and prevention of wound suppuration, including the destruction of germs on instruments, dressings, the hands of the surgeon and his assistants, and everything else in contact with the wound were
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.
clearly elucidated by Lister in the 1870s and remain the inviolate

principles of surgical asepsis today! He used chemicals that were of questionable effectiveness and safety, but the principles were sound.

Actually, the marriage of surgery and thermal sterilization procedures familiar to us today – procedures which are both microbiologically effective and physiologically nonhazardous to the patient – this marriage was arranged by the early microbiologists during the golden years of our profession. In the late 1870s, Robert Koch and his students developed an apparatus for thermal sterilization of culture media. Shortly thereafter, Louis Pasteur's associate, Chamberland, built the first prototype autoclave. And it was Pasteur's genius to recognize the similarity, at least from the point of view of cause and effect, of fermentation and spoilage in the food industry on the one hand and putrefaction and sepsis of wounds on the other hand. In 1878, in a famous address to the Academy of Science in Paris, Pasteur actually suggested that if he were a surgeon, he would use only perfectly clean instruments, and would wash his hands very carefully, and would heat bandages and sponges and irrigating solutions to a sufficiently high temperature (110°-150°C) to kill the germs! There is little doubt that Pasteur's and Koch's published researches encouraged the introduction into surgery of their sterilization techniques. And the success of these techniques in preventing infections initiated the landslide of almost universal acceptance that followed.

In general, surgical sterilization that we would recognize really started in the 1880s. There were some premarital forays that are often overlooked by historians, but this is a good date to serve as a benchmark. And the history of surgical sterilization is an exciting one. The honeymoon was breathtaking! We meet surgeons like Neuber who in 1883 advocated boiled operating gowns and insisted that all participants in operative procedures take frequent baths and wash their arms and hands with soap and warm water before coming into the room. Also, we meet people like Redard who was among the first surgeons to sterilize linens, drapes, gauze, and surgical sponges with heat instead of chemicals. A series of German investigators of great prominence, among them Von Bergmann, his student Davidsohn, and his assistant Schimmelbusch are considered the leaders of the aseptic movement in Europe. And there are others, not the least of whom was F. B. Kilmer whose paper, "Modern Surgical Dressings", published in

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

1897, still stands as a classic in patient, thorough and effective research. From the vantage point of 79 years, some of their work sounds naive. So does NASA’s from the vantage point of less than 7.9 years!

In general, the marriage between the surgeons and the sterilizers was a successful one. Like many marriages, there were some postmarital tensions, some extramarital flirtations, and a lot of second thoughts. Thus we have about fifty years of inconsistency: surgeons wearing masks but no gloves; gloves but no caps and gowns; sterile instruments and open amphitheaters; etc., etc. But this is to be expected in a rapidly developing field. The marriage was successful and the beneficiaries of the marriage are all of us who undergo the ordeal of the surgeon’s knife.

The major handicap to eternal bliss and harmony, however, was an incompatibility between the partners. As in many marriages, the partners made unwarranted demands upon each other, and became frustrated when these demands were unfulfilled.

Some of the tensions and frustrations in surgical sterilization can be understood better by examining the personality traits of the major partners (and their ancestors):

For example, the surgeon is a notoriously poor epidemiologist. His concern is not with statistics or with long-term probabilities, but rather and justifiably he is committed to and has copious compassion for each patient on whom he works. An error to him is not just a probability, it is sometimes a matter of life and death and inevitably a question of pain and inconvenience. A surgeon does not wish to hear about sterility statistics. Instead, he would rather fantasize about the cause of infections; he goes through periods of obsession with air, or with surfaces, or with the nurses’ noses, or with ventilating systems, or whatever you will. It is difficult to convince a person who is working, under real tension, with human life that there are certain probabilities and hazards that are essentially beyond his control. He has to feel that he can control everything and therefore will reach for what some of us might think are irrational straws.

The other partner, the food microbiologist, does not deal with life and death. Like his progenitor Nicholas Appert, he might be concerned with the 12 thousand francs or the 12 million dollars or

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

some other tangible reward. He sterilizes millions of cans of products in a week, not 10,000 patients in a career. To him "n" is so large, and low level probabilities assume practical significance: sterility failures lead to serious economic consequences. Furthermore, the food microbiologist is dealing with a system which is notoriously susceptible to invasion and microbial growth. Cans of dog food and potato soup do not have any lymphocytes or IgG or interferon; and they do not really resist proliferation of spoilage organisms. As a consequence, the food microbiologist is justifiably concerned with a single microbial survivor and particularly with thermal resistant spores which cause spoilage in his particular product. He might not sit up all night with a can of bully beef but he is willing to invest some money in mathematical models and in the study of kinetics of spore inactivation and in the design of sterility processes which will insure economic sterility. But when he reaches the financial trade-off wherein it will cost more than he takes in to kill that last spore, he quits. After all, he is not dedicating his life to sterility!

What has happened in this union, therefore, is a sort of amorphous mixture of incompatible and contradictory goals, derived from both partners:

1) We are dealing with probabilities of survival and deny that any survival is permitted.

2) Our plots on semilog charts do not have a "zero" value and we insist on zero chances.

3) We insist on sterility (absolute) for some devices and instruments and tolerate much less severe standards for others because of expediency.

4) It takes some significant dose of microbes to initiate infections in most humans and we aim for complete kill (or at least, if we find any survivors we consider it a "failure").

5) Infinitesimally few infections are caused by sporeformers and we become paranoid about the survival of an occasional spore.

6) We try to sterilize instruments and devices to the nth degree even when we know that we will expose them to a contaminated and contaminating environment for 4, 5, and 6 hours!

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

And like many marriages in trouble, we seek counsel.

This adds another dimension to the problem: the self-interest of the counselors.

The people we call in for help are all experts and certainly qualified. But with respect to sterility they have their own biases and attitudes which are really meaningful only to them. Now they try to introduce these personal biases into the field of surgical sterilization. For example, we consult microbiologists who are concerned mainly with sterilizing media. We consult pharmacists who are concerned mainly with the infectious hazards of parenteral solutions that are introduced into our circulatory system in fairly large volumes. These specialists prepare pharmacopoeias that give precise instructions for the sterilization of pharmaceutical products; then they suggest that you must use these same techniques for sterilizing everything else that will be used in the hospital. I am not yet certain if this is a valid argument. Do not forget the input of the sterilization industry which is certainly concerned with sterility and safety and decreasing infections in hospitals (and also very concerned with selling sterilizers to as many people as possible and with selling new types of new sterilizers to as many hospitals as possible). And we have consumer groups who are irrationally certain about their own wisdom and always impress us with their humanitarianism by asking questions such as "how much is a human life worth" and how much is ten units of pain worth" and things of that nature – because they know the answer if you do not! While on the subject of consumers, we also have advice from lawyers and advice from government regulatory agencies who are theoretically spaced between the consumer groups and the industry, but who sometimes develop a viability of their own which must be fed by more regulations garnished by pages in the Federal Register.

And in the last 15 years, a new phenomenon entered the sterilization field, called NASA. I honestly feel that the sterilization research sponsored by this agency has pushed back the frontiers of ignorance by light years. But I just wonder sometimes what the application of all this is to surgery.

Above all, the frustrations experienced by the marriage partners, surgery and sterilization, are aggravated by massive failures of communication. I think that the field of surgical

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

sterilization and surgical safety is less confused by technical inconsistencies than it is by semantic nightmares. We have so many inputs and everyone seems to be talking all at once and the sound becomes a little bit garbled and it is becoming more and more difficult to identify the signal and distinguish it from the static.

For example, if you ask a bacteriologist what is sterility, he will almost inevitably give you a textbook definition about "complete absence of living matter". Essentially the bacteriologist is looking at the status of the object under consideration. On the other hand, if you ask a CSS manager what is sterility, he will point to something that came out of an autoclave or an ethylene oxide chamber after having followed a certain protocol. These people equate the status of the object with the procedure to which it was subjected. Ask a statistician about sterility. He will express in mathematical terms a probability figure of finding a real live organism. The epidemiologist is neither concerned with the microbiological status of the product nor the process by which it got there nor the probability of a living organism being there; he is basically interested in the possibility of the organism doing damage. To an epidemiologist, questions of hazard are more important than questions of microbiology. And obviously hazard involves such things as to whom, under what circumstances, and to the number of people, and so forth and so on.

To a surgeon, the word sterility has a different meaning altogether. Unless one has spent some time working in surgery, one does not really quite appreciate what sterility means to a surgeon. It starts with the idea of a person wearing a certain type of clothing, usually green, and standing in front of a sink, and scrubbing hands for a certain period of time. This is all a prerequisite to ultimately being sterile. And if you are wearing the proper clothing and are scrubbing the hands for a certain length of time and then walk confidently into the operating room, some young lady will approach and do a dance around you while enrobing you in one of their magic sterile gowns, also green, which came from the central supply where we trust the manager implicitly. Watch the choreography of surgeon and nurse while he is getting into his uniform. (She was born in it - a sort of immaculate event). And if he does not get into it the proper way, he will not consider himself sterile (I have often felt that the

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

fantasies of the surgeon with regard to clothing and with regard to sterility are wilder than anything we have ever seen in the X-rated movie houses). But after this surgeon is properly garbed and the gloves are snapped on with a certain military sound, he is sterile! And will remain that way indefinitely until somebody touches him in the wrong place or until he accidentally touches something which by definition in the operating room is nonsterile. Now, this relationship of the surgeon's fantasy of sterility has really nothing to do with bacteriological reality or process reality or even infection reality; but it is historical development that is nearly a hundred years old, and we change things rather reluctantly and always slowly.

To an operating room nurse, sterility not only includes what the doctor says but she superimposes on it her own fantasies. She creates an imaginary perimeter around some central location, usually the wound site, which she calls the "sterile field" and she endows the sterile field with certain characteristics that exist only in her imagination. I always get into trouble in the sterile field because I do not know quite where it starts and where it ends and I cannot see much difference between it and the rest of the operating room. But the nurses that still talk to me say that I will develop this acumen as soon as I get my RN license. It is rather a mystical thing… and I'll buy that.

To a hospital administrator, sterility really means immunity from law suit. To a lawyer, sterility depends on whether he is acting for defense or plaintiff. He really only cares about how he can convince a jury that has spent the previous evening watching Marcus Welby who is always sterile. To the government regulator, sterility is still a concept which will require committees and subcommittees and many hours of deliberation. And to the consumer advocate, sterility means absolute safety despite autogenous infection and personal negligence. And if you disagree with him, you are a tool of the industry and a traitor to your class.

I do not think that we shall ever reach a universal definition of sterility. I do not think we really need a universal definition of sterility. It might take longer to get agreement on the subject than it will to actually kill the bugs. Like "love" and "health" and "peace", there are certain words that mean too much subjectively to individuals to give up easily. But like life, and health, and love, and peace, we might have to develop a functional

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

definition for surgical sterility: a definition that is meaningful to those poeple who deal with this particular enterprise and does not necessarily refer to the food industry or the bacteriology laboratory or the courtroom. I know it is a dream. But this is my privilege in the ivory tower of academia. I think we can reach a common ground in semantics, and I think we can reach a common ground in monitoring technology. And I do not think this effort should add considerably to the gross national product.

Now that I have essentially insulted all of my friends and effectively cancelled all future financial support from industry, government, hospitals, or academia, I shall go ahead with my other dream in surgical sterility. Once we can create a functional definition that is meaningful to those who are involved in the field, perhaps we shall also be able to develop a meaningful and functional test for sterility: not a test that will satisfy all the governmental, professional and private agencies which are concerned - like the USP, the FDA, the AAMI, the EPA, the USDA, the DOD, the VA, etc. - and not a test that will be all things to all men. I do not really want to spend my life looking for the last flat sour spoilage organism that might appear on a suture or a scalpel that is going to be used on a person who will not become flat and sour, but rather again, a functional test that will tell us essentially what we want to know about the status of the material and device we are using and the effectiveness of the process that was employed to get there. I do not think we need many new tests. I think we actually have some that are available commercially and on the market right now that are very adequate. (I think that they actually are biased so much in favor of overkill, way beyond the realm of practicality, that if they were not developed at the moment I do not think I would recommend spending another ten dollars to get to the stage we are at now). But we have these things now and it becomes more a question of learning how to use them, and deciding how often to use them and learning a little bit about the interpretation of the data they provide. I think we spend a great deal of time in our ivory towers concerning ourselves with the last injured spore that did not quite go over the river Styx - or where-ever it is that injured spores go when they die! But rather we should learn how to translate the sterility tests in terms of the real world infections hazards.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

And the real world is described very well. But whoever has taken

the trouble to study it? For example, the British workers, Williams and Shooter et al., and Dr. Altemeier and his associates in Cincinnati emphasized years ago that today's sterilization devices and today's sterilizing monitoring systems are quite adequate to preclude infections acquired from surgical instruments and devices employed in the operating room. In this respect I stand foresquare behind the plea of Dr. J. C. Kelsey who unfortunately could not come to this symposium because of ill health. He writes about the "Myth of Surgical Sterility".

> "Although sterility is in theory an absolute term, in practice it may only be regarded as at best relative and at worst misleading. It is a philosophical concept that can never be unequivocally demonstrated in a real world. Experience has shown that it is virtually impossible, even if it is honest, to change the definiton of a term that has been in use for many years; we may need a new term to indicate 'the state of having been sufficiently freed from microorganisms to be deemed safe for some special purpose by some competent body'. The abandonment of the term 'sterility' and the acceptance of some other term would remove confusion and enable the important matter of providing microbiologically safe medical products to be more rationally and realistically considered."

I know that this is the age of the consumer and the public advocate and the demand for absolute safety. I feel, however, that it is time for those who call themselves professionals in health and microbiology and surgical sterility to stop feeding the monsters of semantic obfuscation and legal abstractions. Once and for all, let us eradicate those microbial bogeymen who have no basis in practical infectious disease epidemiology and who have status only in the courts – where the tests of truth and relevance are judged by some bizarre standards. I submit it is time to defuse some noble but unreal dreams in surgical sterility and return to infection control.

I believe that one of the success stories of the last hundred years has been the development of surgical sterility to the point where instruments and medical devices are not really an infection problem to our community. And I think this is a good time to recognize and praise the work of people like Kilmer! Instead of beating this dead horse of surgical sterility, I think that we should admit that we have done something good. Sterility can be achieved. For all practical purposes sterility can be measured.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Let us go find a live horse that needs a little more attention. And let us not make the process much more expensive than it is now.

BIBLIOGRAPHY

Brachman, P. S. and Eickhoff, T. C., Eds. (1970). Proceedings of the International Conference on Nosocomial Infections. American Hospital Association, Chicago, Ill.

Castiglioni, A. (1947). A History of Medicine, 2nd ed. Alfred A. Knopf, New York.

Frazier, W. C. (1958). Food Microbiology. McGraw-Hill, New York.

Kelsey, J. C. (1972). The myth of surgical sterility. Lancet 2:1301-1303.

Kilmer, F. B. (1897). Modern surgical dressings. Amer. J. Pharm. 69:24-39.

Perkins, J. J. (1969). Principles and Methods of Sterilization in Health Sciences. Charles C. Thomas, Springfield, Ill.

Wangensteen, O. H. (1970). Nineteenth century wound management of the parturient uterus and compound fracture: the Semmelweis-Lister priority controversy. Bull. N. Y. Acad. Med. 2nd Series 46:565-596.

Wagensteen, O. H., Wagensteen, S. D., and Klinger, C. F. (1972). Surgical cleanliness, hospital salubrity, and surgical statistics, historically considered. Surgery 71:477-493.

Wagensteen, O. H., Wagensteen, S. D., and Klinger, C. F. (1973). Some pre-Listerian and post-Listerian antiseptic wound practices and the emergence of asepsis. Surg. Gynecol. Obstet. 137:677-702.

Wagensteen, O. H. and Wagensteen, S. D. (1975). The surgical amphitheatre, history of its origins, functions, and fate. Surgery 77:403-418.

Williams, R. E. O., Blowers, R., Garrod, L. P., and Shooter, R. A. (1966). Hospital Infection. Causes and Prevention, 2nd ed. Lloyd-Luke Ltd., London.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

ETHYLENE OXIDE STERILIZATION: A STUDY OF RESISTANCE RELATIONSHIPS

Keith L. West

Castle Co.
Rochester, New York

INTRODUCTION

For over ten years the United States Pharmacopeial Convention has developed, in the chapter “Sterilization”, five sterilization procedures to produce sterile products for the pharmaceutical and disposable device industries. The processes, 1) steam sterilization, 2) dry heat sterilization, 3) gaseous sterilization, 4) sterilization by ionizing radiation, and 5) sterilization by filtration, are described briefly therein. These processes are technically much more complicated than the general information set forth in the United States Pharmacopeia (USP) indicates, and the use of some form of biological indicator (BI) system for monitoring the efficiency of these sterilization processes has been accepted for many years.

USP XVIII [1] has set standards whereby products could be tested for sterility and released by inclusion of inoculated product, inoculated simulated product, or product and simulated product containing BIs (spore strip) given in the chart “Procedural Details for Sterility Tests”, in the chapter entitled “Sterility Tests”.

USP XVIII had also included, in the chapter, “Sterilization”, descriptive information on BIs used in acceptable procedures for the sterility test process, and on some basic limitations and requirements for indicators. Noticeably absent were parametric resistance requirements as presently appears in USP XIX. USP XIX [1] has deleted the option for use of BIs from the sterility test section, Table 2, and has placed detailed information for their use in the section “Biological Indicators”. There are, however, implied references to the use of BIs in the section, “Sterility Testing of Lots”, for example, Paragraph No. 4:

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

"Where biological indicators (see page 710) are substituted for the prescribed samples, the number of indicators and the test conditions specified in Table 2 may be used for the type of product with which they are tested."

Furthermore, USP XIX, First Supplement, actually invites the use of BIs as an alternative method for determining sterility, and gives the above cited Table 2 as reference.

Of significant importance was the incorporation in USP XIX for the first time, in the section "Biological Indicators", of specific suggested requirements for carrier vehicles, control specifications, i. e., lot numbers, expiration dates, storage conditions, and directions for the use of BIs including recovery media, incubation temperatures, disposal procedures, as well as suggested types of organisms to be used.

Most outstanding, however, is Table 1 entitled "Suggested Performance Characteristics of Biological Indicators as Inoculated Carriers". Actual performance criteria have been placed on BIs as related to parametric conditions in resistance qualifications.

This confusion of "on again-off again" utilization of BIs as sterility test monitoring systems for product release, and the inability of the USP to take a firm stand on the use and validity of BIs has helped to precipitate the necessity for development of data on a comparison basis of parameters with resistance relationships of BIs, vis-à-vis naturally contaminated product and inoculated product, and to determine their usefulness as sterility monitors for product release.

Until very recently, biological monitoring systems were used with ionizing radiation sterilization as a product release mechanism. Recently, with substantial historical validation data, the use of dosimetric monitoring alone has been accepted in some cases to show the validity of the sterilization process. We do not, however, believe this could become a standard procedure for ethylene oxide (EtO) sterilization processes because of the many varied parametric conditions necessary and associated with the EtO sterilization process.

Gaseous EtO sterilization is an extremely complex process. At times, the process can be a triphasic composite of five

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

components. The principal considerations are moisture availability and diffusion, for example, the diffusion of EtO, moisture and heat to the contaminated sites. This diffusion or penetration capability is subject to the product and packaging configurations, as well as the various modes of loading in the sterilizer itself.

As shown by Ernst and Doyle [2], there are factors which can either limit or enhance the dynamics of moisture availability and sterilization. These are gaseous stratification, temperature stratification, moisture reducing effects, physical diffusion barriers, chemical reactive barriers, devaporization, and polymerization.

Many research papers, publications, and presentations have clearly defined and indicated relationships as well as postulated theories for the varied parameters associated with EtO sterilization. The basic parametric conditions affecting sterilization with EtO are: temperature, humidity, gas concentration, product and packaging configuration, sterilizer load configuration and density, and penetration or diffusion (temperature, humidity, and EtO). The necessity for adequately monitoring an EtO process, therefore, must incorporate the ability to monitor all factors affecting the process and should integrate all these conditions. At present, with existing technology, only the BI in some system form has the capability of doing this. It is necessary, however, to determine what type of system would be most efficient.

METHODS AND MATERIALS

The first task in this study was to develop and build equipment with consistent reliability to meet the basic parametric conditions to which the different test pieces would be subjected (Fig. 1).

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

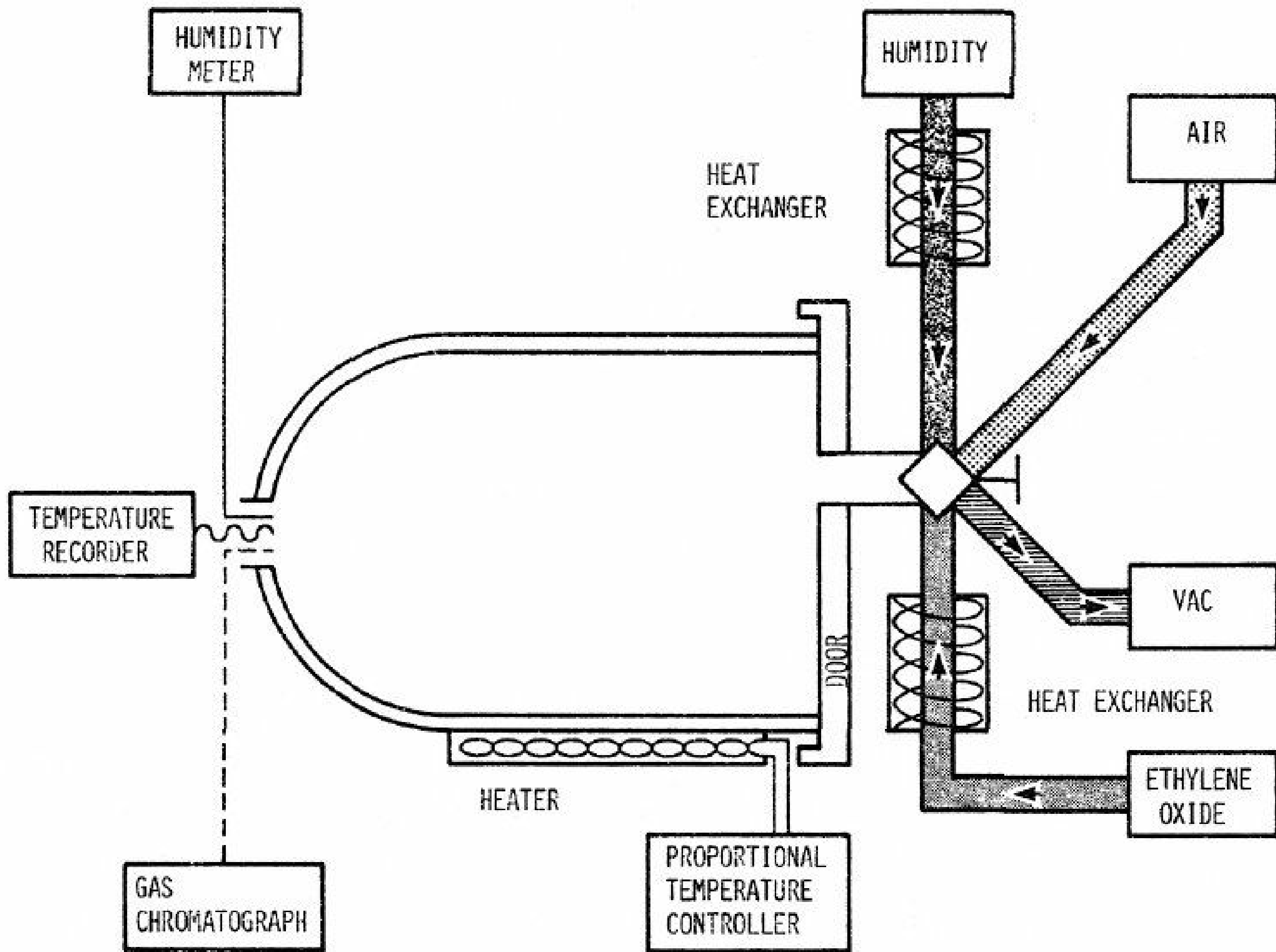


FIGURE 1. Reaction chamber for ethylene oxide studies.

The schematic drawing shows the test chamber, controls, and monitoring systems for the reaction vessels. The jacket of an insulated double-shelled vessel was filled with silicone oil for heat conduction. Heating was accomplished electrically and controlled with a proportional temperature controller using a 1600 watt, 115 volt strip heater.

A quick disconnect valving system was developed for charging the chamber with humidity and EtO through temperature controlled heat exchangers for vaporization of the liquids, for pulling vacuums to desired levels, and for the reintroduction of filtered air to atmospheric conditions. Monitoring and control systems were developed to determine, control, and monitor the parametric conditions throughout each sterilization operation, i. e., temperature, humidity and sterilant concentration.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

A standard operational sequence was developed for the research

effort. This operational sequence was followed precisely to eliminate any possible deviation in data generated due to incongruity of cycling conditions. The operational sequence consisted of the following:

1) Flushing - Four repetitive evacuations and chargings of the test chamber with air to flush out any possible residual gas or water vapor.

2) Equilibration - Test pieces were placed in the chamber and equilibrated to temperature for ten minutes.

3) Prevacuum - A vacuum was drawn to a desired level as required by packaging material.

4) Prehumidification - Humidity was added to the chamber and maintained at 50% relative humidity (RH) level for 20 minutes.

5) Gassing - The test chamber was charged with sterilant at a preselected concentration, and timing began.

6) Timing and Monitoring - Parametric conditions were monitored, maintained, and recorded at selected intervals throughout the cycle.

7) Postvacuum - After the allotted exposure time, the chamber was evacuated to an acceptable negative pressure and recharged with filtered air to atmospheric pressure.

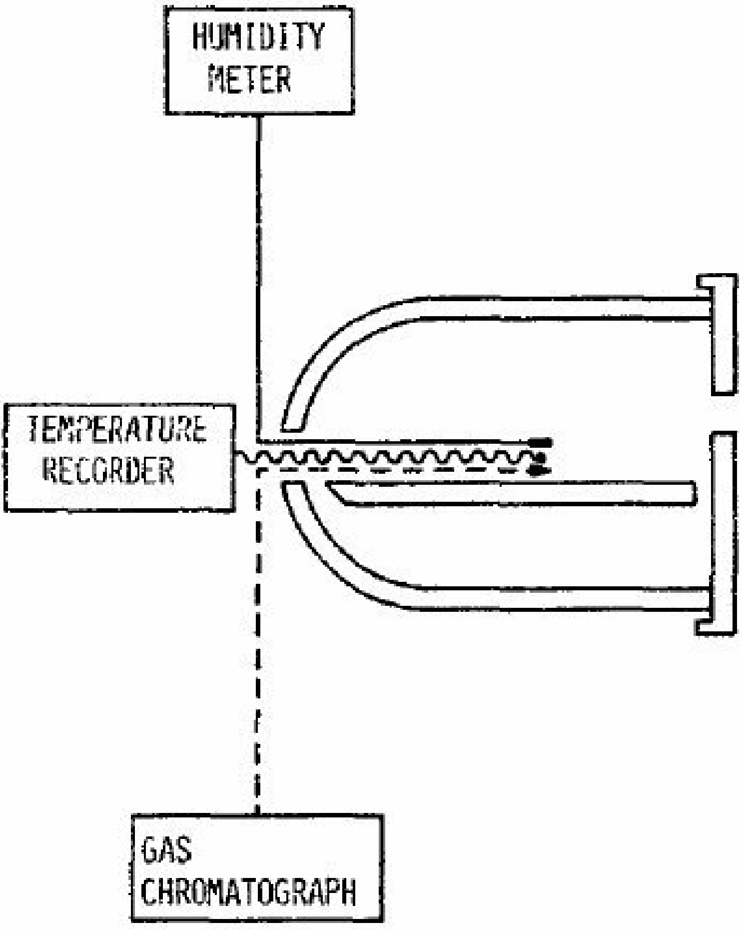
8) Unload - The test pieces were removed from the chamber and immediately transferred to suitable recovery media or assayed using precise sterility tests and sterile techniques. Recovery media for sterility tests consisted of Trypticase soy broth (BBL), while media used for plate counting techniques consisted of Standard Methods Agar (BBL).

Figure 2A shows the position of the monitoring sensors during the operation of a normal sterilization process for parameter studies. The sensors were placed in the sterilized tray in the geometric center of the chamber. Test pieces in the form of filter paper spore strips were placed in an upright position around the edges of polystyrene petri dishes which had previously been tested and found to contain no toxic residue. Each sterilization operation contained only a maximum of forty filter test pieces. Products to be tested under various parametric conditions were
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.
packaged in appropriate packaging material placed around the

sensors in the tray.

The determination of potential temperature stratification was accomplished by placement of the thermocouple probes in the chamber (Fig. 2B). This test was developed to determine the thermal profile of the test chamber. Areas of consideration were hot and cold spots and/or temperature stratification which could lead to a reduction in % RH or gaseous stratification. The sensors were placed in positions x, y, z, a, b, and c, which were on the top rear chamber wall, lower bottom door, lower middle chamber wall, top front chamber environment, geometric center chamber environment, and bottom rear chamber environment, respectively. Sensors a, y, and c were moved in a second set of tests. Sensor a was positioned in the bottom front of the chamber environment; sensor y was positioned on the top front of the door; and sensor c was moved to the top rear of the chamber environment. Table I shows the temperature readings at two temperatures, 130°F and 80°F respectively. Three sets of conditions were tested: 1) ambient (no humidity or gas added to the chamber); 2) charged (humidity and gas added to the chamber); and 3) uncharged (humidity only added to the chamber). Each test cycle consisted of pulling a vacuum to 27″ Hg and adding humidity to 50% when being tested, and/or adding EtO or air to bring the sterilizer to 10″ Hg, atmospheric pressure, or to 5 psig or 15 psig with 12/88 mixture (12% EtO - 88% dichlorodifluoromethane). An equilibration time of one minute was allowed for the environment and equipment to stabilize before readings were taken. The effect of vacuums or presterilization showed essentially no deviation, $\Delta - T = 0.5°F$, from the reading at atmospheric conditions. The total temperature deviation, $\Delta - T$, through the chamber showed a maximum of 4.0°F located on the bottom wall of the chamber directly over the heating source. The maximum temperature deviation, $(\Delta - T_2)$, in the open chamber environment was only 2.0° F. This data indicated essentially no temperature stratification and was acceptable for experimental purposes.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.



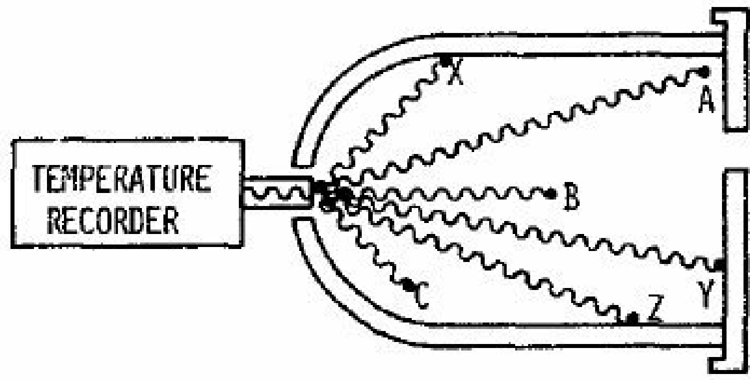


FIGURE 2. A. Sensor position during normal operation. B. Sensor position for temperature stratification studies.

TABLE I

TEMPERATURE STRATIFICATION DETERMINATIONS

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

THERMOCOUPLE POSITIONS	AMBIENT 130°F	AMBIENT 80°F	UNCHARGED 130°F	UNCHARGED 80°F	CHARGED 130°F	CHARGED 80°F
TOP FRONT	130	81	129.5	80	130.5	80
MIDDLE	130	80	130	80	130.5	80
BOTTOM REAR	130.5	81.5	130.5	80	131.5	80.5
TOP WALL	129.5	82	129	79	130	79
BOTTOM DOOR	128	79.5	129.5	79	130.5	79.5
BOTTOM FRONT	131	82	1.3	79	131	81.0
TOP REAR	130	81	1.31	80	131	80.5
TOP DOOR	129	80	1.31	79	129.5	80.0
BOTTOM WALL	128-132	80-83	128-132	79-81	129-132	79-82
Δ - T_1 THROUGHOUT	4.0	3.0	4.0	2.0	3.0	3.0
Δ - T_2 CHAMBER ONLY	1.5	2.0	2.0	1.0	1.0	2.0

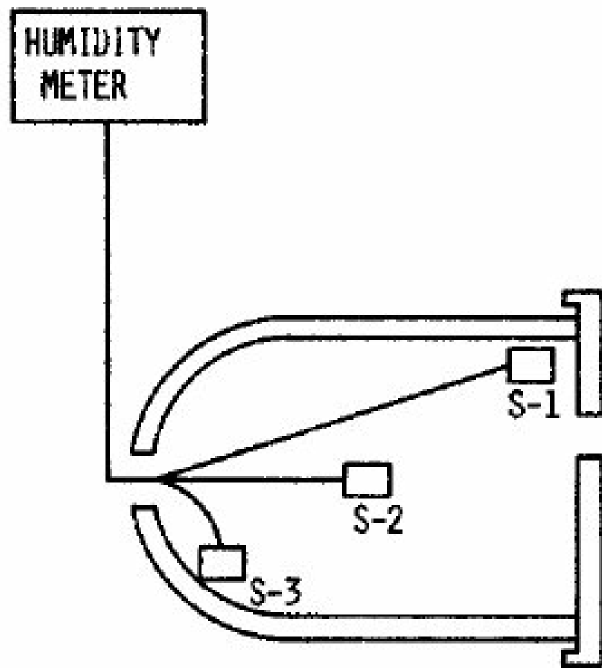
Potential humidity stratification was determined within the chamber environment as seen in Figure 3A. Sensors S-1, S-2, and S-3 were placed in the top front, geometric center, and bottom rear of the chamber, respectively, for the first set of determinations at 130°F and 80°F. Sensors S-1 and S-2 were moved and located in the bottom front and top rear of the chamber environment, respectively, in a second set of determinations at 130°F and 80°F. The temperatures of 130°F and 80°F were temperature targets designed for the parametric condition in this experiment. The target percent relative humidity was 50% RH. The % RH sensors were calibrated and preset by the manufacturer at 130°F and at 75°F. The 75°F sensor required a correction of 1% RH for every 5°F increase in temperature. Calibration tests on the sensors from the manufacturer indicated a deviation in humidity levels of ± 1% to ± 3% RH at the 50% RH level including the correction factor for the sensors at the 75°F temperature. The sensors were precalibrated in a closed system over glycerine and water solutions at prescribed specific gravities to yield environments with constant RH levels

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

over a range of temperatures. Relative humidity determinations were made in the chamber environments with constant RH levels over a range of temperatures. Relative humidity determinations were made in the chamber environment while under vacuum with added humidity, while the chamber was pressurized to atmospheric pressure from 27″ Hg vacuum with 100% ethylene oxide plus humidity, and while the chamber was pressurized to 10 psig with EtO/fluorocarbon mixture plus humidity. The results of the % RH determinations (Table II) indicate essentially no stratification from top to bottom throughout the chamber at either temperature tested. Readings were made every ten minutes for one hour. The chamber humidity monitor read the maximum level within 30 seconds of introduction and maintained this level throughout the test period.

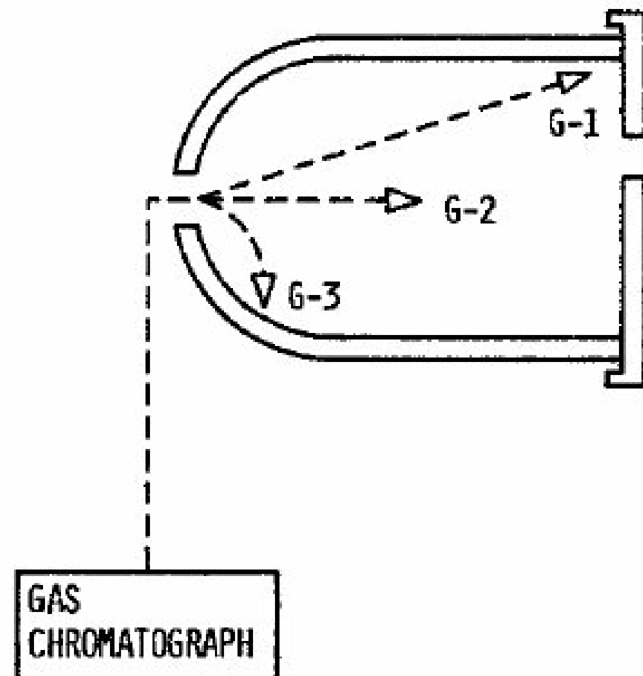
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

A



HUMIDITY STRATIFICATION DETERMINATION

B



STERILANT STRATIFICATION DETERMINATION

FIGURE 3. A. Sensor position for humidity stratification studies. B. Sensor position for sterilant stratification studies.

The maximum deviations from the target of 50% RH at 130°F were +6% RH and -1% RH with a maximum range of 7% RH. The 80°F determination showed a maximum deviation from the 50% RH target of +3% RH and -5% RH with a range of 8% RH. These data indicated no stratification of humidity or variability in the delivery system which could adversely affect the sterilization process since the % RH in the sterilizer atmosphere never dropped below 45% RH or showed a % RH greater than 65% as recommended by Bruch and Bruch [3]. Kaye and Phillips [4] presented data indicating the necessity of water vapor for effectiveness of sterilization with ethylene oxide and indicated 33% RH as being optimum, with decreasing

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

effectiveness with increased relative humidity. Data presented by Ernst and Shull [5], Perkins and Lloyd [6], and Mayr [7] indicated the increase in effectiveness of sterilization with increased relative humidity.

TABLE II

% RELATIVE HUMIDITY DETERMINATIONS

ENVIRONMENT	TEMP. °F	TOP FRONT % R.H.	MIDDLE % R.H.	BOTTOM REAR % R.H.	R.H. RANGE % R.H.	Δ - TARGET % R.H.
VACUUM	130	49	52	54	5	-1/+4
CHARGED-100	130	50	50	51	1	+1
CHARGED-88/12	130	51	50	49	3	-1/+1
VACUUM	80	50	51	52	2	+2
CHARGED-100	80	52	51	50	2	+2
CHARGED-88/12	80	53	51	50	3	+3
		TOP REAR	MIDDLE	BOTTOM FRONT		
VACUUM	130	53	50	49	4	-1/+3
CHARGED-100	130	56	53	50	6	+6
CHARGED-88/12	130	50	50	49	1	-1
VACUUM	80	50	50	50	0	±0
CHARGED-100	80	47	45	45	2	-2/-5
CHARGED-88/12	80	51	49	49	3	-1/+1

A model system was proposed by Ernst and Doyle [8] which would logically explain why an optimal RH is indicated under experimental conditions, whereas a high RH is required in practice. Under the experimental conditions of Kaye and Phillips [4], for example, water and gas molecules are in competition for the active sites of the organism in an equilibrium situation. In a practical situation, this does not exist because the dynamics of moisture and sterilant is primarily toward the sterilization site and increases proportionally with increased relative humidity. Though both situations show the need of moisture in the form of humidity and because of the many parametric conditions to be investigated, we decided to use 50% RH as our humidification level with a ± 10% RH tolerance. The results indicated that our test equipment could perform in this tolerance and that our delivery system was acceptable.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

The determination of possible sterilant (EtO) stratification was accomplished as seen in Figure 3B. Stratification of sterilant must be avoided to prevent a concentration gradient which, in turn, will cause misleading results.

The EtO concentration determinations were made at three target concentration levels: 400 mg/l, 700 mg/l and 1000 mg/l, respectively. Two temperatures, 80°F and 130°F, were studied at these levels. Our experimental protocol called for data comparing 100% EtO and 12/88 mixtures. The concentration of sterilant used in all sterilization cycles was monitored by pressure differential and/or volume metering in the case of pure EtO.

Test cycles performed with 12/88 mixtures require that the mixture in the cylinder be analyzed in order to determine its composition, which may vary by ± 1% EtO. This error can then result in ± 30 mg/l sterilant at 1000 mg/l, if only pressure-sensing is utilized for concentration measurement. However, when the composition of the gas in the cylinder is known, the pressure may be accordingly adjusted to more nearly approach the desired concentration which is then verified by gas chromatographic determinations.

The test chamber was equipped with sampling ports near the top front, geometric center, and bottom rear (Fig. 3B). Two systems for sampling were utilized to determine the concentration of sterilant in the chamber environment.

Under pressurized conditions, sterilant from the chamber was allowed to flow directly through sampling ports maintained at a temperature equivalent to that of the chamber environment, to a gas Chromatograph for analysis against a standard curve.

Sampling the chamber environment at or below atmospheric pressure was accomplished by using an indium tubing trapped gas technique. A section of hollow indium tubing of known volume was fitted with a closure mechanism and placed in the sampling positions described in Figure 3B. After a selected timing period within the gassing phase of the sterilization operation, the tube was closed off mechanically and any gas was trapped therein. The tubing with trapped gas was then recovered from the sterilizer and analyzed by gas chromatography against a standard curve.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

TABLE III

DETERMINATION OF ETHYLENE OXIDE STRATIFICATION

GAS SOURCE	TEMP.	DESIRED	CHAMBER LOCATION			RANGE	Δ - TARGET
			TOP	MIDDLE	BOTTOM		
%	°F	MG/L	MG/L*	MG/L*	MG/L*	MG/L	MG/L
DIRECT FLOW THROUGH GAS SAMPLING VALVE							
12/88	80	400	410	411	402	8	+2/+10
	130	700	695	684	693	11	-5/-16
	80	1000	1023	1031	1015	16	+15/+31
100	130	400	396	388	392	8	-4/-12
INDIUM TUBING TRAPPED GAS							
100	80	700	712	712	718	6	+12/+18
	130	1000	1009	1012	1020	11	+9/+20

* AVERAGE OF 3 DETERMINATIONS

It was determined that within a two-minute exposure period, any concentration gradient present in the chamber had been dissipated and, with the 12/88 mixture, no gradient existed after the chamber had reached the desired pressure. The gas concentration was controlled within a range of 16 mg/l.

Table III shows the results of delivery system sampling techniques and stratification determination using both the 12/88 mixture and 100% EtO at two temperatures (80°F and 130°F) for the desired concentrations. The mg/l value is an average of three consecutive determinations.

The range of stratification was from 6 mg/l to 16 mg/l EtO concentration through the chamber in all conditions studied. This would indicate that no stratification existed with the vessel after a maximum of a two-minute exposure time. The deviation from the target concentration ranged from -16 mg/l to +31 mg/l, which was only a maximum of 4.3% from target concentration. This is acceptable within experimental error.

Table IV shows the specific parametric conditions studied and reported in this paper. There were many varied possible

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

combinations of parameters. The parameters studied were chosen by survey as they most closely duplicate the predominating sterilization conditions utilized by industrial manufacturers. The packaging materials studied and reported were glassine bags or pouches.

In practice there are many types of packaging used by device manufacturers; however, for this investigation, we desired a comparison of product versus indicator system. Using glassine packages of the same type composition eliminated one of the variables which could lead to misleading results.

This research effort, through design, produced a voluminous amount of data. It became necessary to try to develop methods of analysis of these data which could utilize our time-sharing computer. A combination of mathematical models was thus prepared for computerization and consisted of the Stumbo, Murphy and Cochran method, the Spearman-Karber method, and probability equations.

TABLE IV

PARAMETERS INVESTIGATED*

I.	ETHYLENE OXIDE CONCENTRATION	1000 MG/L
	TEMPERATURE	130°F
	GAS SYSTEM	100%
	PACKAGING	GLASSINE
II.	ETHYLENE OXIDE CONCENTRATION	400 MG/L
	TEMPERATURE	130°F
	GAS SYSTEM	12% - 88%
	PACKAGING	GLASSINE
III.	ETHYLENE OXIDE CONCENTRATION	700 MG/L
	TEMPERATURE	130°F
	GAS SYSTEM	12% - 88%
	PACKAGING	GLASSINE
	ETHYLENE OXIDE CONCENTRATION	400 MG/L

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

IV. TEMPERATURE 80°F

GAS SYSTEM 100%

PACKAGING GLASSINE

* CHALLENGE TO PARAMETRIC SYSTEM CONSISTED OF FILTER PAPER STRIPS & ACTUAL PRODUCT

% RELATIVE HUMIDITY TARGETED AT 50% R.H.

The mathematical model incorporates data in the form of most probable number (MPN), fraction-negative (FN), and end point (EP). Utilizing these data, the following information can be obtained through statistical equation models in the form of salient information:

1) D-values = the time for a one log reduction of organism or spore, or a 90% reduction in the total population.

2) F_{t1} time = the time to sterilize a total given population of organisms or spores to one survivor.

3) F_{t6} time = the time to sterilize a total given population, six logs beyond F_{t1} time or a probability of 10^{-6}, which equals the sterilization time necessary to produce one surviving organism in one million pieces of product processed.

4) S-value = the slope of the D-value curve when observing the rate of kill related to the efficiency of the cycle parameters.

5) UFN50 value = the time to sterilize 50% of all the test pieces. This is a projected time when 50% of all the test pieces would be negative under sterility testing in a single cycle as related to a specific set of parametric conditions.

6) DFN50 value = this is a derived D-value as related to the projected time when 50% of all the test pieces would be negative for a single set of parametric conditions.

7) DFNU and DFNL values = these are derived D-values for the upper and lower limits with a 95% confidence level for the derived 50% fraction-negative.

8) EP time = this is the actual end point time determined by the minimum time to sterilize all test pieces in five consecutive

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

cycles having the same parametric conditions and showing no positive cultures.

To develop reliability of the mathematical models, a comparison study was designed to determine relationships among D-value determinations. Table V shows the relationship between the two procedures for arriving at D-values: 1) by subjecting calibrated BIs, in filter paper form, to sublethal kill time for one set of parametric conditions and analyzing by the standard serial dilution-plate count method for reduction studies, and 2) by mathematical models using MPN-FN-EP data with the same calibrated BIs. It will be noted that the total average D-values for each level of organism concentration under both procedures have only a 0.02 minute differential and that the range differential is only 0.17 minutes. This is well within experimental error as the time increments for the average D-value differ by only 1.2%. The maximum and minimum differences in D-value between both processes of analysis for each organism concentration are 10% and 0.6%. The D-value at all organism levels using pure populations under the same parametric conditions should have the same or similar slope or rate of reduction when we observe the mathematical model. We can see only a 15% difference between the maximum and minimum slope. This is minimal in analysis procedures and indicates that the mathematical model is adequate.

Figure 4 shows this relationship graphically. If the different D-values or rate reduction lines from the original concentration of organisms are examined, it is seen that they almost fall upon one another and are essentially parallel, which gives validity to the use of the mathematical model. The data presented justify the use of calculations by MPN-FN-EP data and all results forthcoming will be analyzed by this method.

TABLE V

COMPARISON OF ANALYSIS PROCEDURES FOR D-VALUE DETERMINATIONS

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

III PARAMETERS

EO	700 MG/L
TEMP.	130°F
EO SYSTEM	12-88%
PACKAGING	GLASSINE
% R.H.	50%

ORGANISM CONCENTRATION	ANALYSIS PROCEDURE ASSAY-(A)	SLOPE	CALCULATED-(C)	SLOPE	(A)-(C)	% DIFFERENCE
10^4	1.64	0.61	1.83	0.55	-0.19	10
10^5	1.62	0.62	1.63	0.61	-0.01	0.6
10^6	1.68	0.59	1.53	0.65	+0.15	9
10^7	1.55	0.65	1.56	0.64	-0.01	0.6
AVERAGE	1.62	0.62	1.64	0.61	-0.02	1.2
RANGE	0.13		0.30		-0.17	

DATA POINTS = MINUTES

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

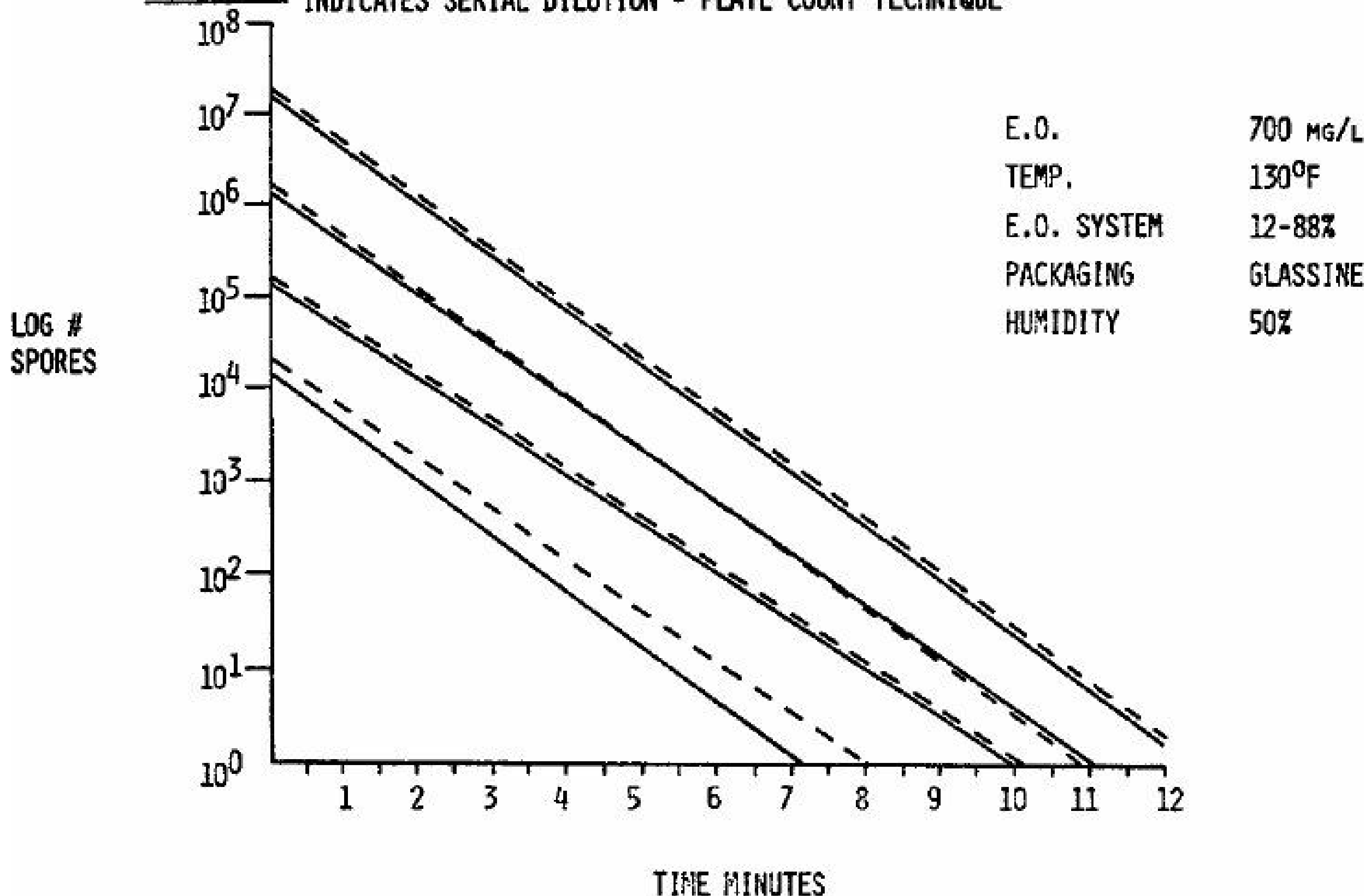


FIGURE 4. Comparison of D-value determination procedures.

The next task in this investigation was to develop a comparison between the four concentrations of organisms versus the four sets of parameters shown in Table IV.

The object of this investigation was to determine the relationship of gas concentrations and temperature, and their effects on BIs (filter paper type). This effort would give our research a baseline from which products other than filter paper could be scrutinized critically, when inoculated with a known concentration of the same spores as those used with the filter paper BIs. This information could also be used to compare results of EtO sterilization parameters with previous research published in the literature.

TABLE VI

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

COMPARISON OF D-VALUES vs PARAMETERS

PARAMETERS*			ORGANISM	= 10^4	10^5	10^6	10^7	AVERAGE D-VALUE ALL
I.	EO TEMP. EO SYS. PACKAGING	1000 MG/L 130°F 100% GLASSINE	D-VALUES	1.46	1.38	1.33	1.31	1.37
II.	EO TEMP. EO SYS. PACKAGING	400 MG/L 130°F 12-88% GLASSINE	D-VALUES	3.04	2.68	2.39	2.26	2.59
III.	EO TEMP. EO SYS. PACKAGING	700 MG/L 130°F 12-88% GLASSINE	D-VALUES	1.83	1.63	1.53	1.56	1.64
IV.	EO TEMP. EO SYS. PACKAGING	400 MG/L 80°F 100% GLASSINE	D-VALUES	23.51	20.73	18.98	17.75	20.24

* RELATIVE HUMIDITY AT 50% ALL CONDITIONS

A baseline study was developed to determine the relationship between the standardized organism (spores) versus the four sets of parametric conditions.

The data given in Table VI and shown graphically in Figure 5 indicate a tremendous effect of temperature on the reaction rate of EtO sterilization when all other parametric conditions are the same for the lower sterilant concentration levels. The 400 mg/l concentration at 130°F is 7.8 times more reactive and efficient than the 400 mg/l concentration at 80°F. The gas concentration relationship at 130°F indicates that the 1000 mg/l concentration is 1.2 times more effective than the 700 mg/l concentration and 1.9 times more effective than the 400 mg/l concentration at the same parametric conditions. This relationship bears out the evidence presented by Ernst and Shull [5], El-Bisi et al. [9], and Opfell et al. [10]. Temperature and gas concentrations are the major factors contributing to sterilization efficiency with EtO at lower sterilant concentrations; however, above a concentration of approximately 450 mg/l EtO, sterilant concentration no longer is a contributing factor in sterilization efficiency, assuming that

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

penetration and diffusion are not factors to be considered. Concentration is important, however, to increase the driving force of sterilant and moisture to overcome diffusion barriers when these are limiting factors.

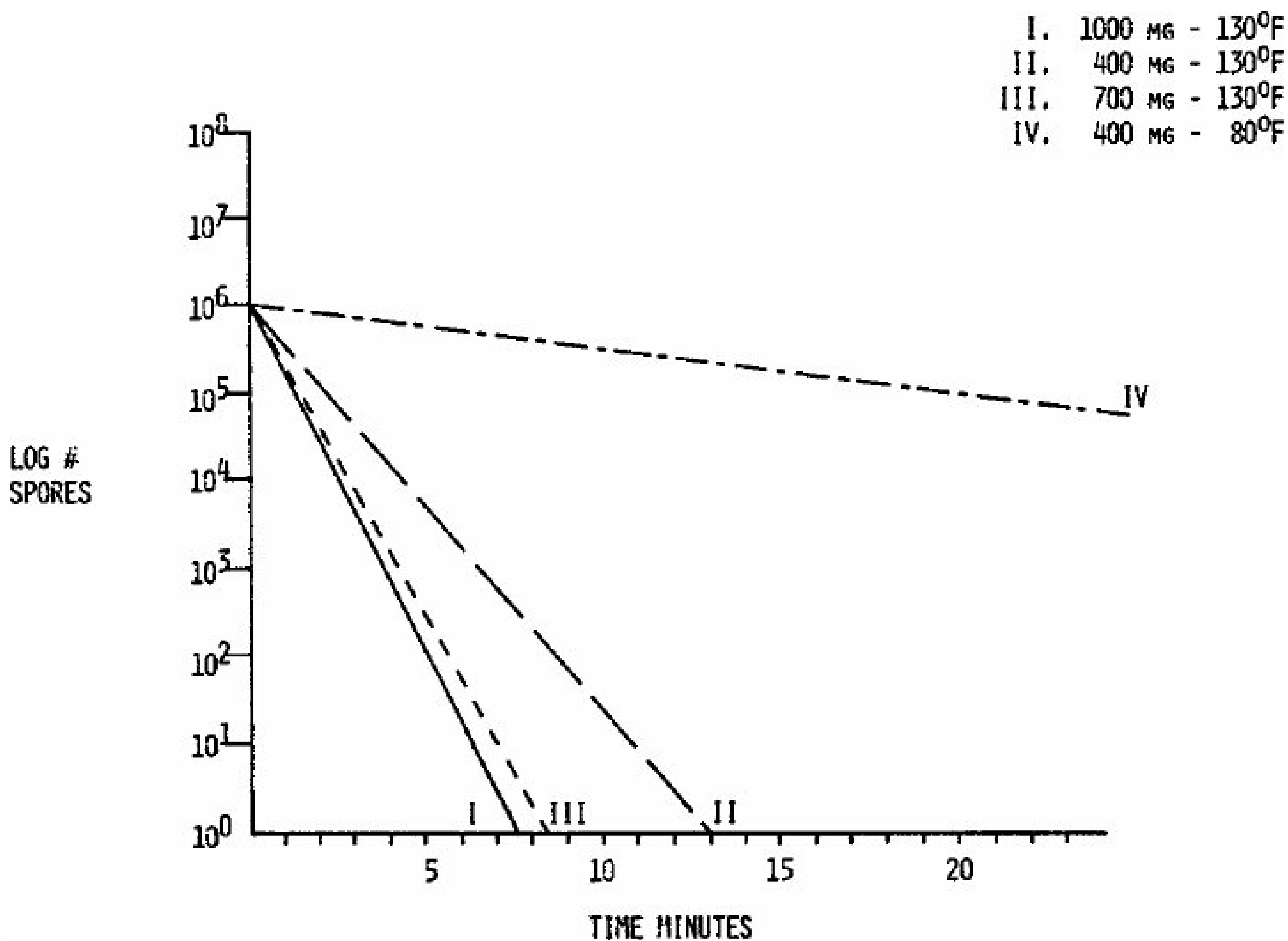


FIGURE 5. Baseline comparison of four parametric conditions.

The scope of the next research effort was to develop resistance data under the four basic sets of parameters using actual products (disposable devices) manufactured for hospital use.

There were many types of products to which this study could be directed. The research effort, unfortunately limited by time and funds, gave us an option of only three products. These products were to be of different composition and configuration in order to provide a wide distribution of types of products to be challenged.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

The following products were investigated.

1) Syringes – 10 ml plastic. This product was investigated for the following reasons:

   a) The plastic construction of this product provides a solid nonabsorbent surface different from filter paper BIs.

   b) In its assembled form, the product had a mated surface which would not allow ready penetration of sterilant but would involve a significant diffusion effect because of its configuration.

   c) This product can be sterilized, cleaned, and readily evaluated for EtO or other toxic residuals and thus reused for future studies.

   d) The material composition of this product lends itself to artificial contamination thanks to its electrostatic properties, thus permitting studies to determine "worst case conditions" of Good Manufacturing Practices (GMP).

2) French Latex Foley Catheters. This product was chosen for challenge research for the following:

   a) Latex has a different diffusion gradient than plastic or filter paper, permitting generation of data with materials of different composition.

   b) In configuration, a Foley catheter consists of two lumina. The drainage lumen which is open at both ends has a channel of 0.100" with a wall size of 0.049". The inflation lumen, closed at the insertion end by a thin-wall inflatable bottom, has an inside diameter of 0.028". This configuration, with the inflation lumen being so small and closed at one end, produces a barrier situation for observation. The microorganisms located in the center of the inflation lumen length may be killed by diffusion as penetration from the open end may be somewhat impeded by entrained air.

   c) This product is very easy to assay and sterility-test by flushing and segmenting the catheters through the inflation lumen and by cross sectioning.

3) Laparotomy Drape – Absorbent Material.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

   This material more closely resembles filter paper BIs;

however, the manufacturing process permits the incorporation of microorganisms into the voids between the fibers. This allows the inoculated test organisms to become absorbed within the product at a much higher concentration, in areas of natural contamination (the voids). Outgrowth of microorganisms from such material may be expected to be similar to outgrowth from filter paper BIs.

These three types of disposable devices were examined using three different contamination situations.

Naturally Contaminated Product: Product directly from the manufacturer was processed through background loading studies for natural manufacturing contamination. The product was also tested for resistance against the four chosen parametric conditions given in Table IV. Information derived from this study gave evidence with regard to the numbers, types and natural resistance of microorganisms in the particular microbiosphere of the product. This study will disclose any artificial protective effect derived from the product itself.

Artificial Contamination: This contamination situation simulated the worst possible case of manufacturing practice. Products to be tested were placed in a highly contaminating environment for several time intervals to simulate a situation in which the product could be manufactured in a grossly inadequate environment. Data obtained in this contamination situation would determine any possible increase in concentration of natural contaminating organisms as related to the three different product types. The effect of such an increase on the resistance was examined under the four sterilization conditions given in Table IV.

Inoculated Product: Product which was previously sterilized, sterility tested, and found to have no toxic residuals was inoculated aseptically with 10^6 spores of Bacillus subtilis var. niger in the most difficult area to sterilize. Syringes were inoculated in the barrel and air-dried in a laminar flow hood. The inoculum was placed in such position in the barrel that, in the assembled syringe, the plunger would cover the inoculum, creating a mated situation. Catheters were inoculated in the inflation lumen in the center of the length of the catheter and allowed to dry for four days in a laminar flow hood. The drape material was inoculated in the center of a 25 sq cm piece of material. The

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

absorbency of the drape material allowed the spore to infiltrate between the fibers with the liquid carrier. The swatches were then air-dried in a laminar flow hood for two days. All material was packaged aseptically in presterilized and aerated glassine envelopes for resistance studies.

Standard assay procedures were developed for each of the products. The assay procedure for syringes consisted of washing them with sterile peptone water containing Tween 80 on a wrist action shaker for one hour and insonated at 20 kHz for two minutes.

Controls were run to determine the insonating effect of 20 kHz for two minutes on both vegetative cells and spores of known concentrations suspended in the wash solution at 10^2 and 10^6 organisms/ml. There was no discernible decrease in count or resistance to condition III described in Table IV, using these organisms on filter paper versus BIs made with organisms not subjected to sonication.

The wash was then aseptically filtered through a 0.2 μ membrane filter. The filter was either overlayed with suitable recovery agar or blended for serial dilution and plated with suitable recovery agar.

High concentration levels of organisms on the syringes were assayed by serial dilution techniques using the NASA procedures for microbiological examination of space hardware [11], with the washing and sonication filtration techniques.

The catheters were assayed using a three-step process. The inflation lumen was washed with 100 ml of previously described washing solution and the washes were collected in a sterile flask. The catheters were split through the inflation and drainage lumina, cut into one-inch segments and placed in the lumen wash solution. The catheters and flasks with washing solution were assayed as described for the syringes at high and low concentrations of organisms by washing, sonication, and filtration. The filter membrane was either overlayed or blended.

The drape material was assayed using the direct overlay technique for colony counting with suitable media, or by direct
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.
blending and serial dilution assay procedures for high

concentrations.

Natural contamination assay procedures for vegetative or spore forming organisms, aerobic or anaerobic organisms, as well as mold or yeast were conducted. Suspected anaerobic spore-forming organisms were subcultured and determinations made to validate strict anaerobes. All spore-forming organisms isolated by heat shock methods were cultured and sporulated to carry out resistance comparisons to parametric conditions.

In no case was any organism, either aerobic or anaerobic isolate, from naturally contaminated product encountered which showed greater resistance than our control organism B. subtilis var. niger. This does not mean that some products could not carry organisms with greater resistance; however, none were encountered here.

All three manufactured products were control-checked for % recovery of microorganisms by inoculation of sterilized and toxicity-checked product with both vegetative cells and spores at both low and high concentrations of microorganisms.

Table VII shows the recovery level for spores from all three products. This indicates that our assay procedure for spore-forming organisms is acceptable for the development of baseline information. The vegetative cells, however, show a very low recovery percentage. This was due to the die-off rate of these organisms during drying and not to the recovery procedure. It is our contention that the spore-forming organism would have greater resistance to ethylene oxide sterilization processes, as evident in much of the literature; therefore, we decided to concentrate primarily on organism-producing spores.

TABLE VII

BIOB URDEN. ASSAY PROCEDURE % RECOVERY CALIBRATION

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

ORGANISM	AVERAGE % RECOVERY			
	HIGH		LOW	
	VEGETATIVE	SPORES	VEGETATIVE	SPORES
SYRINGES	41	86	52	92
CATHETERS	43	94	53	91
DRAPE	52	95	49	94
ORGANISM CONCENTRATION	10^4		10^2	

The next task in this research effort was to simulate a condition of possible grossly inadequate GMP. This condition was achieved by placing product in a highly contaminating environment. The determination of microbial increase was carried out by using previously described methods for each product, incorporating percentage recovery data for accurate concentration levels.

Samples were selected at different time intervals and microorganism concentrations determined as shown in Table VIII. The maximum concentration of microorganisms stabilized at about four weeks' exposure to the contaminating environment as seen in Figure 6.

TABLE VIII

BIOBURDEN. ASSAY AND EXPOSURE SEQUENCE AT VARIOUS TIME INTERVALS

	AVERAGE # ORGANISMS/PIECE		
	SYRINGES	CATHETERS	DRAPES
EXPOSURE PERIOD			
IMMEDIATE	8	14	75

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

2 DAYS	367	66	105
4 DAYS	1570	128	2700
2 WEEKS	5700	450	3700
4 WEEKS	7600	650	4300
8 WEEKS	7200	680	4100

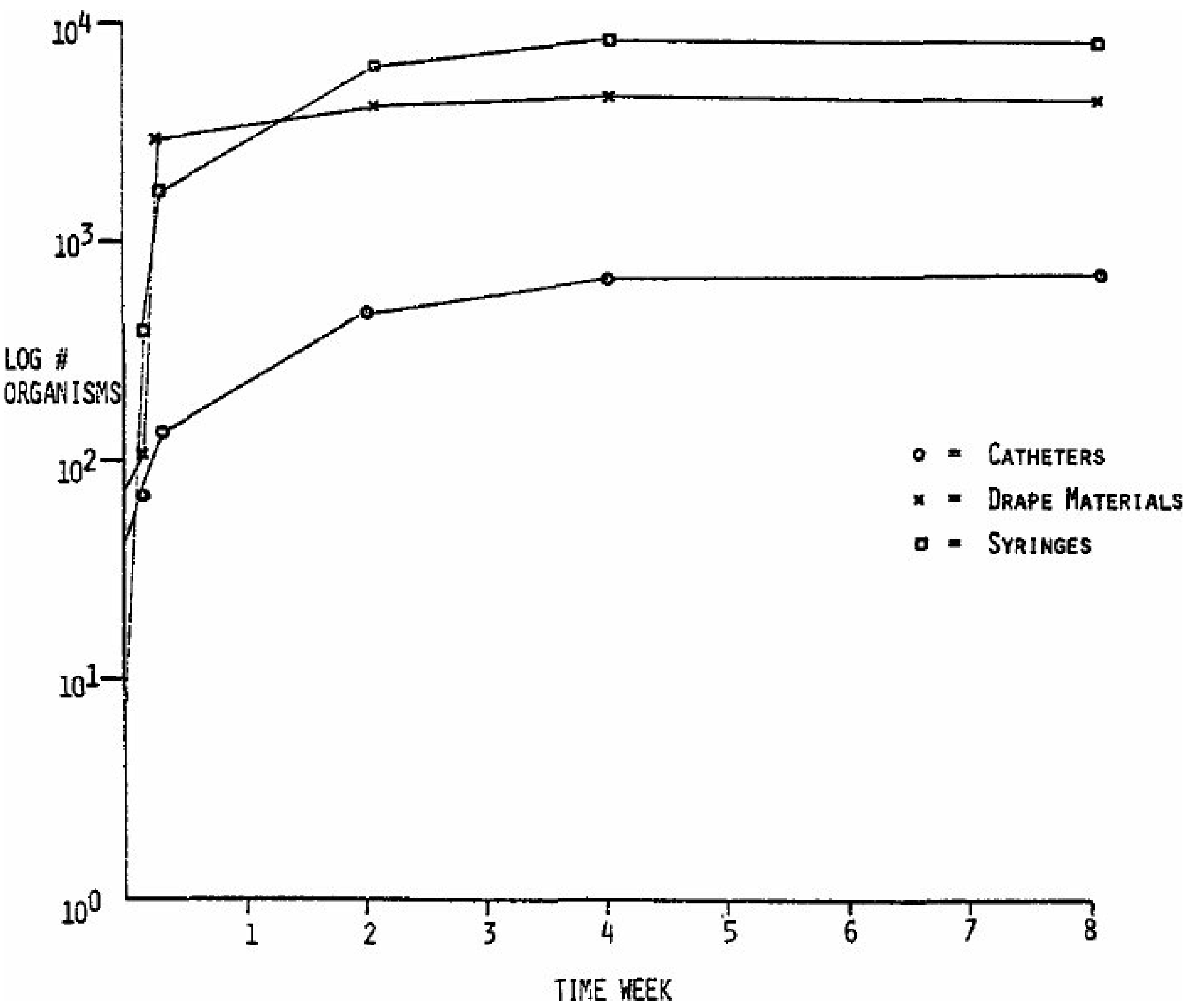


FIGURE 6. Bioburden recovery versus time of exposure to environment.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

These data indicated that the following two contaminating

exposure times could be used for resistance studies against our four basic parametric conditions:

1) Unexposed direct from the manufacturer.

2) Exposed four weeks in a contaminating environment.

A very low concentration of microorganisms was present on the product direct from the manufacturer, both in the case of syringes and catheters. The drape material studied, however, had a higher concentration of microorganisms in a 25 sq cm piece, when compared with the other products.

This was undoubtedly due to the manufacturing process. The total size of the absorbent drape from which the sample pieces were sectioned was 3087 sq cm. Assuming there are 75 microorganisms/25 sq cm and this is uniform throughout the entire drape, there would be approximately 1×10^4 microorganisms/drape. There was a greater percentage of vegetative cells on the drape material than was found on the catheters or syringes. This is probably due to the absorptive ability of the drape which holds larger quantities of moisture and thus impedes desiccation and destruction of vegetative cells.

The number of microorganisms present on the exposed syringes increased rapidly and consisted primarily of spore-formers. The plastic composition of the syringes, having high electrostatic potential, would readily attract microorganisms bearing a charge or charged particles containing microorganisms. The leveling off effect is probably due to neutralization of the charges on the particles and loss of adherence to the syringe. The predominance of spores is probably due to desiccation and rapid destruction of vegetative cells, as well as the environment having very high concentrations of spores.

The catheters did not display as rapid an increase in microorganism population because of their configuration, as well as not being as highly electrostatic. The predominance of spore forms again was evident, and was due to both the destructive effect on vegetative cells and environment.

The high concentration of spores on the artificially contaminated product may also be attributed to the laboratory environment where operations are performed with mostly spore-

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

forming organisms.

Using the MPN-FN-EP calculation procedures described previously, the following data points for the actual products studied were developed:

1) D-value times
2) End point times
3) Survivor probability time at 10^{-6}

Products and filter paper strips were inoculated at 10^{6} spore concentrations and resistance was studied using MPN-FN-EP calculations to develop the following data points:

1) D-value times
2) End point times
3) Survival times for one spore/test with ten samples in a single sterilization process

Historical research has shown that the thermochemical destruction of microorganisms with EtO is exponential. Assuming that this is valid, the D-value determination can be used to analytically evaluate resistance studies. D-value is defined as the decimal reduction time, or the time required for a survivor curve to transversely drop one log cycle. The D-value is actually the reciprocal of the slope of the survivor curve in minutes. The term "decimal reduction" arises from the fact that there is a reduction in the number of viable organisms which is expressed by moving the decimal point one place to the left, i. e., reduction from 1000 to 100 or 0.01 to 0.001. D-values for a particular study with constant parameters must, therefore, produce a one log reduction for every equal time increment.

Another observation is that the D-value may be considered as the time to reduce the viable population of microorganisms by 90%. If the total population of microorganisms present is of identical species with the same resistance, a kill curve plotted on semilog paper would produce a straight line function, if the ordinate were logarithmic and the abscissa, linear. In a mixed population of microorganisms with different resistances to thermochemical destruction, a straight line function could not be derived because of the different destruction rates. If, however, this mixed population were sampled statistically with our previously mentioned mathematical models, we could develop a D-value in

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

conjunction with end points to determine a straight line function for plotting on semilog paper, assuming the most resistant organisms would be killed last. The time scale of exposure for each set of parametric conditions must be of equal increments as dictated by the mathematical model for MPN-FN-EP analysis in order to function properly and produce a straight line function.

Figure 7 is a graphic expression of the combination of an idealized D-value and probability curve. Starting with 10^6 or 1,000,000 organisms with a D-value of one minute, in that first minute of exposure or D = 1 there would be 100,000 or 10^5 survivors. In six minutes or D = 6, there would be one surviving organism. D = −1 then would produce 0.1 surviving organisms. Since a fraction of an organism could not survive or be detected, we would be operating in the probability part of the D-value curve. D = −1 would have a probability of one survivor in ten samples processed. D = −3 would project 0.001 surviving organism or one survivor in 1000 samples processed. D = −6 or D = 12 would estimate 0.000001 survivor organisms or would show a probability of one surviving in 10^6 samples. D = 1, D = 6, D = −1, D = −3, and D = −6 would require exposure times of 1, 6, 7, 9, and 12 minutes respectively.

PRODUCT RESISTANCE TESTING

Fraction-Negative Data

The end point is designated as the minimum time within the exposure time scale for each set of parametric conditions which would deliver five consecutive sterilization processes (times) producing <u>no</u> positive cultures in ten replicate samples when sterility-tested.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

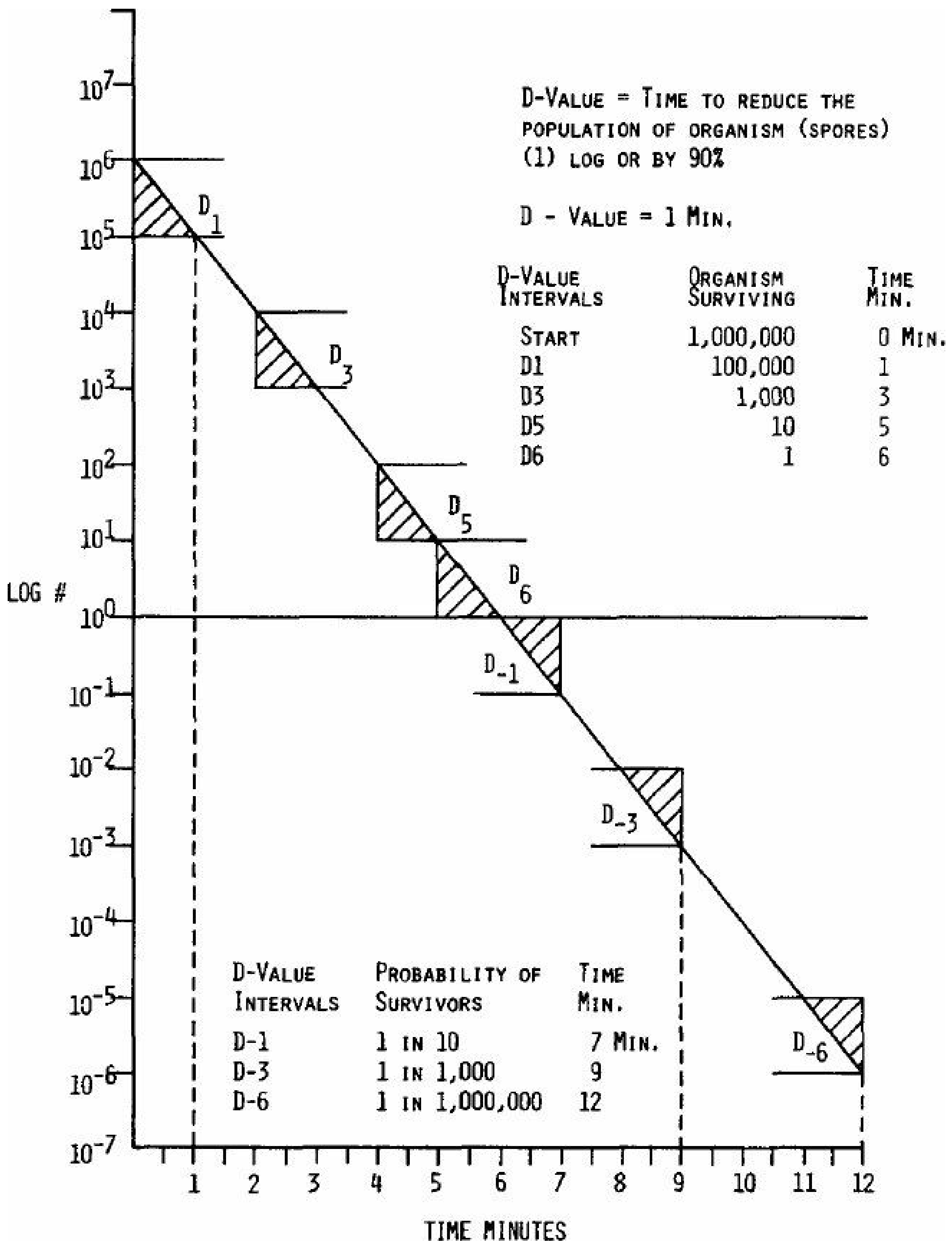


FIGURE 7. Idealized probability curve.

Tables IX-XII indicate the maximum time within the time scale for each set of parameters to produce all positive (+) cultures upon sterility testing. Also included are the previously described end point and time increment intervals to produce data from all

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

positive (+) to all negative (−) cultures upon sterility testing (fraction-negative sterilization zone). The concentration of microorganisms present and the D-values derived for each set of parametric conditions are also presented as data points in these tables.

It is evident that the inoculated product monitors at 10^6 concentration of spores had a proportionally larger partial survivor range or “resistance window” than either naturally or artificially contaminated syringes or catheters. The drape material on the other hand had a narrower partial range or “window” for inoculated product when compared with the naturally and artificially contaminated materials at the same parameters, and compared with the syringes and catheters. This led to the development of the concept of a microbiosphere protection effect of naturally contaminated products. The manufacturing process for various products produces contamination which, because of the product configuration, protects the microorganism in the microbiosphere against the sterilization process at resistance levels which would be much greater than the natural resistance of the microorganism by itself.

The D-values in many cases are greater with naturally contaminated product than with artificially contaminated product and in some cases greater than inoculated product. D-value determinations in such instances may be misleading as a result of the microbiosphere concept in addition to the very low concentration of microorganisms found on the product. The natural resistance cannot be duplicated by either artificially contaminated or inoculated product at the same concentration levels of spores of B. subtilis var. niger.

TABLE IX

FRACTION-NEGATIVE DATA

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

	SYRINGES					CATHETERS					DRAPE				
*	+	-	T	D	C	+	-	T	D	C	+	-	T	D	C
NATURAL CONTAMINATION	5	31	2	15.71	8	3	18	1	8.70	14	2	11	1	2.78	1
ARTIFICIAL CONTAMINATION	10	95	5	12.01	7600	5	19	1	3.54	650	3	13	1	1.91	4300
INOCULATED PRODUCT MONITORS 10^6 SPORES	70	170	10	16.48	2.47	80	150	10	17.77	1.78	9	16	1	1.97	1
FILTER PAPER MONITORS															
10^4 SPORES	4	9	1	1.46	1.57										
10^5 SPORES	4	10	1	1.38	1.59										
10^6 SPORES	6	11	1	1.33	1.47										
10^7 SPORES	6	12	1	1.31	1.46										

E.O. CONC. = 1000 MG/L
TEMP. = 130°F
E.O. SYSTEM = 100%
PACKAGING = GLASSINE
% R.H. = 50%

* + = TIME (MIN.) ALL POSITIVES RECOVERED
- = TIME (MIN.) ALL NEGATIVES RECOVERED
T = TIME (MIN.) INCREMENTAL INTERVALS
D = D-VALUES (MIN.)
C = ORGANISM CONCENTRATION

TABLE X

FRACTION-NEGATIVE DATA

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

	SYRINGES					CATHETERS					DRAPE				
	* +	-	T	D	C	+	-	T	D	C	+	-	T	D	C
NATURAL CONTAMINATION	21	47	2	33.12	8	5	32	2	13.33	14	3	19	1	4.96	75
ARTIFICIAL CONTAMINATION	10	150	10	18.35	7600	9	36	2	6.41	650	4	22	1	3.13	4300
INOCULATED PRODUCT MONITORS 10^6 SPORES	100	290	10	27.70	2.47	60	480	60	39.26	1.76	15	27	1	3.20	1.49
FILTER PAPER MONITORS															
10^4 SPORES	10	17	1	3.30	1.60										
10^5 SPORES	10	18	1	2.63	1.53										
10^6 SPORES	10	19	1	2.39	1.47										
10^7 SPORES	12	23	1	2.26	1.57										

E.O. Conc. = 400 mg/L
Temp. = 130°F
E.O. System = 12-88%
Packaging = Glassine
% R.H. = 50%

* + = Time (Min.) all positives recovered
− = Time (Min.) all negatives recovered
T = Time (Min.) incremental intervals
D = D-Values (Min.)
C = Organism Concentration

TABLE XI

FRACTION-NEGATIVE DATA

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

	SYRINGES					CATHETERS					DRAPE				
*	+	-	T	D	C	+	-	T	D	C	+	-	T	D	C
NATURAL CONTAMINATION	10	35	2	16.80	8	3	20	1	7.55	14	3	14	1	3.44	75
ARTIFICIAL CONTAMINATION	10	100	10	12.74	7600	5	23	1	4.05	650	3	16	1	2.30	4300
INOCULATED PRODUCT MONITORS 10^6 SPORES	80	210	10	22.01	2.47	80	240	20	22.90	1.78	10	18	1	2.19	1.49
FILTER PAPER MONITORS															
10^4 SPORES	6	11	1	1.32	2.01										
10^5 SPORES	6	12	1	1.63	1.55										
10^6 SPORES	6	14	1	1.53	1.44										
10^7 SPORES	8	15	1	1.56	1.56										

E.O. CONC. = 700 MG/L
TEMP. = 130°F
E.O. SYSTEM = 12-88%
PACKAGING = GLASSINE
% R.H. = 50%

* + = TIME (MIN.) ALL POSITIVES RECOVERED
- = TIME (MIN.) ALL NEGATIVES RECOVERED
T = TIME (MIN.) INCREMENTAL INTERVALS
D = D-VALUES (MIN.)
C = ORGANISM CONCENTRATION

TABLE XII

FRACTION-NEGATIVE DATA

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

	SYRINGES					CATHETERS					DRAPE				
	* +	-	T	D	C	+	-	T	D	C	+	-	T	D	C
NATURAL CONTAMINATION	160	180	10	227.10	8	30	150	10	51.80	14	20	130	10	36.00	75
ARTIFICIAL CONTAMINATION	180	1200	60	142.60	7600	30	160	10	29.0	650	30	160	10	22.30	4300
INOCULATED PRODUCT MONITORS 10^6 SPORES	360	1560	120	136.20	2.47						110	200	10	23.90	1.49
FILTER PAPER MONITORS															
10^4 SPORES	80	125	5	23.50	1.67										
10^5 SPORES	90	135	5	20.70	1.53										
10^6 SPORES	100	145	5	19.00	2.08										
10^7 SPORES	110	160	5	17.70	2.36										

E.O. Conc. = 400 mg/L
Temp. = 80°F
E.O. System = 100%
Packaging = Glassine
% R.H. = 50%

* + = Time (Min.) all positives recovered
- = Time (Min.) all negatives recovered
T = Time (Min.) incremental intervals
D = D-values (Min.)
C = Organism concentration

To compensate for this inability to utilize D-value determinations, one could rely on inoculated products which produce longer end point times, though in many cases, also smaller D-values. D-values may be considered as precisely valid tools only when related to the purity and concentration of the types of organisms present and their resistance to the sterilization processes.

It should be noted that the D-values as well as the end point for the filter paper monitors are <u>not at all</u> comparable with those for the catheter or syringe material. However, they are very close when compared with the drape material at each of the separate sets of parameter conditions. This would indicate a similarity of materials.

Probability Relationship

The sterilization procedures involving decimal reduction determinations and survivor curves are methods for analyzing the data as well as extrapolations to probabilities. The utilization of the above methods generated for naturally and artificially

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

contaminated product will project exposure times which would be required to produce one survivor in one million (10^{-6} probability). Survivor curves from naturally contaminated product with low concentration levels would be valid information and necessary for determining probabilities. Exposure times may also be projected for various types of monitors which would give one survivor/ten replicate test samples at each set of the sterilizing conditions.

This information is presented in Tables XIII-XVI for each set of parameters tested.

Probability Graphs

The combination of data points from the fraction-negative data tables and the probability relationship data tables can then be combined to produce the necessary-probability graphs (Figs. 8-18) to show how well monitors perform against the projected 10^{-6} probability times.

TABLE XIII

PROBABILITY DATA

	SYRINGES			CATHETERS			DRAPE		
	D-VALUES	10^{-6} PROBABILITY	(1) SURVIVOR	D-VALUES	10^{-6} PROBABILITY	(1) SURVIVOR	D-VALUES	10^{-6} PROBABILITY	(1) SURVIVOR
NATURAL CONTAMINATION	15.71	124.16	29.90	8.70	70.84	18.66	2.78	24.68	7.99
ARTIFICIAL CONTAMINATION	12.01	130.70	58.63	3.54	34.72	13.49	1.91	20.30	8.84
INOCULATED PRODUCT MONITORS 10^6 SPORES	16.48	220.65	121.80	17.77	235.47	128.85	1.97	25.91	14.11
FILTER PAPER MONITORS 10^6 SPORES	1.33	17.47	9.48						

DATA = TIME (MIN.)

E.O. CONC. = 1000 MG/L
TEMP. = 130°F
E.O. SYSTEM = 100%
PACKAGING = GLASSINE
% R.H. = 50%

TABLE XIV

PROBABILITY DATA

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

	SYRINGES			CATHETERS			DRAPE		
	D-VALUES	10^{-6} PROBABILITY	(1) SURVIVOR	D-VALUES	10^{-6} PROBABILITY	(1) SURVIVOR	D-VALUES	10^{-6} PROBABILITY	(1) SURVIVOR
NATURAL CONTAMINATION	33.12	261.75	63.03	13.33	108.62	28.62	4.96	44.04	14.27
ARTIFICIAL CONTAMINATION	18.35	199.67	89.57	6.41	62.87	24.43	3.13	33.24	14.49
INOCULATED PRODUCT MONITORS 10^6 SPORES	27.70	370.96	204.77	39.26	520.17	284.63	3.20	42.21	22.98
FILTER PAPER MONITORS 10^6 SPORES	2.39	31.36	17.09						

DATA = TIME (MIN.)

E.O. CONC. = 400 MG/L
TEMP. = 130°F
E.O. SYSTEM = 12-88%
PACKAGING = GLASSINE
% R.H. = 50%

TABLE XV

PROBABILITY DATA

	SYRINGES			CATHETERS			DRAPE		
	D-VALUES	10^{-6} PROBABILITY	(1) SURVIVOR	D-VALUES	10^{-6} PROBABILITY	(1) SURVIVOR	D-VALUES	10^{-6} PROBABILITY	(1) SURVIVOR
NATURAL CONTAMINATION	16.80	132.76	31.97	7.55	61.54	16.21	3.44	30.55	9.90
ARTIFICIAL CONTAMINATION	12.74	138.61	62.18	4.05	39.77	15.46	2.30	24.42	10.64
INOCULATED PRODUCT MONITORS 10^6 SPORES	22.01	294.70	162.68	22.90	303.48	166.06	2.19	28.84	15.70
FILTER PAPER MONITORS 10^6 SPORES	1.53	20.23	10.98						

DATA = TIME (MIN.)

E.O. CONC. = 700 MG/L
TEMP. = 130°F
E.O. SYSTEM = 12-88%
PACKAGING = GLASSINE
% R.H. = 50%

TABLE XVI

PROBABILITY DATA

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

	SYRINGES			CATHETERS			DRAPE		
	D-VALUES	10^{-6} PROBABILITY	(1) SURVIVOR	D-VALUES	10^{-6} PROBABILITY	(1) SURVIVOR	D-VALUES	10^{-6} PROBABILITY	(1) SURVIVOR
NATURAL CONTAMINATION	227.11	1794.91	432.22	51.76	421.63	111.08	35.96	319.16	103.39
ARTIFICIAL CONTAMINATION	161.58	1758.09	788.63	29.04	284.98	110.73	22.26	236.72	103.15
INOCULATED PRODUCT MONITORS 10^6 SPORES	136.25	1823.45	1006.53				23.90	314.83	171.43
FILTER PAPER MONITORS	18.98	252.73	138.87						

DATA = TIME (MIN.)

E.O. CONC. = 400 MG/L
TEMP. = 80°F
E.O. SYSTEM = 100%
PACKAGING = GLASSINE
% R.H. = 50%

The inoculated filter paper and inoculated product monitor curves were developed by using a known concentration of spores inoculated on presterilized product and plotted against the time for survival of one organism per test cycle to 10^0 intercept.

The natural and artificial contamination curves were developed by plotting known concentrations of microorganisms through assay procedures, against calculated survival times for a probability of one survivor in a million samples (1/1,000,000).

The line-up from the 10^{-6} point on the probability curve to the 10^0 intercept indicates the time needed for a 1/1,000,000 safety factor for naturally and artificially contaminated product.

The plot of inoculated product and filter paper monitors from their respective concentrations to the 10^0 intercept indicates how well the monitors performed within the one in a million probability.

The filter paper biological monitors for catheters and syringes show less resistance than the product, either naturally or artificially contaminated. This is indicated by the relationships of all curves at 10^0 intercept. The filter paper monitor in all cases never approached the 10^{-6} probability of survivor times. This evidence indicates that filter paper monitors at 10^6 concentration of viable resistant spores are not acceptable as an indication of sterility and can not be used for product release.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

The catheter probability graphs (Figs. 14, 8, and 11) for 400, 1000, and 700 mg/l sterilant concentrations, respectively, at 130°F indicated that the inoculated product produced from 1.82 to 2.70 times greater resistance levels beyond the 10^{-6} probability for sterilization of naturally contaminated product. In this situation, over-sterilization is observed as the inoculated product produced probabilities of 10^{-11} to 10^{-16}. All of the catheter data indicate that the level of inoculation necessary to produce the desired probability of 10^{-6} may be at a much lower concentration of resistant organisms than 10^{6} spores.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

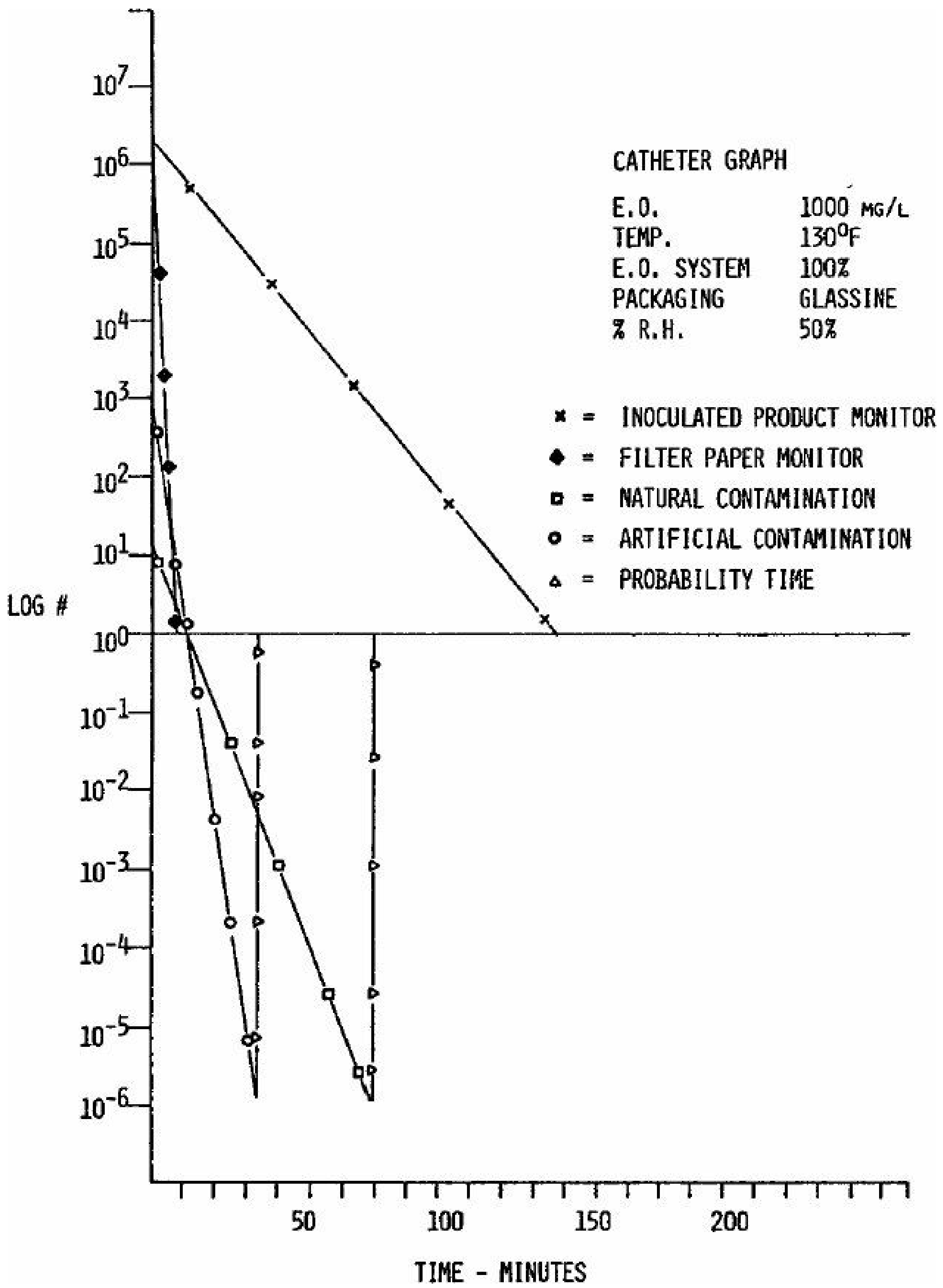


FIGURE 8. Probability graph for catheters.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

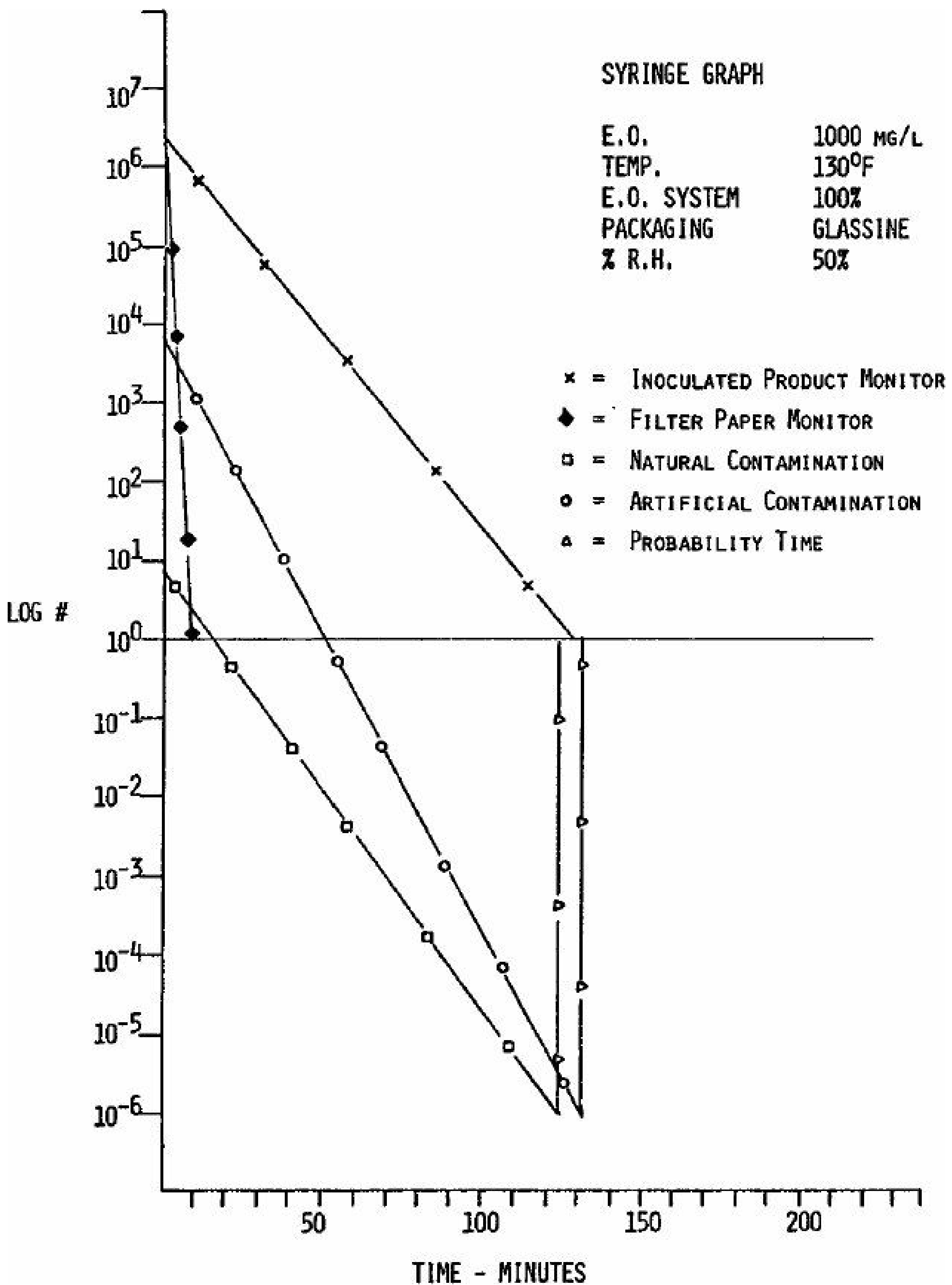


FIGURE 9. Probability graph for syringes.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

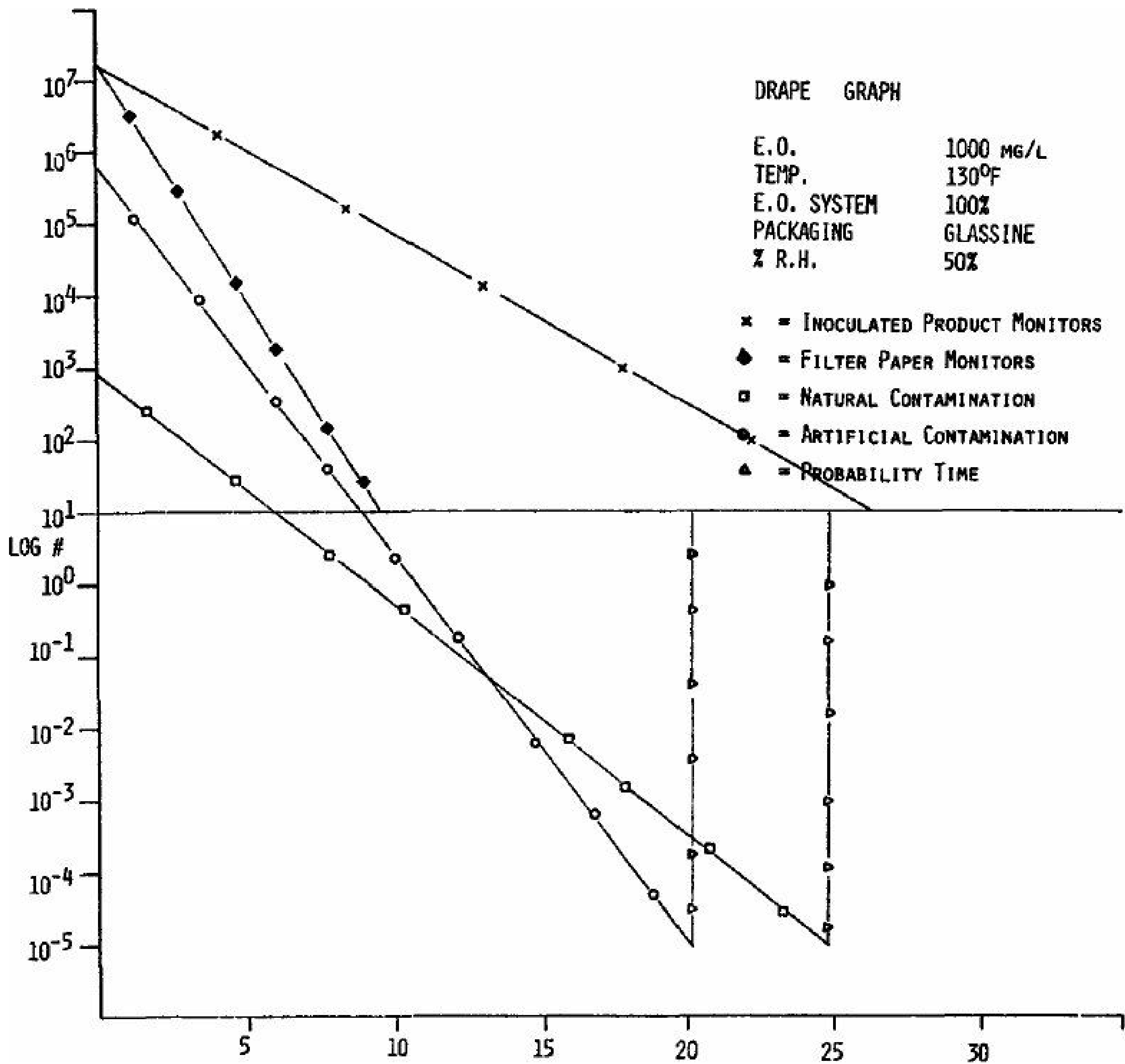


FIGURE 10. Probability graph for drape material.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

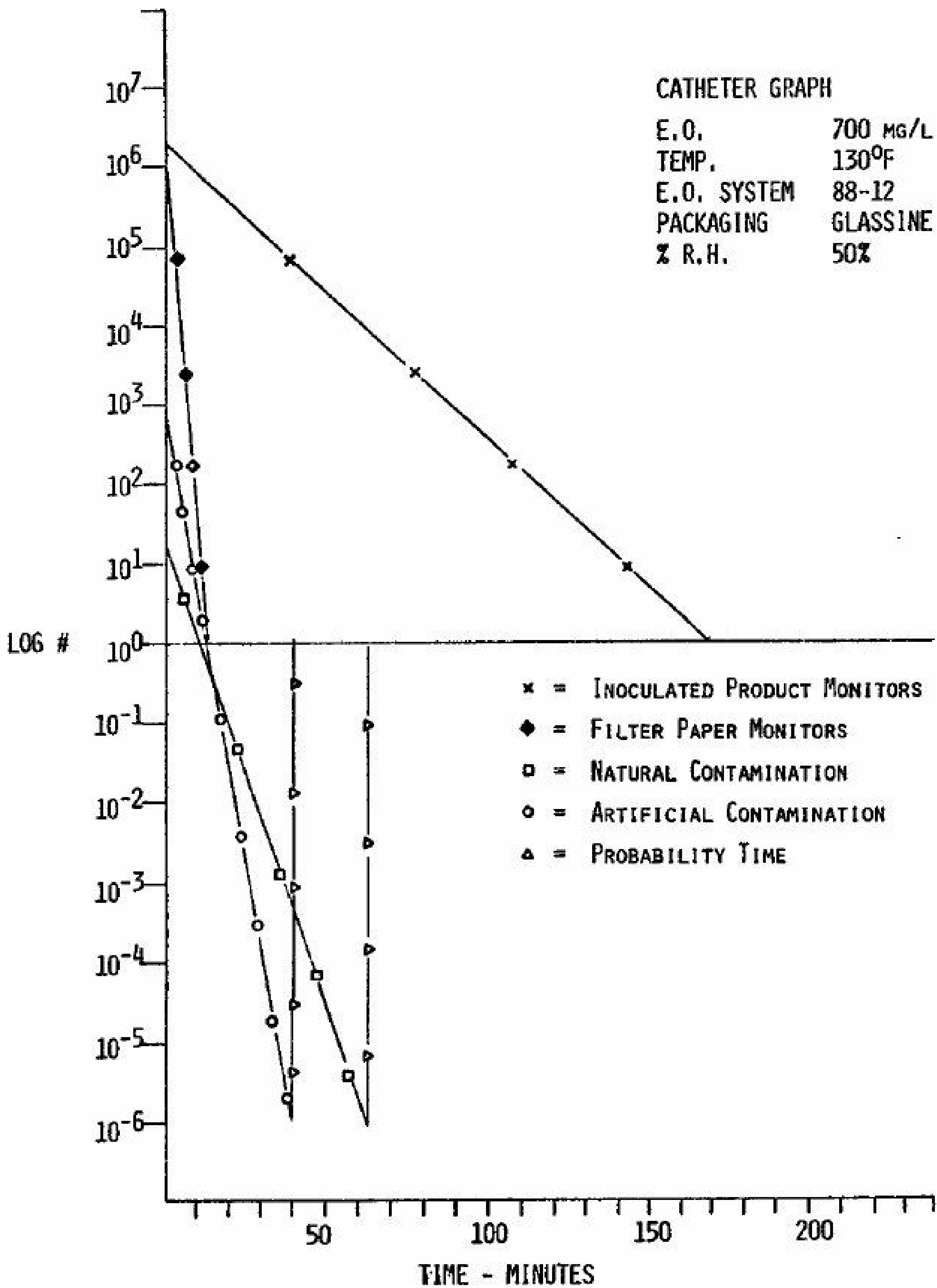


FIGURE 11. Probability graph for catheters.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

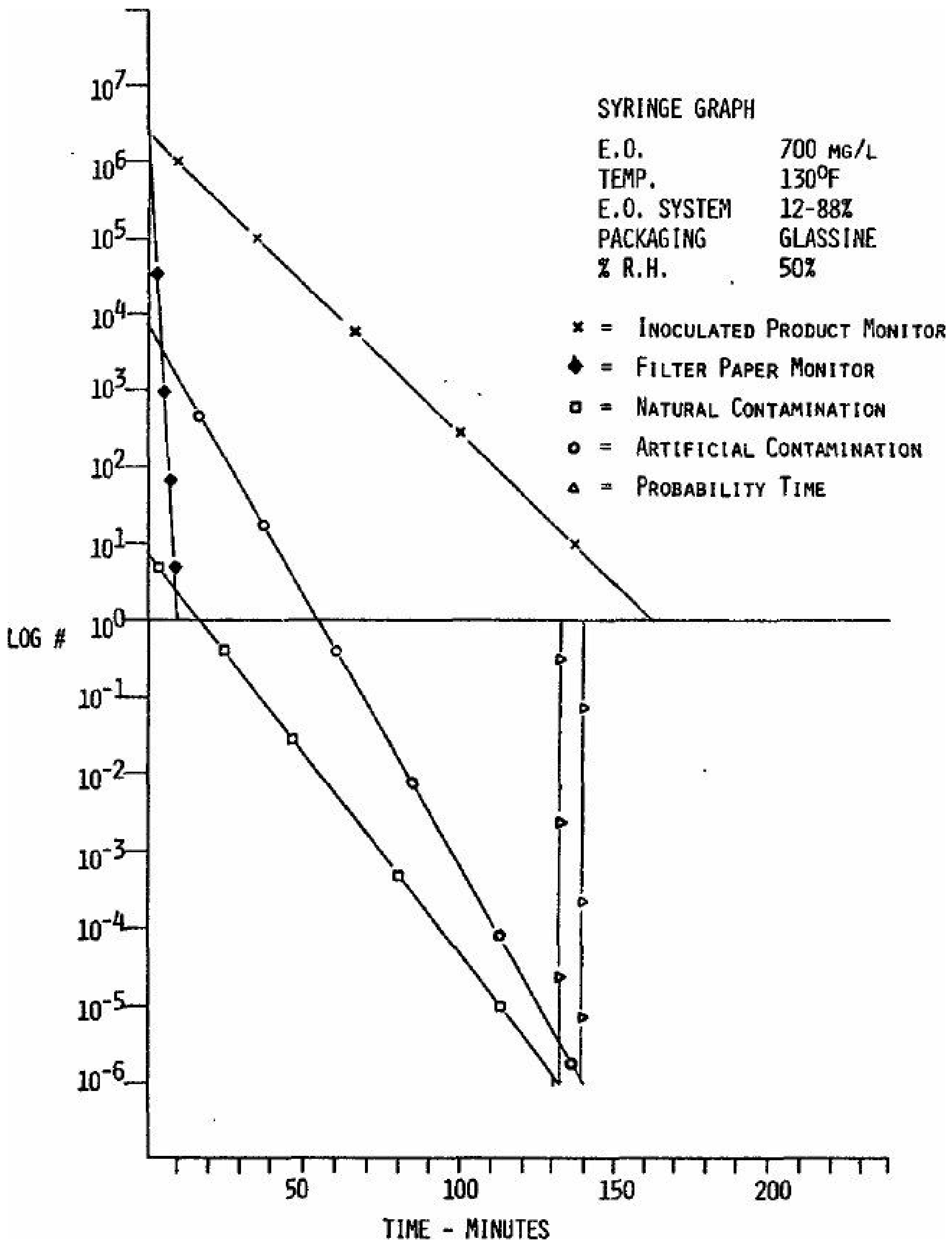


FIGURE 12. Probability graph for syringes.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

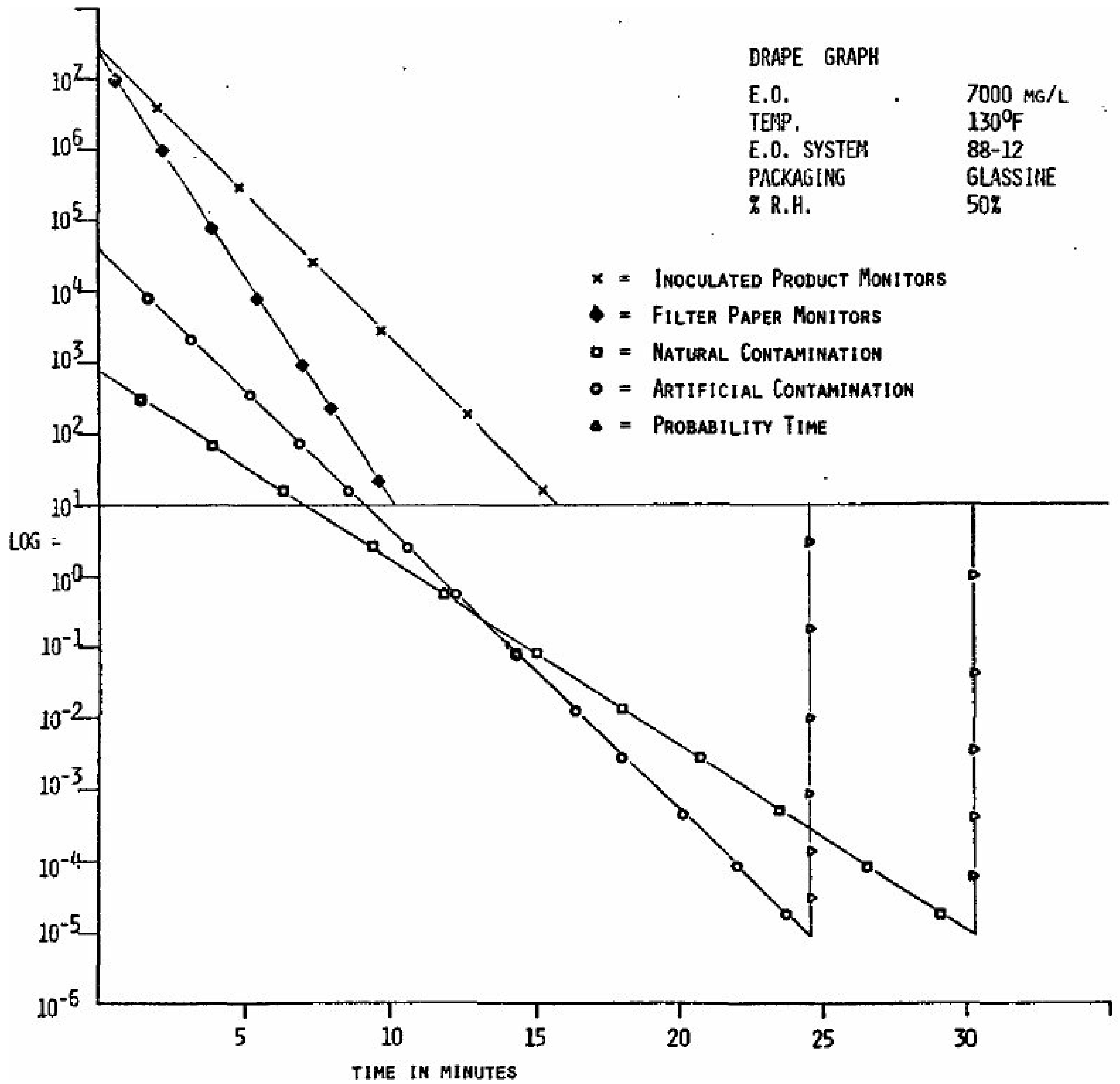


FIGURE 13. Probability graph for drape material.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

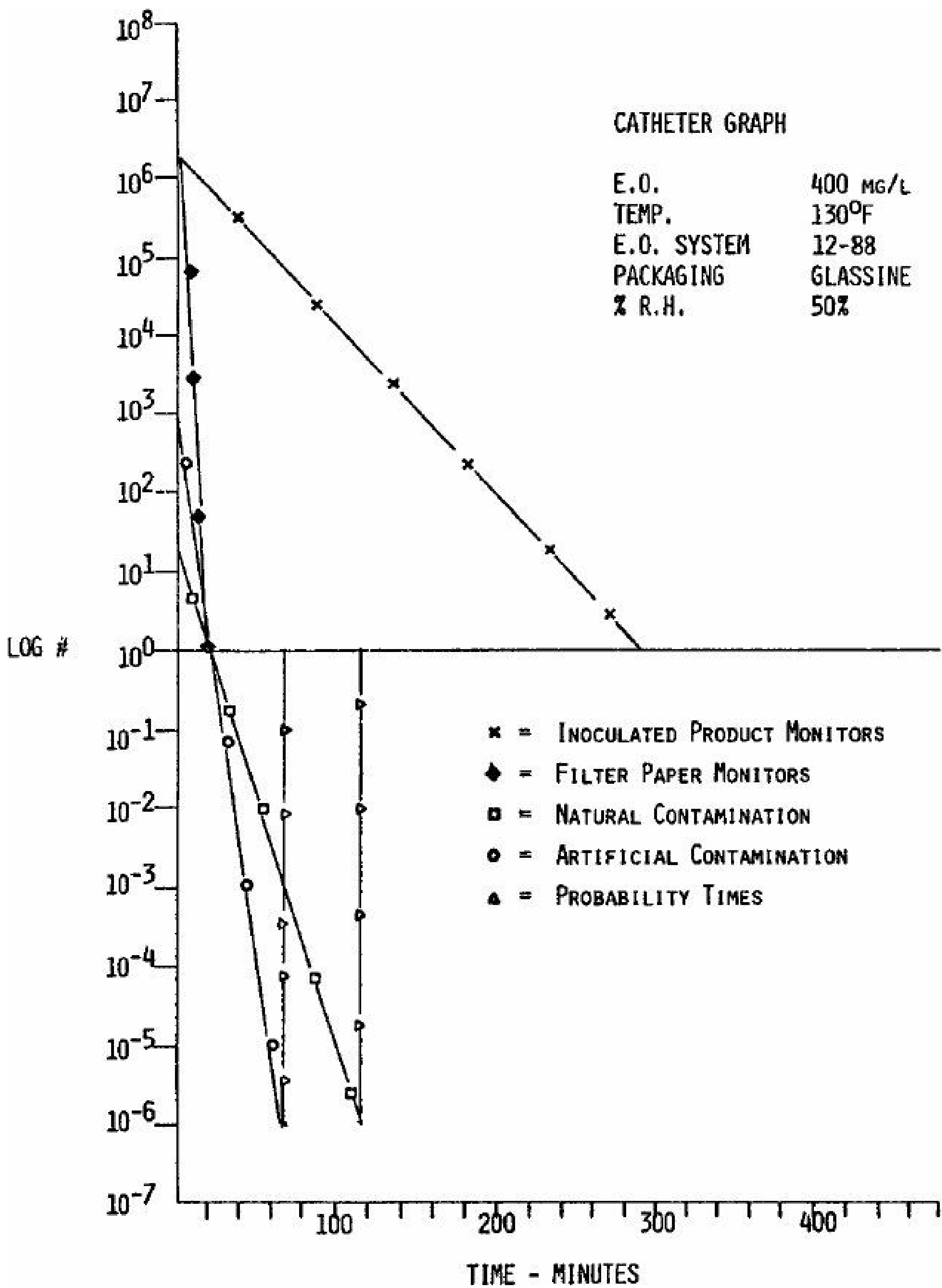


FIGURE 14. Probability graph for catheters.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

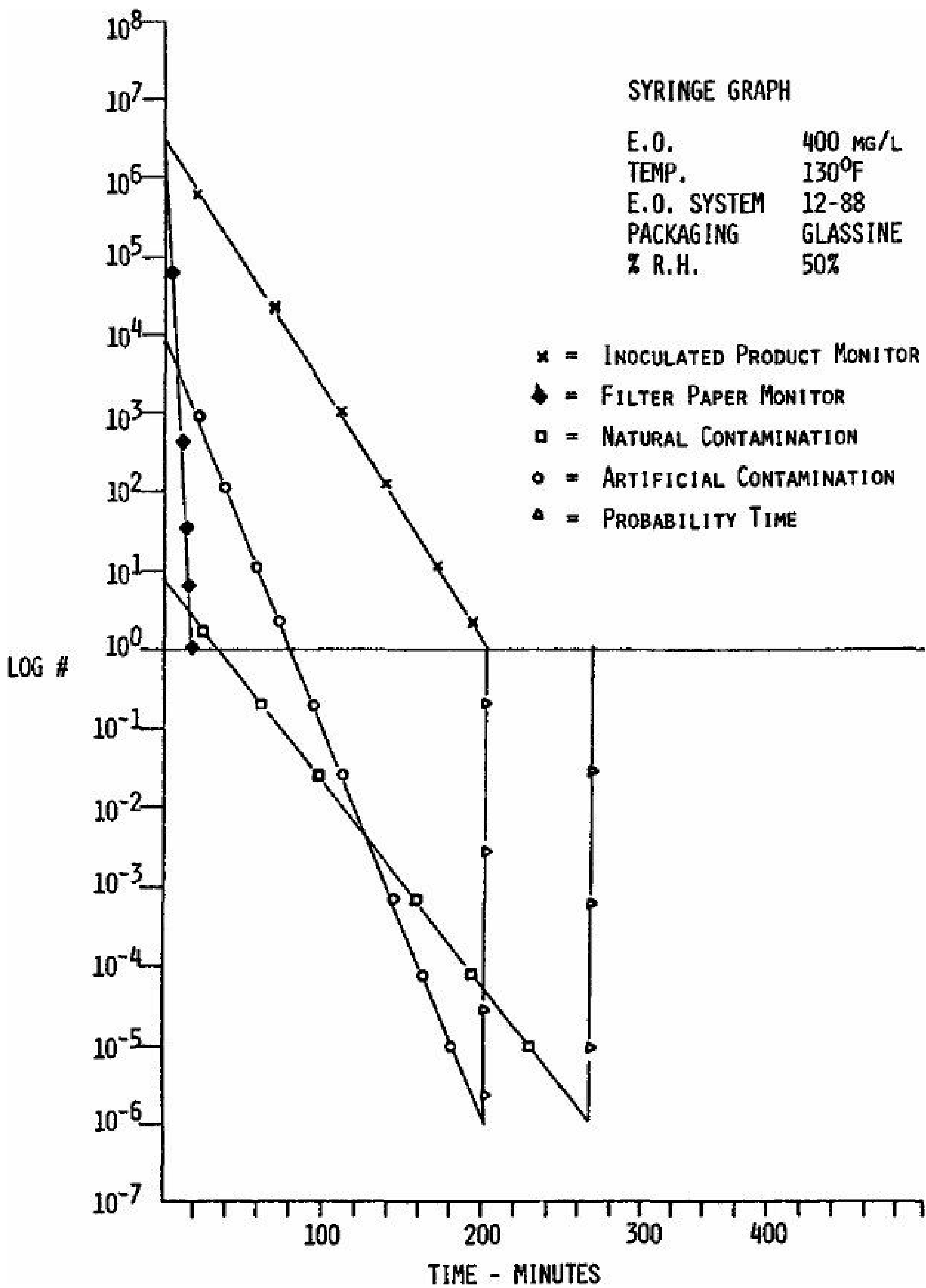


FIGURE 15. Probability graph for syringes.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

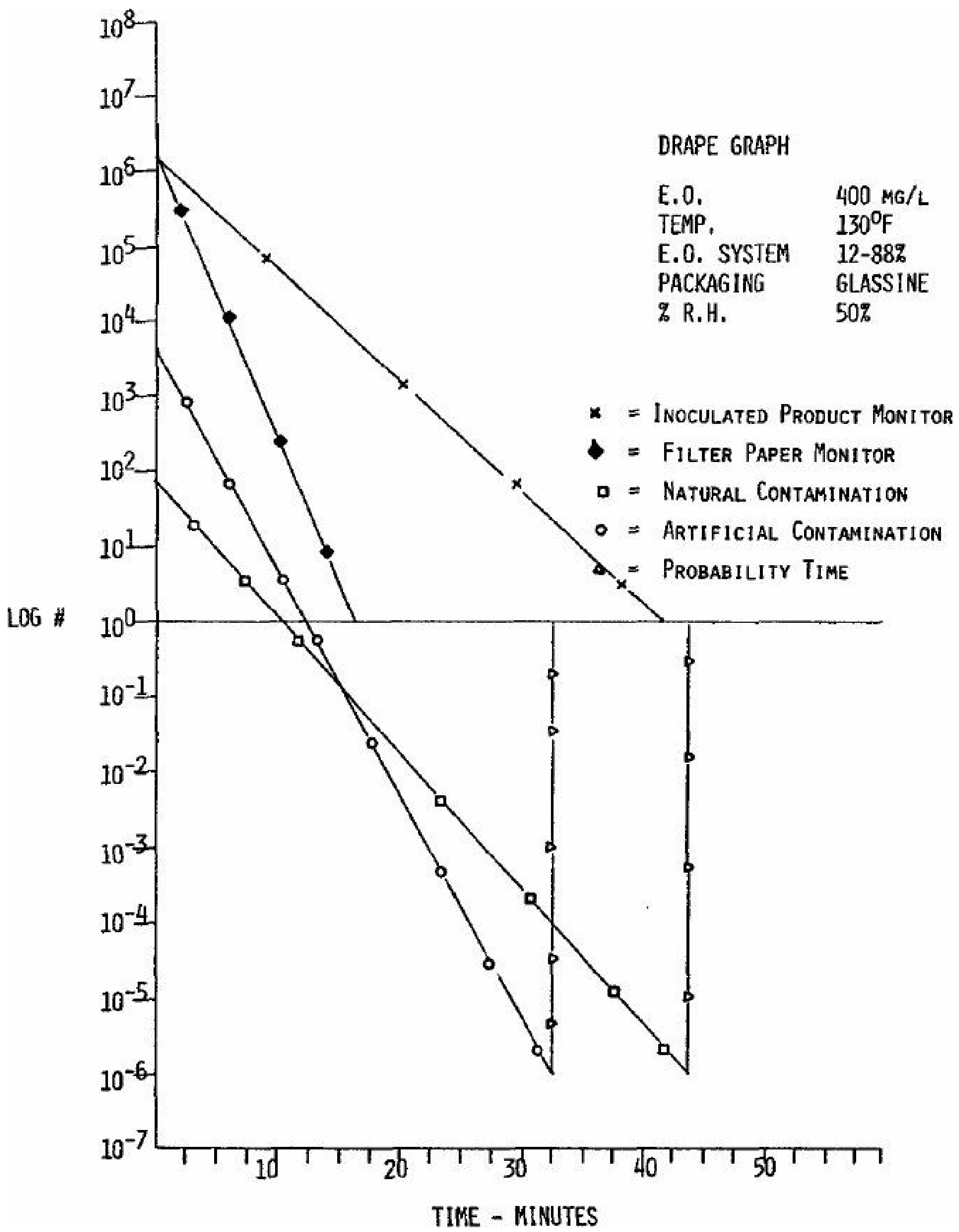


FIGURE 16. Probability graph for drape material.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

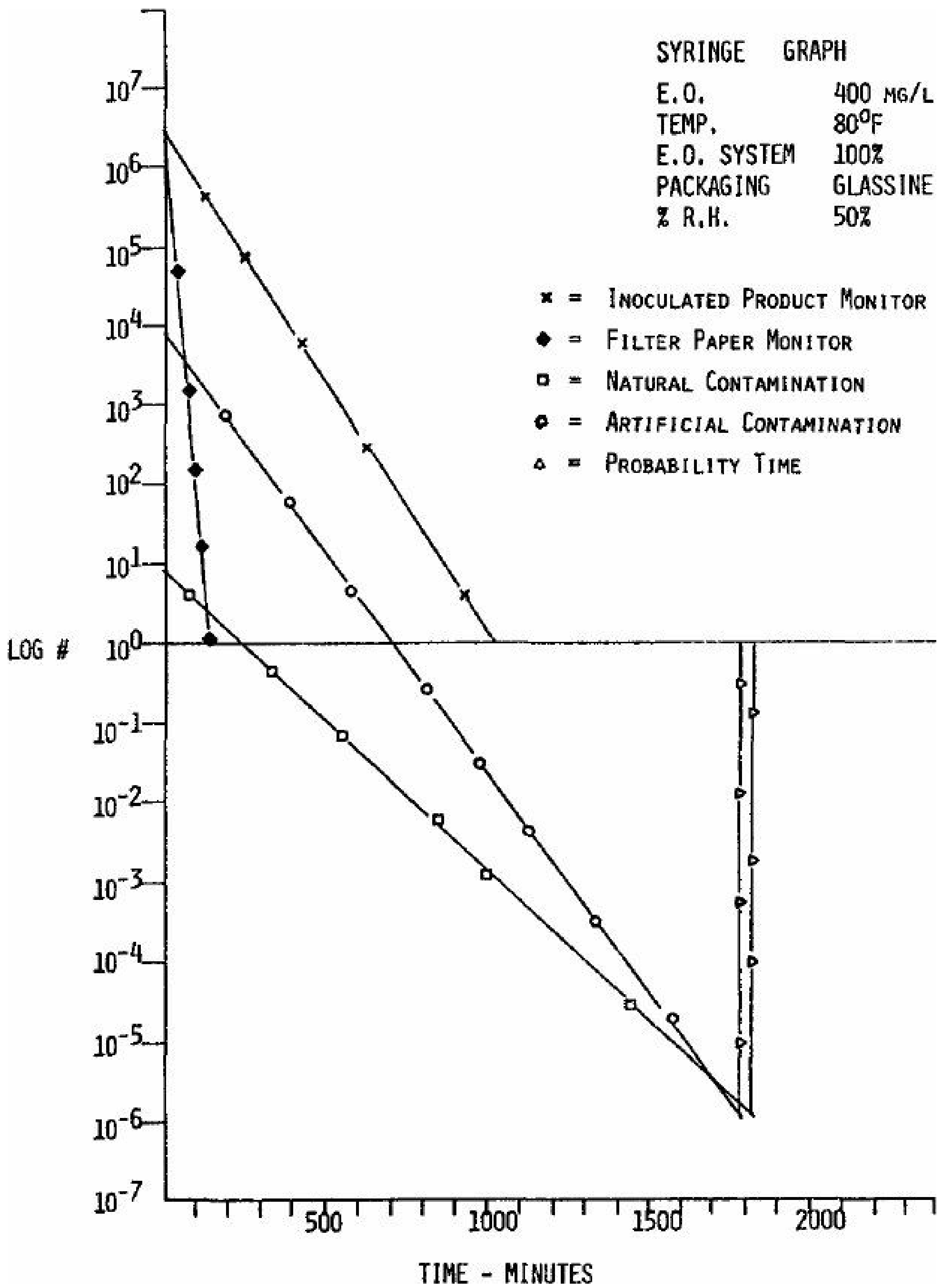


FIGURE 17. Probability graph for syringes.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

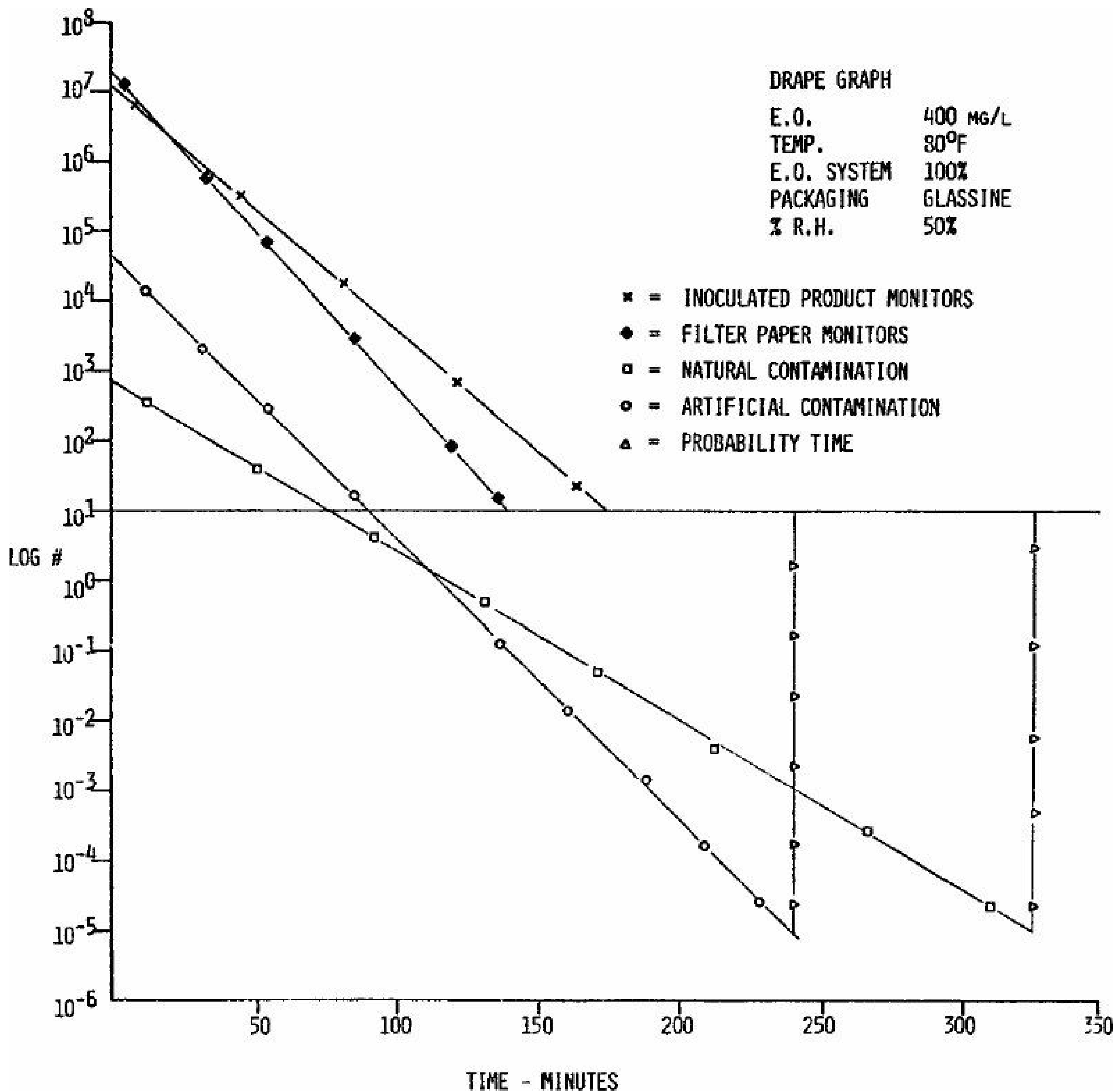


FIGURE 18. Probability graph for drape material.

Unfortunately, the trend observed with the catheter did not continue with the drape or syringes. The syringe probability graphs in Figures 9 and 12 for 700 and 1000 mg/l sterilant at 130° F indicated that the inoculated product at 10^6 concentration of spores produced probabilities at or very near the 10^{-6} probability targeted.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

The syringe probability graph of Figure 15 for the 400 mg/l sterilant at 130°F also was very close to the 10^{-6} probability, but fell short by one probability unit or predicted 10^{-5}, or one survivor in 100,000. The syringes processed at 400 mg/l sterilant concentration at 80° F, however, only gave a probability of 10^{-3} or half the desired 10^{-6} targeted probability. This evidence indicates that the 130°F temperature is very significant at the higher concentration of sterilant and the inoculated product at 10^{6} spore concentration can produce the desired probability levels.

Figures 10, 13, 16, and 18 for the drape material probability studies showed that the product produced a varied relationship in probability comparisons. Figures 10 and 16 for 1000 mg/l and 400 mg/l at 130° F showed good correlation while Figures 13 and 18 for 700 mg/l at 130°F and 400 mg/l at 80°F did not produce the required results. This points to a precise requirement for parametric conditions for types of products to be sterilized and may be indicative of other variables not studied in this paper.

CONCLUSIONS

From the generated data, we have determined the following criteria for sterilization with ethylene oxide:

1) Naturally contaminated products vary in resistance initially because of structure and composition.

2) Good Manufacturing Practices are of the utmost importance to reduce natural contamination to its lowest possible level. The manufacturer must maintain strict control over his GMP if his operation is not to become suspect by maintaining the same sterilization parameters.

3) It is possible, through assay and resistance studies, to develop biological monitoring system for the sterilization process to give the manufacturer a suitable safety factor. This, however, can be accomplished only after the manufacturer has developed enough data in background bioburden and in the natural resistance of his product to his particular set of sterilization parameters.

4) The most significant result of this investigation is the fact that, under none of the conditions studied, were filter paper

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

monitors at 10^6 spore population adequate to successfully give the manufacturer any assurance of sterility beyond one chance in 100 of a survivor, and in most cases they were not as resistant as the product which was naturally contaminated at a very low level.

We, therefore, recommend that the manufacturer of disposable devices 1) utilize inoculated product to monitor the sterilization conditions of his equipment, 2) maintain a tight control of manufacturing practices, and 3) develop resistance studies against the product to determine the necessary level of safety factors for his sterilization processes.

REFERENCES

1. U. S. Pharmacopeia, XVII (1965); XVIII (1970); XIX (1975). Mack Publishing Company, Easton, Pa.

2. Ernst, R. R. and Doyle, J. E. (1968). Limiting factors in ethylene oxide gaseous sterilization. In Developments in Industrial Microbiology, 9:293-296.

3. Bruch, C. W. and Bruch, M. K. (1970). Gaseous disinfection. In Disinfection. M. A. Bernarde, Ed. Marcel Dekker, Inc., New York; pp. 149-206.

4. Kaye, S. and Phillips, C. R. (1949). The sterilizing action of gaseous ethylene oxide. IV. The effects of moisture. Amer. J. Hyg. 50:296-306.

5. Ernst, R. R. and Shull, J. J. (1962). Ethylene oxide gaseous sterilization. II. Influence of method of humidification. Appl. Microbiol. 10:342-344.

6. Perkins, J. J. and Lloyd, R. S. (1961). Applications and equipment for ethylene oxide sterilisation. In Recent Developments in the Sterilisation of Surgical Materials. Pharmaceutical Press, London, England; pp. 76-90.

7. Mayr, G. (1961). Equipment for ethylene oxide sterilisation. In Recent Developments in Sterilisation of Surgical Materials. Pharmaceutical Press, London, England; pp. 90-97.

8. Ernst, R. R. and Doyle, J. E. (1968). Sterilization with

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.
gaseous ethylene oxide - a review of chemical and physical

factors. Biotechnol. Bioeng. 10:1-31.

9. El-Bisi, H. M., Vondell, R. M., and Esselen, W. B. (1963). Kinetics of bactericidal activity of ethylene oxide in vapor phase. I. Effect of sterilant temperature and pressure. Bacteriological Proceedings of the 63rd Annual Meeting of the American Society for Microbiology, Cleveland, Ohio. Abstract A51; p. 13.

10. Opfell, J. B., Wang, Y. -L., Louderback, A. L., and Miller, C. E. (1964). Penetration by gases to sterilize interior surfaces of confined spaces. Appl. Microbiol. 12:27-31.

11. NASA Standard Procedure for the Microbiological Examination of Space Hardware. (1968). NHB5340.1A.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

RESISTANCE LEVELS FOR BIOLOGICAL INDICATORS FOR USE IN STERILIZATION BY IONIZING RADIATION

Robert Berube

Corporate Quality Assurance
Becton, Dickinson & Company
Research Triangle Park, North Carolina

The Microbiological Quality Assurance Department of Corporate Quality Assurance, at Becton, Dickinson & Company, was granted Contract FDA 73-214. The contract was entitled the “Determination of Resistance Parameters for Biological Indicators Used in Sterilization of Medical Supplies by Ionizing Radiation”, and a primary objective was to develop a reliable method to assure product sterility.

On practical levels, finished product sterility tests can never assure sterility or be used to assess the probability of a survivor in the sterilization process. The sterilization process, however, can be defined as a probability function. In a sterilization process, the probability of a survivor of less than 1 in 10^6 (an arbitrarily selected minimum) can be estimated through the use of calibrated challenges, i. e., through the use of biological indicator (BI) controls.

The work scope of the contract required that the performance and certain characteristics of BIs be defined. To that end, the work scope of the contract was organized into component objectives so that the necessary consequential and complementary activities could be scheduled. These objectives are listed in Table I.

TABLE I

OBJECTIVES OF EXPERIMENTS FOR CONTRACT NO. FDA 73 - 214

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Experimental	Objective
Preparatory studies	Selection of cultures and growth techniques
1	With spores of B. pumilus: establish a baseline dose-response curve to ionizing (gamma) radiation.
2	With spores of B. pumilus: enhance the ionizing (gamma) radiation resistance of the spores by treatment with various protective substances.
3	With spores of B. pumilus: determine if immature spores are more resistant to ionizing (gamma) radiation than mature spores.
4	Determine the resistance to ionizing (gamma) radiation of M. radiodurans and/or S. faecium.
5	With spores of B. pumilus: develop, compare, and assess the resistance to ionizing (gamma) radiation of carrier-borne spores versus product-borne spores.
Composite experiment	Demonstrate that, when using the "best" biological indicator system devised from the experimental data, the comparison of the radiation resistance of the product microbial load to the radiation resistance of biological indicators can lead to the assessment of levels of probability of sterility.

The first objective was to select the appropriate biological indicator organisms, techniques of cultivation and purification, and storage conditions. Spores of Bacillus pumilus E601 (ATCC 27142), and vegetative cells of either Streptococcus faecium (ATCC 19580) or Micrococcus radiodurans (ATCC 13939), or a contractor-developed organism, could be used, provided that six-month storage stability could be demonstrated for wet cell preparations and provided that there was no decrease in the radiation resistance of wet cell preparations.

The radiation resistance was to be measured in D-value units. (A D-value is the dose needed to reduce the population by 90%, or one log cycle when the log number of survivors is graphically plotted). Three levels of radiation resistance were sought, 0.15 Mrad, 0.25 Mrad, and 0.35 Mrad, and these levels of radiation resistance could be attained by the use of different organisms, or by protective systems, or both.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

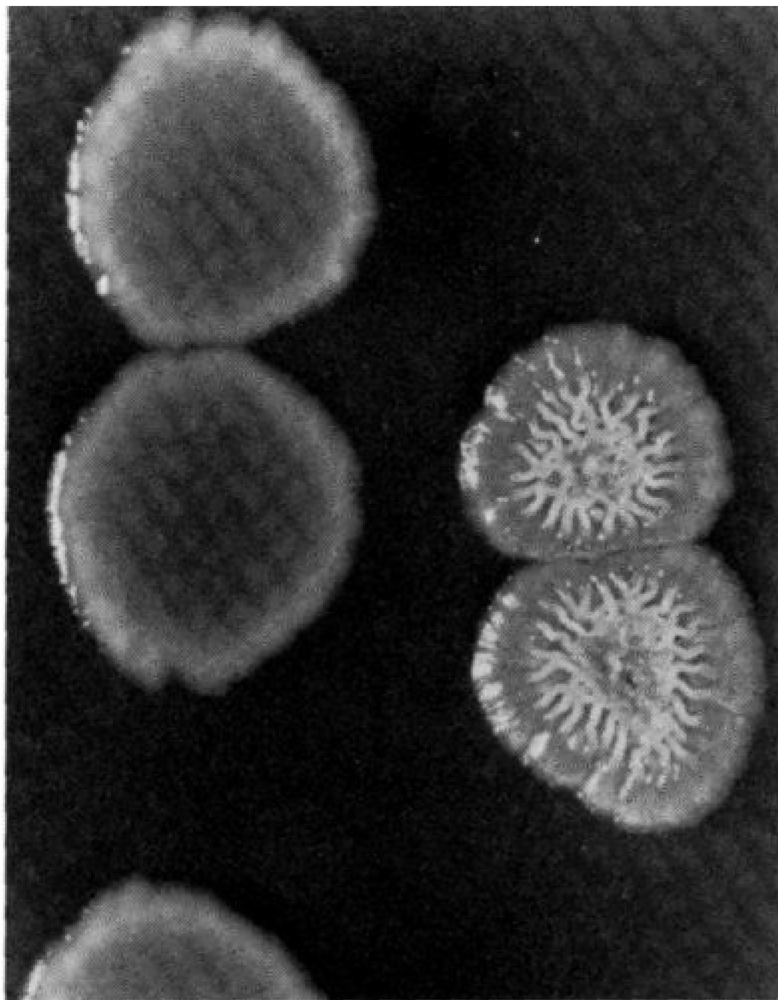


FIGURE 1. Rough and smooth colonies of _Bacillus_ _pumilus_ E601.

The stock culture of _B_. _pumilus_ E601 occasionally showed two types of colonies, one rough in appearance and one smooth in appearance (Fig. 1). A new culture of _B_. _pumilus_ E601 (ATCC 27142) also showed rough and smooth colonies, and isolates of each type were repurified until each gave only its type of colony. The rough type was designated BP-1, the smooth was designated BP-2, and the original rough type organism was labeled BP-3. Each culture was cultivated in two spore production systems; each spore crop was used to prepare biological indicators, and the resistance to ionizing radiation of the BIs was determined. The data show that the nutritional environment does indeed influence spore crop yield, but it does not affect the radiation resistance of the spores (Table II). Results of the preparatory studies carried out with _M_. _radiodurans_, _S_. _faecium_, and the contractor-selected organism are consolidated with other data developed on those organisms, and are presented later.

The second objective was to prepare biological indicators with
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.
spores of _B_. _pumilus_ E601 (ATCC 27142) because it is an organism

that is used to monitor ionizing (gamma) radiation systems. The paper BIs were examined for a predictable, reproducible dose-response to ionizing radiation that would be the baseline dose-response pattern in the study.

When the BIs were equilibrated to three concentrations of water vapor, to three different equilibrium relative humidity levels (25%, 50%, and 75% RH), there was no apparent influence upon the radiation resistance of the BIs. When three calibrations of BIs were prepared from the same spore crop of B. pumilus E601, that is, the BIs carried 1×10^5, or 1×10^6, or 1×10^7 spores/carrier, the radiation resistance was not greatly influenced by the concentration of spores on the carrier. When the BIs were stored in hermetically sealed pouches, the viable titer of the BIs remained stable over 36 weeks (Table III).

TABLE II

SPORE CROP YIELDS AND D_{Mrad}-VALUES FOR STRAINS OF BACILLUS PUMILUS E601 (ATCC # 27142)

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Culture*	Media Combination					
	A**			B***		
	Final volume (ml)	Final titer (spores/ml) ($\times 10^{10}$/ml)	D-value (Mrad) ****	Final volume (ml)	Final titer (spores/ml) ($\times 10^{10}$/ml)	D-value (Mrad) ****
BP-1 (rough)	20	6.7	0.25	50	14.0	0.25
BP-2 (smooth)	<1.0	-	-	45	10.0	0.26
BP-3 (rough)	<1.0	-	-	20	1.7	0.28

*BP-1 (rough) and BP-2 (smooth) are isolates of B. pumilus E601 (ATCC #27142). BP-3 (rough) is B. pumilus E601 (ATCC #27142), a culture that was in the stock culture collection.

**Medium A combination: starter culture medium contained (g/l): Trypticase soy broth (BBL), 35 g; soluble starch (Difco), 1 g; yeast extract (BBL), 2 g; and distilled water, 1 l. Sporulation medium contained (g/l): Trypticase soy broth (BBL), 35 g; agar (BBL), 18 g; soluble starch (Difco), 1 g; yeast extract (BBL), 2 g; and distilled water, 1 l.

***Medium B combination: starter culture medium contained (g/l): tryptone (Difco), 10 g; yeast extract (BBL), 5 g; K_2HPO_4, 2 g; and distilled water, 1 l. Sporulation medium contained (g/l): Trypticase (BBL), 5 g; phytone (BBL), 3 g; beef extract (BBL), 5 g; $MnSO_4 \cdot 2H_2O$, 0.15 g; $CaCl_2$, 0.01 g; agar (BBL), 20 g; and distilled water, 1 l.

****Dose rate: 0.15 Mrad/hr, ^{60}Co.

TABLE III

STABILITY OF ASSAY TITER OF BIOLOGICAL INDICATORS OF BACILLUS PUMILUS E601 SPORES DURING STORAGE

BI lots*	Assay titers after storage intervals (weeks)			
	0	4	12	36
bp-1×	3.4×10^6	3.8×10^6	2.9×10^6	3.8×10^6
bp-1	4.6×10^6	5.1×10^6	6.1×10^6	4.4×10^6
bp-2	5.7×10^6	9.0×10^6	7.9×10^6	1.5×10^6
bp-3	3.4×10^6	7.1×10^6	7.1×10^6	5.9×10^6

*Biological indicators (BI) of B. pumilus E601 (ATCC #27142) spores were prepared from spore crops BP-1×, BP-1, BP-2, and BP-3 on August 29, 1973

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

When the same spore crop (BP-1) was used to prepare a new lot of BIs (bp-1), and a new spore crop (BP-7) was prepared to produce a new lot of BIs (bp-7), and both were compared to the original lot of BIs (bp-1, old), the radiation resistance of the original lot was stable in storage, the radiation resistance of the new lot prepared from the same crop was similar to the original and had storage stability, and the radiation resistance of a new lot of BIs prepared from a new crop had storage stability and radiation resistance similar to the original lot of BIs (Table IV). When the same lot of BIs was exposed to different dose-rates, the rate of kill varied (Table V).

The third objective was to determine if the resistance to ionizing radiation could be enhanced by treating spores of B. pumilus E601 with various protective substances.

TABLE IV

D_{Mrad}-VALUE OF BIOLOGICAL INDICATORS OF BACILLUS PUMILUS E601 SPORES

Dates of exposure	D_{Mrad}-value** (0.15 Mrad/hr)		
	Biological indicator lots*		
	bp-1 (old)	bp-1 (new)	bp-7
3/11/74	0.27	0.29	0.25
4/16/74	0.26	0.25	0.18
4/18/74	0.28	0.22	0.22
5/15/74	0.20	0.21	0.21
5/28/74	0.21	0.22	0.20
6/14/74	0.22	0.22	0.22
6/28/74	0.26	0.26	0.23

*Biological Indicators (BI) were prepared from spore crops of B. pumilus E601. BI lots bp-1 (old) and bp-1 (new) were prepared from spore crop BP-1 on August 29, 1973, and on February 25, 1974 respectively. BI lot bp-7 was prepared on February 11, 1974, from another spore crop.

**The quantity of radiation at a stated dose rate required to kill 90% of the population.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

TABLE V

INFLUENCE OF DOSE RATE ON D_{Mrad}-VALUE OF BIOLOGICAL INDICATORS OF <u>BACILLUS</u> <u>PUMILUS</u> E601 (ATCC #27142) SPORES

BI lot	BI titer	Average D_{Mrad}-value of dose rate (Mrad/hr)		
		0.15*	0.22**	0.80*
bp-1	6.1×10^6	0.24	0.19	0.17

*Average of 7 D-value determinations.
**Average of 2 D-value determinations.

TABLE VI

EFFECT OF SPORE TREATMENT ON THE D_{Mrad}-VALUE OF BIOLOGICAL INDICATORS OF <u>BACILLUS</u> <u>PUMILUS</u> SPORES E601 (ATCC #27142)

BI lots*	D_{Mrad}-value	
	0.15 Mrad/hr	0.8 Mrad/hr
bp-1 (old)	0.27	0.19
bp-1 (new	0.29	0.18
bp-1 (control)	0.26	0.17
bp-1 (cystine)	0.27	0.17
bp-1 (horse serum)	0.31	0.18
bp-1 (sorbitol)	0.25	0.18
bp-7	0.25	0.18

*BI lots bp-1 (old), bp-1 (new), and bp-7 were prepared from crops BP-1 and BP-7 on August 29, 1973, February 25, 1974 and February 11, 1974, respectively. BI lots bp-1 (control), bp-1 (cystine, 0.0025%), bp-1 (horse serum, 0.5%), bp-1 (sorbitol, 0.5%) were prepared from spore crop BP-1 on January 30, 1974.

A portion of spore crop BP-1 was used to prepare four spore suspensions, each to the same titer but containing either cystine, or sorbitol, or horse serum, or water. BIs were produced from these suspensions and the D_{Mrad}-value of each BI lot was

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

determined. These substances did not markedly alter the radiation resistance of the BIs (Table VI).

The fourth objective was to determine if immature spores were more resistant to ionizing radiation than mature spores, and if immature spores could be stabilized. When a vegetative cell sporulates, the content of sulfurcontaining amino acids increases, and sulfurcontaining amino acids are reputed to give some resistance to ionizing radiation. By manipulation of the constituents in nine growth media, the ratio of endosporangiospores to mature spores to vegetative cells was affected, and there was an optimal harvest time when the yield of endosporangiospores was at a maximum. However, the premise that the higher sulfur content of the immature spores would increase their resistance to radiation was not supported by the data (Table VII).

TABLE VII

D_{Mrad}-VALUE OF BIOLOGICAL INDICATORS OF BACILLUS PUMILUS E601 ENDOSPORANGIOSPORES AND BACILLUS PUMILUS E601 SPORES

BI lots*	D_{Mrad}-value**
bp-1×	.25
bp-1	.20
bp-2	.20
bp-3	.21
bp-endosporangiospore	.16

*BI lots bp-1×, bp-1, bp-2, and bp-3, and bp-endosporangiospore were prepared from B. pumilus E601 (ATCC #27124) spore crops BP-1×, BP-1, BP-2, and BP-3, on August 29, 1973, and from a crop of endosporangiospores on October 19, 1973, respectively.

**Dose rate was 0.15 Mrad/hr.

The fifth objective in the scheduled activities was to determine the radiation resistance of M. radiodurans, of S. faecium, and of the contractor-selected biological indicator organism; and also to compare the radiation resistance of these organisms to the radiation resistance exhibited by spores of B. pumilus E601. A new

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

culture of M. radiodurans (ATCC 13939) was obtained from the American Type Culture Collection, and cell crops were produced from two media - tryptone-glucose-yeast extract broth and Trypticase soy broth. The cells were cleaned and stored at 4-6°C in a minimal amount of sterile 1% peptone water. The yield from each medium was similar, but only one crop was stable for up to nine months (Table VIII). BIs prepared from cell crops of M. radiodurans were stable through seven months (Table IX), but the radiation resistance of the BIs was variable, showing a decrease in resistance with length of time of storage (Table X).

TABLE VIII

EFFECT OF REFRIGERATED STORAGE ON CELL CROP VIABLE STABILITY OF MICROCOCCUS RADIODURANS (ATCC #13939)

Date of test	Cell crop (cells/ml)			
	MRTGY-1	MRTGY-2	MRTSB-1	MRTSB-2
8/30/73	4.4×10^9	*	1.5×10^9	*
9/22/73	1.4×10^9	2.3×10^9	1.4×10^9	2.1×10^9
9/29/73	**	1.0×10^9	**	1.1×10^9
10/2/73	1.5×10^9	1.3×10^9	1.5×10^9	1.4×10^9
12/4/73	2.2×10^9	1.4×10^9	0.9×10^9	1.7×10^9
12/31/73	2.6×10^8	4.9×10^8	1.3×10^9	7.9×10^7
5/8/73	1.1×10^6	**	4.0×10^9	**

MRTGY-1 and MRTGY-2 cell crops were grown in Tryptone-glucose-yeast extract broth, harvested, suspended in 0.1% peptone water, and refrigerated at 4°C. MRTSB-1 and MRTSB-2 cell crops were grown in Trypticase soy broth, harvested, suspended in 0.1% peptone water, and refrigerated at 4° C. MRTGY-1 and MRTSB-1 cell crops were prepared August 30, 1973, and MRTGY-2 and MRTSB-2 cell crops were prepared September 22, 1973.

*Cell crop not prepared.

**Viable titer not determined.

The cell crops of S. faecium (ATCC 19580) showed a slow decrease in titer during storage. Also, the BIs prepared from the cell
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.
crops of S. faecium exhibited a constant decrease in titer during

90-day storage, and had a D-value of 0.90 Mrad or less. On the basis of these results, we discontinued study of the organism.

The contractor-selected organism was Bacillus stearothermophilus (ATCC 7953). Two lots of BIs were prepared from a spore crop of B. stearothermophilus, and their resistance to radiation was determined and compared to that of BIs of B. pumilus E601. BIs of B. stearothermophilus had a greater D_{Mrad}-value than BIs of B. pumilus (Table XI).

The sixth objective was to evaluate and to compare the resistance to ionizing radiation of spores on paper carriers and spores on three types of disposable items: 10 cc plastic syringes, intravenous catheters, and needles. In order to compare the radiation resistance of spores on paper carriers with spores on selected types of plastic disposable medical products, the inoculum put onto the various carriers (products) had to be recoverable.

TABLE IX

EFFECT OF STORAGE ON BIOLOGICAL INDICATORS OF MICROCOCCUS RADIODURANS (ATCC #13939)

Storage time*** (days)	Biological indicators from cell crop (cells/carrier)	
	MRTGY-1*	MRTSB-1*
0	7.6×10^6	5.7×10^6
2	6.2×10^6	5.2×10^6
11	6.2×10^6	6.9×10^6
20	5.5×10^6	6.5×10^6
72	2.6×10^6	7.6×10^6
87	5.6×10^6	5.2×10^6
204	3.4×10^6	5.0×10^6
218	2.2×10^6	5.6×10^6
240	**	3.5×10^6

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

*MRTGY-1 and MRTSB-1 are designations of cell crops: MR, M. radiodurans; TGY, tryptone-

glucose-yeast extract; TSB, Trypticase soy broth; 1, grown on August 30, 1973.

**BIs of cell crop MRTGY-1 were depleted.

***BIs were stored at room temperature (72 ±4°F) in hermetically sealed aluminum-plastic laminate pouches.

TABLE X

D_{Mrad}-VALUES OF BIOLOGICAL INDICATORS OF _MICROCOCCUS_ _RADIODURANS_ (ATCC #13939) AT TWO DOSE RATES

Date	0.15 Mrad/hr		0.80 Mrad/hr	
	MRTGY-1	MRTSB-1	MRTGY-1	MRTSB-1
9/14/73	0.48	1.48	*	*
12/11/73	0.55	1.20	*	*
3/15/74	0.38	1.20	0.30	0.82
4/16/74	0.18	0.84	0.29	0.36
4/18/74	0.23	*	0.18	*
5/15/74	*	0.74	*	0.42
5/28/74	*	0.74	*	0.42

MRTGY-1 and MRTSB-1 are designations of cell crops: MR, _M_. _radiodurans_ (ATCC # 13939); TGY, tryptone-glucose-yeast extract broth; TSB, Trypticase soy broth; 1, grown August 30, 1973.

*Exposures not done.

The three types of products that were used, intravenous catheter tubes, 10 cc plastic syringes, and needles, were inoculated with a spore suspension of _B_. _pumilus_ E601. By adding Tween 80 to the suspension used to inoculate the product, immersing the product into sterile water containing Tween 80, and then vortexing and insonating the immersed product, alternately, three times, better than 95% of the inoculum could be recovered.

Then, the radiation resistance of these inoculated carriers (Product-BIs) was compared with that of product-containing paper BIs (BIs-in-Product). As Table XII shows, inoculated product

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

(Product-BIs) were not more resistant to radiation than the paper

BIs in the product (BIs-in-Product). These data do not demonstrate a valid decrease in resistance. After we began this series of exposures in the 0.80 Mrad/hr exposure system, the source materials were rearranged at a time between the first and subsequent exposures.

TABLE XI

D_{Mrad}-VALUES OF BIs OF BACILLUS PUMILUS E601 (ATCC #27142) AND BACILLUS STEAROTHERMOPHILUS (ATCC #7953) AT TWO POSTIRRADIATION PERIODS AND AT TWO DOSE RATES

BI lots*	D_{Mrad}-value (Mrad/hr)			
	Immediate testing**		Two-week storage***	
	0.15	0.80	0.15	0.80
bp-1 (old)	0.27	0.19	0.37	0.24
bp-1 (new)	0.29	0.18	0.33	0.25
bs (old)	0.49	0.37	0.42	0.30
bs (new)	0.48	0.32	0.50	0.33

*BI lots bp-1 (new) and bp-1 (old) were prepared from spore crop _B. subtilis_ E601, BP-1, on February 25, 1974 and on August 29, 1973, respectively. BI lots bs (old) and bs (new) were prepared from a spore crop of _B. stearothermophilus,_ BS-1, on October 4, 1973 and on February 28, 1974, respectively.

**Assays to determine the D_{Mrad}-value of the BIs were initiated within 36 hr of irradiation of the sealed pouches.

***Assays to determine the D_{Mrad}-value of the irradiated BIs were initiated only after two-week storage of the sealed pouches at room temperature (72 ± 4°F).

Then, using the accumulated data to design the composite experiment, the goal was to demonstrate that, with the "best" biological indicator system devised from the experimental data, the comparison of product resistance to biological indicator resistance can lead to an assessment of the level of probability of sterility of the sterilization process. The radiation resistance of the contaminant microbial load had to be related to the radiation resistance of BIs.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

As step one, the contaminant microbial load on naturally contaminated product taken from a production line in a manufacturing environment was determined. For the work scope of the contract, the contaminant microbial load on dissembled product, deliberately and exaggeratedly-exposed for five weeks in a manufacturing environment, in a manner that would enhance accumulation of a microbial load, was also determined. The contaminant microbial load on naturally contaminated disposable 10 cc plastic syringes was estimated as 2.1 aerobic colony forming units (CFU)/product; the load on exaggeratedly contaminated similar product was estimated as 167.3 aerobic CFU/product.

TABLE XII

STORAGE STABILITY OF D_{Mrad}-VALUES OF SEEDED PRODUCT AND PAPER BIs

Product***	D_{Mrad}-value (0.8 Mrad/hr)			
	Storage intervals (days) between exposures*			
	0	45	75	110
bp-1 (old)**	0.26	0.16	0.14	0.15
bp-1 (new)**	0.26	0.22	0.15	0.17
Paper strip	0.24	0.14	0.18	0.14
Paper strip (Tween 80)	0.27	0.14	0.17	0.16
Hardpak needles	0.23	0.16	0.14	0.14
10 cc plastic syringes	0.15	0.15	0.16	0.16
IV catheter tubes	0.20	0.15	0.14	0.15

*The dates of exposure of the products to ionizing radiation were: March 11, 1974; April 25, 1974; May 25, 1974; and June 29, 1974. Day 0 (March 11, 1974) was the starting day of the study on the storage stability of the D_{Mrad}-value of the BI lots. Storage was conducted at ambient (72 ± 4°F) temperature.

**BIs of bp-1 (old) and bp-1 (new) were prepared from spore crop BP-1 on August 29, 1973 and February 25, 1974, respectively. They were included as a control on continuity of BI response.

***These products were inoculated with inocula from spore crop BP-7 on February 11, 1974, and were stored at room temperature in hermetically sealed aluminum-plastic laminate pouches.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

TABLE XIII

QUANTAL RESPONSE RESULTS*** AFTER EXPOSURE OF NATURALLY CONTAMINATED SYRINGES** AND EXAGGERATEDLY CONTAMINATED SYRINGES TO TWO DOSE RATES

Dose* (Mrad)	0.15 Mrad/hr		0.80 Mrad/hr	
	Naturally contaminated	Exaggeratedly contaminated	Naturally contaminated	Exaggeratedly contaminated
0.01	10/10	10/10	2/10	10/10
0.05	1/10	10/10	1/10	10/10
0.10	0/10	10/10	0/10	10/10
0.20	0/10	10/10	0/10	10/10
0.40	0/10	6/10	0/10	1/10
0.80	0/10	0/10	0/10	0/10

*Dose, in Mrad, calculated from the average dose rate profile across the exposure cannister within the exposure chamber. Calculations made by Nuclear Energy Services, N. C. S. U.

**Packaged 10 cc plastic, disposable syringes.

***Sterility test medium in Trypticase soy broth (TSB). Sterility tests were incubated 30 days at 30-35°C.

Then, naturally contaminated syringes, and exaggeratedly contaminated syringes, and BIs were exposed to various total doses of ionizing radiation. After exposure, the syringes were subjected to sterility testing in Trypticase soy broth for ten days at 30-35°C. The results were scored as growth/no growth, and the fraction-negative responses were tabulated (Table XIII).

By calculation and inference, the level of probability of sterility can be estimated. Of the three lots of BIs that were used to monitor the exposure of syringes to radiation, BI lot bp-7 with a D_{Mrad}-value of 0.21 Mrad was used to assess the level of probability of a survivor in the composite experiment. With an assay titer of 4.5×10^6 spores/carrier, the number of D-value multiples (increments) necessary to reduce the titer from 4.5×10^6 spores/carrier to 1×10^0 spores/carrier was 6.62 – equivalent to a

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

dose of radiation of 1.39 Mrad. When the fraction-negative response of BI lot bp-7 was studied, some BIs survived a dose of 1.2 Mrad – this was less than the 1.39 Mrad that was calculated – but none survived 1.6 Mrad (Table XIV).

Then the fraction-negative response of the sterility-tested, naturally contaminated syringes was compared to the fraction-negative response of the BIs that had been simultaneously exposed. The estimated number of CFU/packaged syringes was 5.2 and this load was sterilized with a 0.1 Mrad dose. (A D-value on products cannot be determined. The titer and homogeneity of the contaminant microbial load on individual product is an unknown; however, given a dose of 0.1 Mrad, the naturally contaminated product did test sterile). If the resistance to ionizing radiation of the load is assumed to be as high as the D_{Mrad}-value of the BI, in this instance as high as 0.21 Mrad, an estimate of the level of probability of a survivor of less than 1 in 10^7 at a dose of 1.6 Mrad (Fig. 2) is obtained.

TABLE XIV

FRACTION-NEGATIVE RESPONSE OF BI LOTS USED TO MONITOR NATURALLY CONTAMINATED SYRINGES

BI lots	Dose (Mrad) received at 0.15 Mrad/hr		
	0.8	1.2	1.6
bp-1	20/20	20/20	5/20
bp-1 (new)	20/20	20/20	0/20
bp-7	20/20	20/20	0/20

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

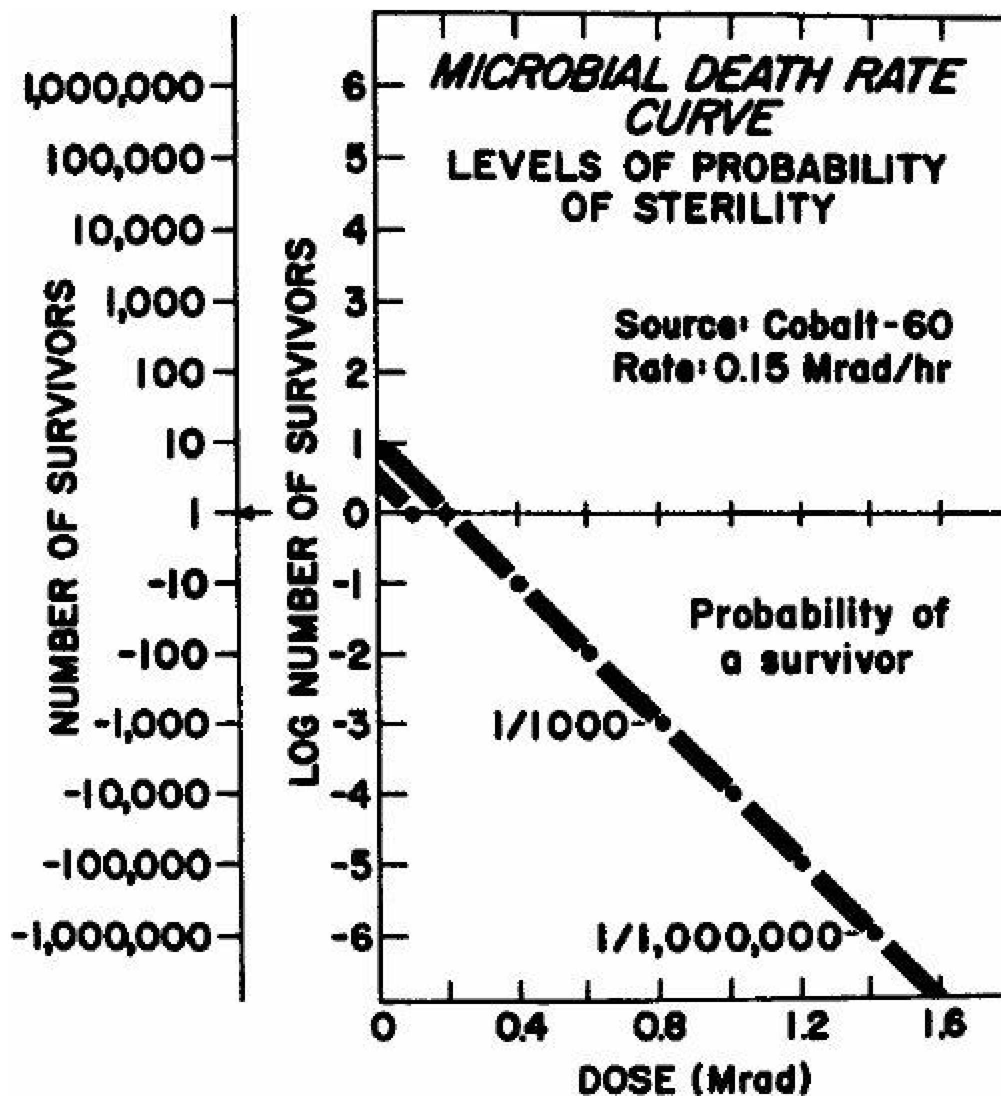


FIGURE 2. Microbial death rate curve: levels of probability of sterility.

We can infer from the data of the composite study that, by using production process control during the manufacture of a product wherein the contaminant microbial load on the product and the radiation resistance of the contaminant microbial load are known and are within defined boundaries, a total dose of radiation can be selected that will provide the desired level of probability of a survivor.

ADDENDUM

On the Criticality of Knowing the Dose Rate at the Site of Exposure of the BI to Ionizing (Gamma) Radiation

The cause(s) for the variation in the fraction-negative

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

responses of BIs exposed to the ionizing (gamma) radiation of cobalt-60 were studied. A special holder of small containers was designed for the exposure system that received an estimated averaged dose rate of 0.10 Mrad/hr ionizing (gamma) radiation. In each exposure, the holder was placed into the same geometric position, and into the same alignment with the radiation source material. Consequently, the small containers in the numbered sites in the holder were exposed to the same dose rate at each exposure trial, as shown by replicated Fricke dosimetry results (Table XV).

There is a dose rate associated with each site of exposure, and the dose rates at some sites are quite different from those at other sites. This difference in dose rate at the numbered sites did not greatly affect the D_{Mrad}-values of the BIs exposed at those sites (Table XVI). The quantal response of the BIs, however, does reflect the total accumulated dose received at each site (Table XVII).

TABLE XV

FRICKE DOSIMETRY IN 0.1 Mrad/hr FACILITY

Exposure site	Mrad/hr at 304 mμ wavelength	
	Trial 1	Trial 2
11	0.0831	0.0820
12	0.0851	0.0845
13	0.0856	0.0845
14	0.0856	0.0848
15	0.0856	0.0838
16	0.0910	0.0906
17	0.0900	0.0905
21	0.0870	0.0877
22	0.0902	0.0900
23	0.0916	0.0899
24	0.0902	0.0912
25	0.0906	0.0896
26	0.0953	0.0951

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

27	0.0968	0.0948
31	0.0867	0.0842
32	0.0921	0.0886
33	0.0876	0.0859
34	0.0916	0.0897
41	0.0918	0.0910
42	0.0971	0.0946
43	0.0926	0.0946
44	0.0989	0.0978

TABLE XVI

D_{Mrad}-VALUES OF BIs OF BACILLUS PUMILUS E601 (ATCC #27142) IN THE 0.10 Mrad/hr FACILITY

Site* (locus)	Trial 1		Trial 2	
	Mrad/hr	D_{Mrad}-value	Mrad/hr	D_{Mrad}-value
12	0.0851	0.25	0.0845	0.24
14	0.0856	0.25	0.0848	0.23
16	0.0910	0.26	0.0906	0.25
22	0.0902	0.25	0.0900	0.24
24	0.0902	0.24	0.0912	0.24
26	0.0953	0.26	0.0951	0.24
34	0.0916	0.26	0.0897	0.25
42	0.0971	0.26	0.0946	0.24
44*	0.0989	0.26	0.0978	0.24

*In both trials, Site #44 was chosen from the Fricke dosimetry data as the control site for determining exposure times to achieve doses of 0.1, 0.2, 0.4, 0.6, and 0.8 Mrad. Doses at other sites were determined from the time exposures at the control site and the dose-rate of the specific site.

TABLE XVII

QUANTAL RESPONSE OF BIs OF BACILLUS PUMILUS

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

E601 SPORES AT SELECTED SITES IN THE 0.10 Mrad/hr FACILITY

Site	Actual dose rate* (Mrad/hr)	Estimated dose** (Mrad)	Actual dose*** (Mrad)	Quantal response+
		1.0	0.86	20/20
14	0.0848	1.2	1.04	16/20
		1.4	1.21	4/20
		1.0	0.93	20/20
24	0.0912	1.2	1.12	5/20
		1.4	1.31	1/20
		1.0	0.91	20/20
34	0.0897	1.2	1.10	20/20
		1.4	1.28	1/20
		1.0	1.0	17/20
44	0.0978	1.2	1.2	1/20
		1.4	1.4	0/20

*Dose rate at each selected site monitored by Fricke dosimeter.

**Dose and exposure time based on dose rate of Site #44.

***Doses calculated from dose rate at the site multiplied by the exposure time of Control Site #44.

+Number of positives/total number of tubes.

BIBLIOGRAPHY

Berube, R. and Jones, L. A. (1974). Final Report: Determination of resistance parameters for biological indicators used in sterilization of medical supplies by ionizing radiation. Contract No. FDA 73-214.

Borick, P. M. and Fogarty, M. G. (1967). Effects of continuous and interrupted radiation on microorganisms. Appl. Microbiol. 15:785-789.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Burt, M. M. and Ley, F. J. (1963). Studies on the dose requirements for the radiation sterilization of medical equipment. I. Influence of suspending media. J. Appl. Bacteriol. 26:484-489.

Christensen, E. A. and Shested, K. (1964). Radiation resistance of Streptococcus faecium and spores of Bacillus subtilis dried in various media. Acta Pathol. Microbiol. Scand. 62:448-458.

Christensen, E. A. and Kjems, E. (1965). The radiation resistance of substrains from Streptococcus faecium selected after irradiation of two different strains. Acta Pathol. Microbiol. Scand. 63:281-290.

Duggan, D. E., Anderson, A. W., and Elliker, P. R. (1963). Inactivation of the radiation-resistant spoilage bacterium Micrococcus radiodurans. I. Radiation inactivation rates in three meat substrates and in buffer. Appl. Microbiol. 11:398-403.

Duggan, D. E., Anderson, A. W., and Elliker, P. R. (1963). Inactivation of the radiation-resistant spoilage bacterium Micrococcus radiodurans. II. Radiation inactivation rates as influenced by menstruum temperature, preirradiation heat treatment, and certain reducing agents. Appl. Microbiol. 11:413-417.

Gould, G. W. and Hurst, A., Eds. (1969). The Bacterial Spore. Academic Press, New York.

Hugo, W. B., Ed. (1971). Inhibition and Destruction of the Microbial Cell. Academic Press, New York.

Morgan, B. H. and Reed, J. M. (1954). Resistance of bacterial spores to gamma irradiation. Food Res. 19:357-366.

Murrell, W. G. and Scott, W. J. (1966). The heat resistance of bacterial spores at various water activities. J. Gen. Microbiol. 43:411-425.

Pflug, I. J. and Schmidt, C. F. (1968). Thermal destruction of microorganisms. In Disinfection, Sterilization and Preservation. C. A. Lawrence and S. S. Block, Eds. Lea and Febiger, Philadelphia, Pa.; pp. 63-105.
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

THE USE OF BIOLOGICAL INDICATORS FOR MONITORING WET HEAT STERILIZATION PROCESSES

Irving J. Pflug and Geraldine M. Smith

University of Minnesota
Minneapolis, Minnesota

A primary objective of this report is to present data on the performance of bacterial spores as biological indicators for monitoring wet heat sterilization processes when the spores are deposited on paper carriers. We believe that to understand and appreciate the attributes and limitations of bacterial spore strips we need to know more about the behavior and performance of bacterial spores. To give a more complete picture of monitoring sterilization processes, performance of bacterial spores and spore strips, we are including background material on both monitoring wet heat sterilization processes and on characteristics of bacterial spores. We have organized this report into the following sections: 1) monitoring wet heat sterilization processes, where we will discuss the nature of the wet heat sterilization process and the requirements for monitoring; 2) some characteristics of bacterial spores that must be considered in their use as biological indicators, where we will discuss the attributes, limitations, uniqueness and sensitivity of these biological entities; 3) methods of using spores as biological indicators, where both end point and count reduction methods will be discussed; and 4) performance of spore strips designed for monitoring steam sterilization processes, where we will present data on the reproducibility of commercial spore strips, examine lot-to-lot variation of spore strips, observe the effect of temperature on spore strip performance, report on some effects of storage on spore strip performance, and present data on the effects of test conditions on spore strip performance.

MONITORING WET HEAT STERILIZATION PROCESSES

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Why are biological indicators used for monitoring wet heat sterilization processes? In the health industries, it is usually agreed that the probability of a nonsterile unit should be less than one in one million (less than 10^{-6}) for products or devices that are marketed as being sterile. To achieve this near zero level of nonsterile units requires sterilization processes that are carefully designed, controlled and monitored. Confidence in the performance of the sterilization process is obtained by monitoring to insure that the sterilization process has been delivered to the product. In monitoring a wet heat sterilization process, a physical, chemical or biological monitoring system can be used.

The destruction of microbial contamination by wet heat is a function of both time and temperature. The temperature effect is exponential as shown in the equation below:

$$F(T_B, z) = \int 10^{\frac{(T(t) - T_B)}{z}} dt \qquad (1)$$

The sterilizing value F is the equivalent time at the base temperature T_B, usually 250° F. T(t) is the temperature measured as a function of time (t) at the slowest heating zone in the container of product or of the object. The z-value, measured in degrees of temperature, is the temperature coefficient of microbial destruction and is the time for the D-value or sterilization time of the microorganisms to change by a factor of ten. The values of T_B, T(t) and z must all be in the same units of temperature, either degrees Centigrade or Fahrenheit.

When T_B equals 250° F and z equals 18°F, Equation (1) becomes the widely used General Method Equation (2) shown below:

$$F^{250}_{18} = \int 10^{\frac{T(t) - 250}{18}} dt \qquad (2)$$

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

To measure the sterilizing value physically, we measure the temperature as a function of time at the slowest heating point on the object or in the unit during the sterilization process and then sum up or integrate the temperature effect to obtain the sterilizing value.

A problem in monitoring sterilization processes using temperature and time measurements is that these data must be integrated according to Equation (1) to arrive at the final sterilizing value. The monitoring system must evaluate the effect of the sterilization process on an exponential basis to be meaningful. Since we are interested in a sterilization probability of 10^{-6} at all points in a product or for all products, we must be certain that the process we are monitoring or the temperature we are measuring is at the point in the product that receives the lowest sterilizing value, and that this product is positioned in the autoclave at the location receiving the lowest sterilizing value. Because of the exponential effect of temperature on the sterilizing value, the temperature must be known accurately.

The biological monitoring of sterilization processes using calibrated microorganisms is a direct method but requires about 48 hours of incubation time before results are available. The exponential destruction rate is unique to microorganisms and some physical and chemical systems; this attribute can be utilized by using a calibrated microorganism to monitor a sterilization process. Monitoring methods that mimic the exponential destruction of microorganisms employing chemicals such as thiamine are not in general use, although systems are under development.

Sterilization processes are designed and specified on the basis of physical characteristics [1]. For wet heat sterilization processes, the basic unit is time at a specified temperature. Therefore, in many ways, it would be logical to monitor sterilization processes on the basis of physical variables. To carry out this monitoring procedure, it would be necessary to place a temperature sensing unit at the slowest heating zone of the product in that part of the autoclave that heats most slowly and cools most rapidly. In nonagitating processes, it is difficult to insert thermocouples in products in remote parts of baskets in the autoclave. In agitating types of processing equipment, it is practically impossible to have thermocouples in place in a product during a sterilization process. There have been major efforts to

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

develop temperature measuring equipment where information is transmitted by a microwave radio from the container to a receiver. These systems are only in limited use today and those actually employed fail frequently. At the present time, chemical and biological monitors are the only practical methods of monitoring continuous processes.

The biological indicator using calibrated bacterial spores has an important place in monitoring wet heat sterilization processes. Most wet heat biological indicators use spores of some strain of Bacillus stearothermophilus. The spores may be directly inoculated onto a product if it is a solid, dispensed into a liquid product such as a parenteral solution, or placed on pieces of filter paper, usually referred to as spore strips, for use in measuring sterilization of hospital packs. The bacterial spores integrate time and temperature with a temperature coefficient that is of the same order as the contaminating microorganisms. No wires, strings or other connections are required; therefore, the biological indicators can be placed inside an object, pack or inoculated into the product and this unit can be located at any point on a rack in the autoclave.

As a monitoring device and quality control tool, the biological indicator is totally separate from the physical system. If a sterilization process is to be monitored by physical means, there is considerable opportunity for the monitoring system and the control system of the physical conditions in the autoclave to be on the same basis. The biological indicator system is a totally independent monitoring unit in that it can be placed in the product before the product is loaded into the autoclave, and recovered from the product after the product leaves the autoclave; it is therefore completely separate not only on a physical basis but also as far as the personnel involved in putting the biological indicator in place and later recovering and assaying the indicator are concerned.

Consideration of the requirements in the way of personnel, equipment and facilities to obtain a given level of precision for biological monitoring leads to some interesting observations; a trained technician can utilize calibrated biological indicators with relatively simple equipment to accurately monitor sterilization processes. To physically monitor sterilization

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

processes requires reasonably sophisticated measuring equipment

and trained operating personnel. It is possible that, if the quality control technicians at the plant level who are operating the physical monitoring system and the biological monitoring system have the same level of ability and training, the results from the use of calibrated biological indicators will be more reliable than those from the use of a physical monitoring system.

SOME CHARACTERISTICS OF BACTERIAL SPORES THAT MUST BE CONSIDERED IN THEIR USE AS BIOLOGICAL INDICATORS

A biological indicator is a calibrated device designed to measure a specific sterilization condition. Almost all biological indicators utilize bacterial spores. Bacterial spores are often the most resistant microorganisms available; however, an additional important criterion is stability. Bacterial spores are normally much more stable than vegetative cells. For an organism to be useful in a biological indicator system, it must be possible to produce the spore crop, calibrate it and if it is going to be used in spore strips, produce the spore strips, evaluate their performance, and then distribute them for use either for an in-company situation or as a commercial product.

Spores of B. stearothermophilus are widely used for monitoring wet heat sterilization processes. It is possible to grow B. stearothermophilus spores that have high heat resistance. B. stearothermophilus is an aerobic, non-pathogenic organism; it grows at 55°C and therefore the problem of interfering contamination is minimized. The heat resistance of any specific strain of B. stearothermophilus can vary widely when either or both the spore production techniques or recovery techniques are altered. The D(121.1° C)-value can vary from near zero to more than twelve minutes. The effect of some common parenteral solutions and buffer solutions on the heat destruction characteristics of B. stearothermophilus spores are shown in Figure 1; the data is taken from Pflug and Smith [2].

Bacterial spores have the ability of responding to small variations in the environment. The sensitivity of bacterial spores to small variables is an attribute in their monitoring role; however, it also imposes limitations on the use of spores.
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.
Presented below are some characteristics and attributes of

bacterial spores that affect their performance. The effect of some of these characteristics and attributes will be shown experimentally in the latter part of this report.

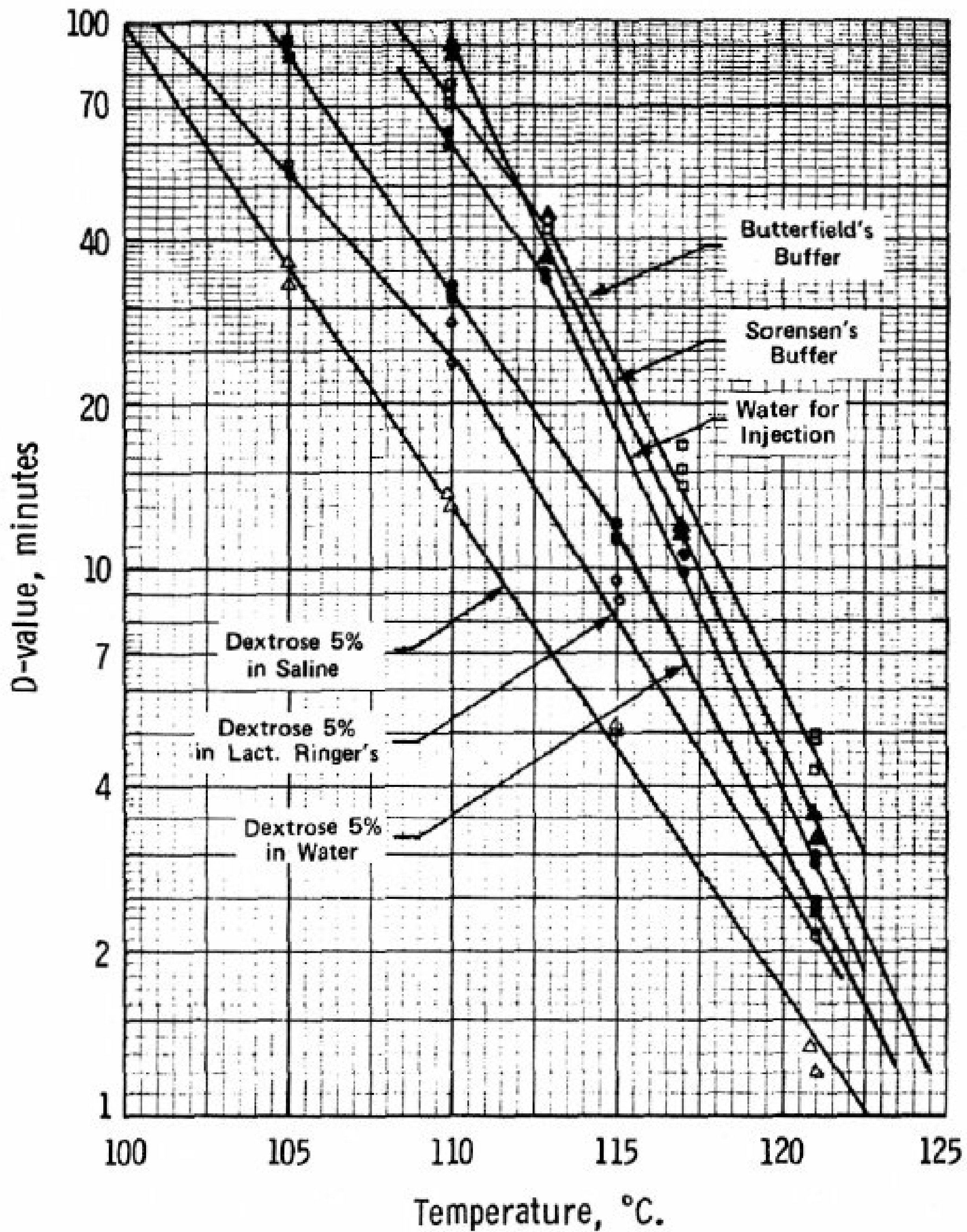


FIGURE 1. z-Value analysis for Bacillus stearothermophilus spores heated in six solutions.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

in Biological Indicator Systems

1) The survival rate of bacterial spores subjected to a lethal environmental stress condition is affected by a great many physical and chemical factors. The effect of a specific factor in a test system cannot be determined other than by measuring the effect of that factor on the survival rate.

2) Physical and chemical factors at activity levels or concentrations that may not be generally measurable in the microbiological laboratory can measurably alter the survival rate of the microorganisms.

3) The effects of the physical and chemical variations in the test system may be difficult to differentiate from the environmental conditons that are influenced by day-to-day changes in laboratory conditions, including habits of laboratory personnel or alternation or rotation of laboratory personnel.

4) Microorganisms are used in the analytical world to carry out microbiological assays of certain complex nutrients present in low concentrations in food and drug products. In these assays, the ability of microorganisms to respond quantitatively to the level of certain complex nutrients present in low concentrations is a further indication of the sensitivity of microorganisms to environmental conditons.

5) When we deal with microorganisms, we are dealing with biological entities where we utilize their ability to reproduce and form colonies as our measuring end point. This ability to survive under environmental stress conditions and later outgrow is an integrated measure of the total conditions that have affected the particular organism. The effect of the heat process does not necessarily have to be an immediate destruction of the cell, because in order for the cell to make itself countable at a later time, it must be able to be functionally intact and viable to the point where it will produce a visual or countable number of progeny.

Attributes and Limitations of Individual Spore Crops

The production of resistant bacterial spore crops is an art:

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

1) When spore crops are grown in an identical manner under close

laboratory control, all spore crops will probably be similar but not identical in response to an environmental stress.

2) The only way to know how a spore crop will respond to an environmental stress condition is to test the spore crop under the environmental stress condition.

3) The only way we can determine the effect of a change in a spore production step, nutrient or test system is to evaluate the performance of spores grown or tested with and without the changed conditions.

4) Bacterial spores are so sensitive to environmental conditions that both the spores and the environment must be evaluated using appropriate controls; otherwise we will not know if a variation in results is due to changes in the spores or if the spores are measuring a change in the environmental conditions.

5) The resistance of a spore crop may change as it ages. Crop stability is a function of time and storage condition.

METHODS OF USING SPORES AS BIOLOGICAL INDICATORS FOR MONITORING STERILIZATION PROCESSES

When bacterial spores are used as biological indicators, they can either be placed on paper carriers as in the conventional spore strip or they can be deposited directly on or in a product. There are two methods of assaying the biological indicators: 1) the spores may be used in an end point method of analysis where the biological indicator unit is simply placed in a recovery medium and scored as being either positive or negative; or 2) a count reduction procedure can be used where the number of bacteria recovered after the treatment is used with a calibration graph to numerically determine the sterilizing value. We believe that it is important to understand these concepts when utilizing biological indicators in wet heat processes; therefore, they will be discussed briefly below.

Survival-Kill vs Numerical Survival Numbers

In using bacterial spores as biological monitors we assume that the spores have known, reproducible destruction rates. Two

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

alternative methods are available as far as spore recovery is concerned; we can design the indicator either for a "survival-kill" or for a "number of survivors" type of analysis. The graph in Figure 2 has been prepared to illustrate the relative areas of the semilogarithmic survivor curve used in the end point and in the plate count analysis methods.

The spores used in the example in Figure 2 have a D(121.1°C)-value of 2.0 minutes and the N_o is 1.0×10^4. If biological indicators are prepared with spores that have this N_o and D-value and are subjected to sterilizing values between zero and six minutes, the plate count method would be used to enumerate the number of survivors. For sterilizing values greater than six minutes the end point method of analysis would be used.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

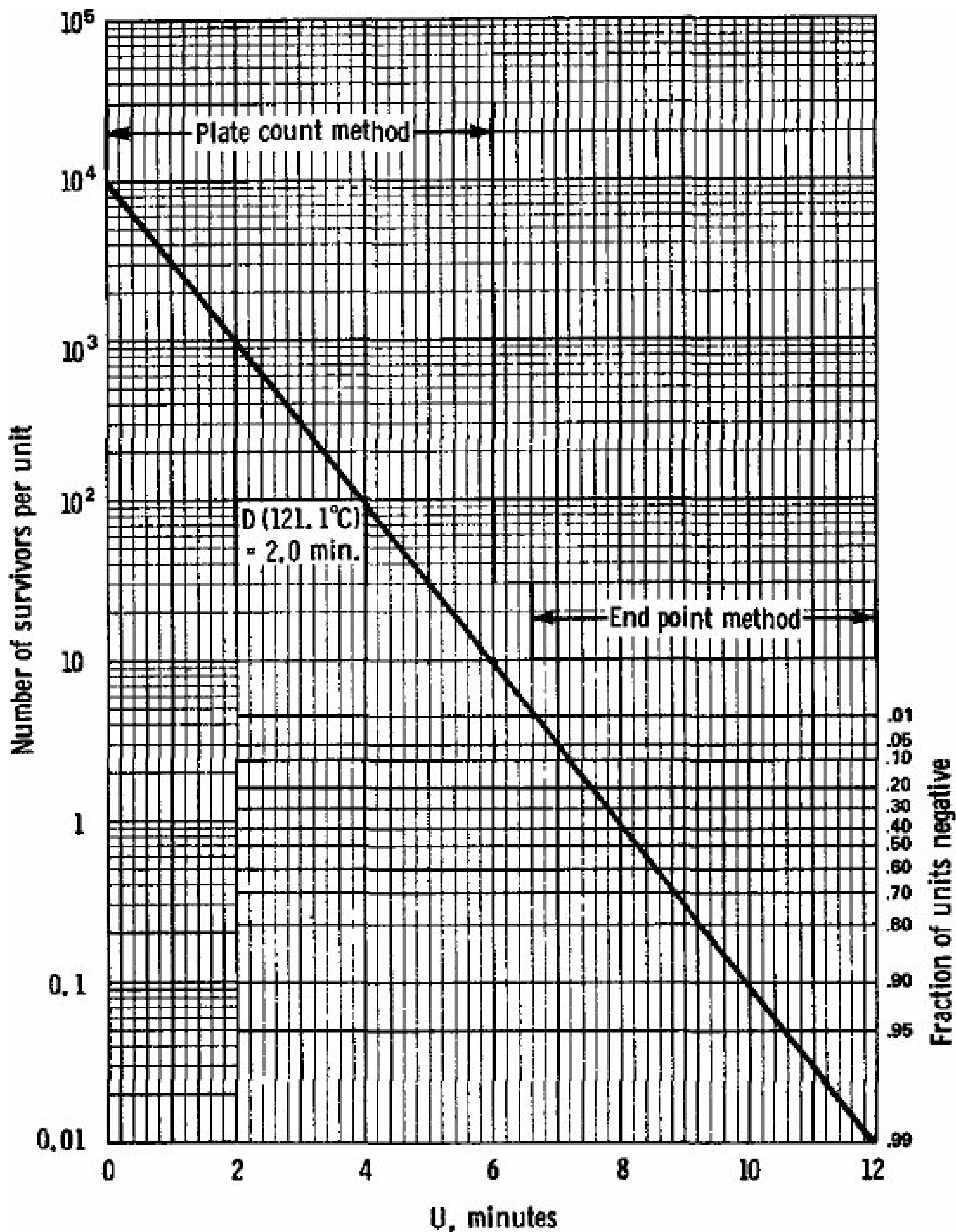


FIGURE 2. The fraction negative or quantal area of response and the plate counting area of response for bacterial spores where the initial number N_o is 1×10^4 and the D(121.1° C)-value is 2 minutes.

The use of a biological indicator in a survival-kill mode is, in general, more comfortable to the user because we like to equate

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

sterility with the absence of life. This may be satisfying but it is not necessarily meaningful. Microbial death takes place on an exponential basis and the time required to reach a specified low number of survivors is a function of the initial number of microorganisms present and their resistance to the applied stress. Practical sterilization processes are designed on the basis of a specified initial number of microorganisms with an assumed maximum resistance, contaminating the product to be sterilized. The right combination of species or strain of microorganism, the number of organisms per indicator and the type of carrier or support unit for the organisms can yield a biological indicator that will survive almost any sterilization process. The survival of microorganisms on a biological indicator that is designed to survive the sterilization cycle should not be an uncomfortable concept to the user. In fact, a great deal more information can be obtained from a biological indicator that results in a measured number of surviving spores than from a survival-kill indicator.

When several survival-kill indicators are used, there can be three types of results: 1) all units heated show spore survival; 2) no units heated show spore survival; or 3) some units show survival and some do not. Some examples of expected results can be drawn from the survivor curve in Figure 2. When 20 replicate biological indicators with this N_o and D-value are used in a survival-kill mode, they will have a survival (P = 0.05, where P is the probability of a negative result) time of 7.0 minutes and a kill (P = 0.95) time of 10.5 minutes.

If 20 replicate spore strips are heated and upon recovery all are found to have spore survival, it is probable (but not certain) that the sterilizing value delivered for conditions shown in Figure 2 was less than 7.0 minutes where P = 0.05. When all units show spore survival, there is only a probable upper limit on the sterilizing value and no direct estimate of its magnitude. All that can be inferred from the data is that the sterilizing value was most likely less than 7.0 minutes.

If, after heating, none of the 20 replicate spore strips result in spore survival, then there is a probable lower level for the sterilizing value. The delivered sterilizing value from Figure 2 would probably be greater than 10.5 minutes where P = 0.95. All sterilizing values of 10.5 minutes and more would likely lead to the result observed.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

To illustrate the third type of result, we will assume that in a single test, 12 of the 20 replicate units heated are positive for spore recovery. For this type of data, we now have both probable upper and lower limits on the magnitude of the sterilizing value. The sterilizing value most likely lies between 7.0 minutes and 10.5 minutes; however, we do not have enough data to refine this estimate. For data of this kind, where the results are neither all positive nor all negative, a better estimate of the sterilizing value can be obtained.

In the previous examples, a total of 20 replicate spore strips were used to monitor each sterilization process. When 20 replicates are used, one has a reasonable idea of which of the three survival regions (less than 7.0 minutes, 7.0 to 10.5 minutes, greater than 10.5 minutes) the data are attempting to describe. The amount of information that the data provide depends on the number of replicates. If the number of replicates is decreased from 20 to less than 5, then there will be a considerable decrease in the amount of information available.

On the basis of a single test, regardless of the number of replicates, we can never directly obtain a more definitive result than determination of the biological indicator region into which the sterilizing value falls. When small numbers of replicate spore strips are used to monitor a single sterilization process, the chances are great that the biological indicator region selected on the basis of the results will be the wrong region.

In contrast to the information provided by a survivor-kill indicator, if only a single biological indicator is used where the results are in the plate count range (we actually obtain a numerical sterilization value result), we will obtain more information from this single biological indicator result than we could obtain from a larger number of biological indicator units used in the survivor-kill mode. If biological indicators are used that produce a sterilizing value result, the sterilization value of the process is measured each time the biological indicator is used. The resulting data makes it possible to estimate variation of the delivered sterilization value among sterilizer loads and to identify trends in the magnitude of the delivered sterilization process.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

One of the most widely used biological indicator units is the spore strip. Perkins [3] describes the preparation of spore strips. Filter paper is the material of choice and is inoculated with a measured amount of the spore suspension to give the desired performance. Producing spore strips is a relatively simple process. However, since we are dealing with a biological material which must perform according to prescribed standards, the production of spore strips is a highly developed art. Spore strips are usually placed in glassine envelopes or in some other carrier device that allows for distribution, use, and later recovery without contaminating the spore strips.

The major use of spore strips is in monitoring the the sterilization of surgical packs in the hospital. The spore strip is designed to give a survival or kill reading. After the spore strip has been placed in the load and subjected to the sterilization cycle, the envelope containing the spore strip is recovered and the spore strip is aseptically transferred to a tube of culture medium. After an appropriate incubation time, the tube is examined and scored for either growth or no growth. Obviously, growth implies inadequate sterilization and no growth implies satisfactory sterilization. The spore strip, by its very nature, is really not usable in any way other than as a positive or negative measure.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

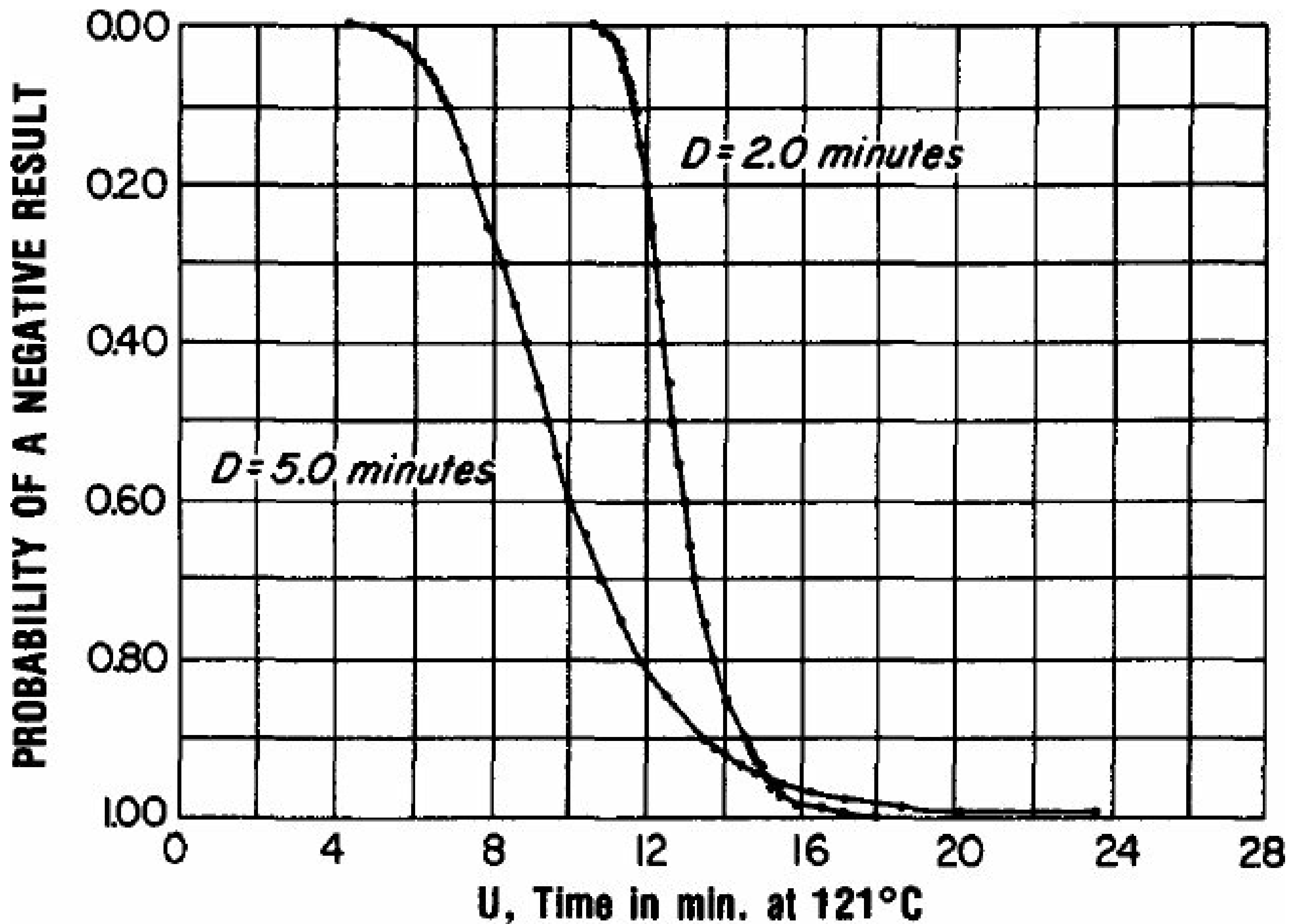


FIGURE 3. The probability of a negative unit P as a function of the sterilization value over the quantal range (P = 0.01, P = 0.99); The N_o-values used were: D-value of 2.0 min, N_o = 1.62E6; D-value of 5.0 min, N_o = 50.

The growth-no growth approach has one major drawback, which is that a rather wide time span or window exists between the smallest probability of finding a negative result to the highest probability of finding a negative result. If we assume that the death of the individual spores of a spore strip containing a homogeneous quantity of spores proceeds on an exponential basis, then the time required for sterility will be a function of the number of spores present. Mathematical analysis of the destruction function leads to the curves shown in Figure 3 where we show the relative probabilities of finding a negative result as a function of the heating time. In this figure are shown curves for two different D-values demonstrating that the D-value of the spores

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

has an effect on the time between when a probability of 0.01 and 0.99 occurs. This is often referred to as the survival-kill window.

Spores Deposited Directly on or in a Product

In the sterilization of drugs and devices, it is often more desirable to deposit the spores directly on the object if it is a piece of hardware, or in the product if it is a liquid such as a parenteral solution.

A piece of hardware can be analyzed for survival or kill by placing the object in a container of broth and incubating. The National Aeronautics and Space Administration [4], in their planetary quarantine program, developed a method for the assay of the number of organisms on piece-parts for space hardware. The object is aseptically placed in 50 ml of Tween 80, insonated for two minutes using an ultrasonic bath, and plating aliquots of the eluate. The piece-part is then placed on a layer of solidified agar in a 150 mm diameter plate and sufficient molten agar added to completely cover the part. The size of the object obviously limits the feasibility of plating directly.

In the case of a liquid such as a parenteral solution, we can evaluate for survival or kill by either adding a quantity of the solution to a broth culture or by filtering the entire quantity of solution through a bacteriological filter and adding the filter to a broth such as Trypticase soy broth.

In many situations, especially with liquid products, it is just as easy to perform the final recovery using a plate count procedure. If we go the plate count route, then we can use count reduction with a calibration curve to arrive at an answer that is not just survival or kill but will indicate the magnitude of the sterilization value. When in the monitoring analysis the numbers of surviving organisms are determined by plate count, a calibration graph similar to that shown in Figure 4 is used to arrive at the sterilization value. As far as information regarding the performance of the sterilization process is concerned, this procedure provides much more information than a positive or negative type of analysis.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

PERFORMANCE OF SPORE STRIPS DESIGNED FOR MONITORING WET HEAT STERILIZATION PROCESSES

In this section of our report, we shall look at five different aspects of the performance of bacterial spore strips. These are: 1) the reproducibility of results for commercial spore strips within a given lot; 2) the lot-to-lot variation of spore strips from the same manufacturer; 3) the effect of temperature on spore strip performance; 4) the effect of storage condition and time on spore strip performance; and 5) the effect of the test conditions on spore performance.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

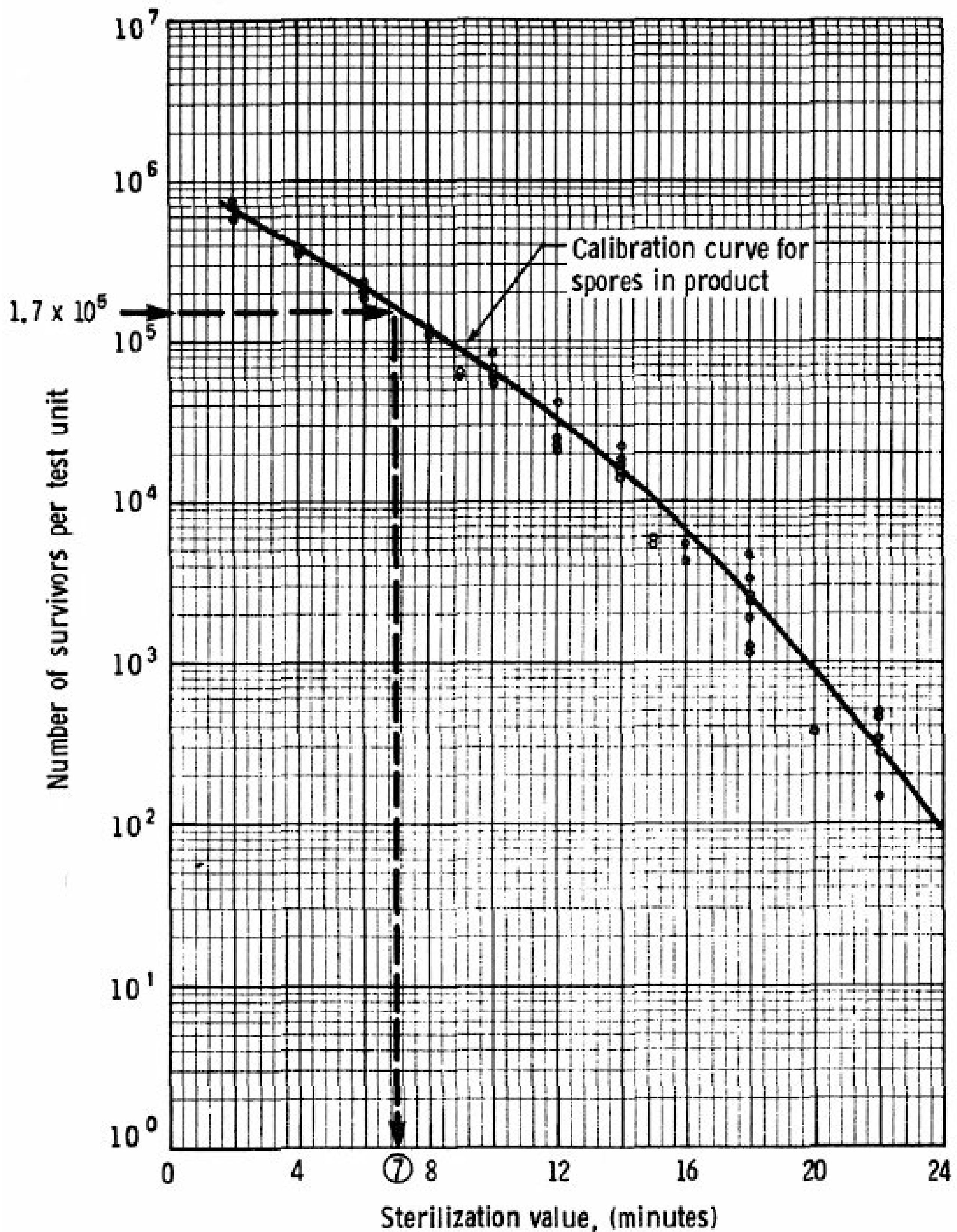


FIGURE 4. Heat destruction calibration curve for <u>Bacillus stearothermophilus</u> spores in water for injection, heated at 121.1°C. When these spores were used as biological monitors of a water for injection sterilization process, 1.7×10^5 spores per unit were recovered indicating that a sterilization value of 7 min at 121.1°C was received by the product.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Reproducibility of Commercial Spore Strips within a Manufactured Lot

Spore strips from three different lots from a single manufacturer were evaluated; codes for the three lots were QHOM, QHPM, and QHQM. Three tests, each on different days, were carried out to evaluate the performance of the spore strips.

In each experiment, ten spore strips were heated at each of four to eight heating times. Heating was in a saturated steam atmosphere in a miniature retort. The majority of the tests were carried out at 121°C. After each set of ten indicators had been heated for the specified time, they were removed from the miniature retort and transferred to an adjacent laminar airflow hood. Using aseptic techniques, the spore strips were removed from the glassine envelopes and transferred to the recovery media tubes: Trypticase soy broth (TSB) with 0.04% bromcresol purple (BCP). The recovery tubes were incubated for fourteen days at 55°C. After incubation, the number of tubes showing no growth out of the ten tested was recorded for each of the heating times.

The fraction-negative or quantal data were analyzed using the Spearman-Karber method described by Pflug and Holcomb [5]. The Spearman-Karber procedure is a method of estimating the heating time we expect to observe before a sample of N_o organisms becomes sterile. It is the expected time or mean time for sterility of a spore strip and can be calculated from quantal or fraction-negative data. The Spearman-Karber equation is given below:

$$U_{SK} = \sum_{i=1}^{k-1} \left(\frac{U_{i+1} + U_i}{2} \right) \left(\frac{r_{i+1}}{n_{i+1}} - \frac{r_i}{n_i} \right) \qquad (3)$$

where r_i is the number of sterile replicates out of n_i heated for time U_i and k is the number of heating times.

If tests of successive heating times differ by a constant time value d and all heating times have the same number of replicates n, the Spearman-Karber Equation (3) reduces to:

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

$$U_{SK} = U_k - \frac{d}{2} - \frac{d}{n} \sum_{i=1}^{k-1} r_i \qquad (4)$$

The Spearman-Karber value is nothing more than the quantal version of an arithmetic mean. The heating interval (U_1, U_2, … U_k) should be chosen to cover completely the quantal region. The first time, U_1 should show zero sterile replicates or $r_1 = 0$. The last time interval U_k should have all sterile replicates or $r_k = n_k$. If a set of experimental data has been gathered, the first U_1 should be chosen so that no times less than U_1 have replicates that are all sterile. Similarly, the time U_k should be chosen so that no replicates at times longer than U_k show growth. The D-values have also been calculated using the procedure outlined by Pflug and Schmidt [6], using the D-value equation below:

$$D = U_{SK}/(\log N_o + 0.2507) \qquad (5)$$

TABLE I

PERFORMANCE OF THREE DIFFERENT LOTS OF COMMERCIAL SPORE STRIPS

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Heating time min	QHOM			QHPM			QHQM		
	173B	207D	212B	199C	207C	212A	255A	260B	268A
5.00	0			0			0		0
5.75		2	0	0	0	0			
6.00							0	1	3
6.50		2	3	0	1	0			
7.00	5						6	7	8
7.25		6	2	3	0	0			
8.00		7	9	0	2	0	8	9	10
8.75		10	10	3	8	6			
9.00	10						9	10	10
9.50		10	9	5	7	10			
10.00							10	10	10
10.25				10					
11.00							10	10	10
12.00							10		
13.00	10								

These raw data are the number of negative tubes among the ten tubes tested at 121.0°C.

TABLE II

SPEARMAN-KARBER TIMES AND THEIR 95% CONFIDENCE INTERVALS (CI) FOR COMMERCIAL SPORE STRIPS

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Experiment	Spearman-Karber times, min	95% CI (S-K)
QHOM		
N_o = 2.5E4		
173B	7.13	6.69-7.57
207D	7.28	7.02-7.74
212B	7.00	6.33-7.67
$\bar{x}$	7.14	
QHPM		
N_o = 2.2E4		
199C	9.05	8.64-9.46
207C	8.49	8.12-8.86
212A	8.68	8.44-8.92
$\bar{x}$	8.74	
QHQM		
N_o = 2.15E4		
255A	7.20	6.74-7.66
260B	6.80	6.38-7.22
268A	6.40	6.00-6.80
$\bar{x}$	6.80	

Spore strips were heated in steam at 121.0°C.

The results of the three replicate tests with commercial spore strips QHOM, QHPM, and QHQM are shown in Table I. Spearman-Karber times and their 95% confidence intervals (CI) for the nine tests are shown in Table II. The results for spores QHOM show overlapping 95% CI suggesting that the three replicates are not different. For spores QHPM there is no difference in results between tests 207C and 212A. Although the Spearman-Karber times of 207C and 212A fall within the 95% CI of 199C, the Spearman-Karber time for 199C falls outside of the 95% CI of both 207C and 212A, suggesting that the results for test 199C may be different from 207C and 212A. For spores QHQM there is no apparent difference

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

between the results of experiments 255A and 260B or between 260B and 268A; however, the results suggest that there is a difference between 255A and 268A as measured by the 95% CI. In summary, the results suggest that there is fair reproducibility upon replication within each of the three commercial lots of spores evaluated.

The specifications for the three lots of commercial spore strips are shown in Table III. To determine how well spores QHOM, QHPM, and QHQM conform to specifications, the times for a 0.01 and 0.99 probability of sterility (one out of 100 or 99 out of 100) were calculated. This calculation can be carried out if we have the initial number (N_o) and the Spearman-Karber time (U_{SK}) for a set of quantal data. We can calculate both the survival [P(t) = 0.01] and the kill time [P(t) = 0.99]. The calculation is made using the equation of Pflug [7]:

$$t = \frac{[\ln N_o - \ln(-\ln P)]\, U_{SK}}{[.57722 + \ln N_o]} \quad (6)$$

The calculated survival and kill times are shown in Table IV. Comparing the results in Table II with the specifications in Table III, we observe that all three lots of spores fall within the specification range. Spores QHOM and QHQM tend toward the low side of the range whereas spores QHPM tend toward the high side.

TABLE III

COMMERCIAL BIOLOGICAL INDICATOR SPECIFICATIONS

Heating temp °C	Time, min	
	All indicators nonsterile	All indicators sterile
132.2	0.33	2.0
121.1	5.0	13.0

Resistance data when heated in saturated steam.

Organism: Bacillus stearothermophilus.
Number of spores per strip: 10,000.
Expiration date: January, 1975 (all tests were completed before the expiration date).

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

TABLE IV

CALCULATED PROBABILITY OF STERILITY TIMES AT 121.0°C

Spores	Spearman-Karber times, min	Calculated probability of sterility times, min	
		0.01	0.99
QHOM	7.14	5.7	9.8
QHPM	8.74	7.0	12.1
QHQM	6.80	5.4	9.4

Manufacturer's calibration specifications at 250°F:
All strips positive (prob. of sterility = 0) at 5 min.
All strips negative (prob. of sterility = 1.00) at 13 min.

The Lot-to-Lot Variation in Commercial Spore Strips

The data in Tables II and IV show the wide lot-to-lot variation that is possible within the overall manufacturing specifications for these commercial spore strips. The survival to kill time window, calculated from the data in Table IV, was 4.0 minutes for spores QHQM, 4.1 minutes for QHOM and 5.1 minutes for QHPM. Reviewing the specifications of survival at 5 and kill at 13 minutes for these commercial spores (Table III) leads to the conclusion that we not only have the window that will be formed by going from a survival time of 0.01 to 0.99 but also provision is undoubtedly made for variations in calibration and perhaps change in calibration during distribution and storage prior to use.

Effect of Temperature on the Performance of Spore Strips

Spore strips from the commercial lot QHQM (previously described) were used to carry out a study at 120.0°C, 121.0°C, and 122.1°C. The raw data from these tests are shown in Table V. The data are summarized in Table VI. The mean Spearman-Karber times for the 120.0°C, 121.0°C, and 122.1°C data were analyzed for a z-value which was found to be 10.7°C.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

There is nothing surprising in this result in that it is widely accepted that B. stearothermophilus spores have z-values in the range of 7°C to 12°C (12.6°F to 21.6°F). However, this effect of temperature on the response of bacterial spores is often overlooked. A change in temperature of 1°C produces a major change in the response of the biological indicator units. We could have proceeded to calculate the 0.01 and 0.99 survival times and these would, of course, reflect the effect of the temperature change.

TABLE V

PERFORMANCE OF COMMERCIAL SPORE STRIPS QHQM AT VARIOUS TEMPERATURES

Heating time, min	120.0°C		121.0°C			122.1°C	
	262B	269A	255A	260B	268A	267A	270A
3						0	0
4						0	0
5	0		0		0	3	3
6	0	0	0	1	3	8	9
7	0	1	6	7	8	10	10
8	1	6	8	9	10	10	10
9	7	7	9	10	10	10	10
10	10	10	10	10	10	10	
11		10	10	10	10		
12			10				

These raw data are the number of negative tubes among the ten tubes tested.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

TABLE VI

SPEARMAN-KARBER TIMES AND THEIR 95% CONFIDENCE INTERVALS (CI) FOR QHQM SPORES

Temperature	Experiment	Spearman-Karber times, min	95% CI (S-K)
120.0°C	362B	8.70	8.34-9.06
	269A	8.10	7.61-8.59
	$\bar{x}$	8.40	8.08-8.72
121.0°C	255A	7.20	6.74-7.66
	260B	6.80	6.39-7.22
	268A	6.40	6.00-6.80
	$\bar{x}$	6.80	6.56-7.04
122.1°C	267A	5.40	5.00-5.80
	270A	5.30	4.94-5.66
	$\bar{x}$	5.35	5.08-5.62

Effect of Storage Conditions on the Performance of Biological Indicators

A study reported by Smith, Pflug, and Chapman [8] was carried out to determine the effect of storage time and conditions on biological indicators. The objectives were: 1) to determine the performance of biological indicators when tested repeatedly (three times over a three-week period); 2) to determine the effect of long-term storage on the resistance parameters of laboratory-prepared and commercial biological indicators; 3) to compare the performance of commercial and laboratory-prepared biological indicators.

B. stearothermophilus spores PBBF that were grown in our laboratory were used in the preparation of 3,250 spore discs. Commercially prepared spore strips, 1,200 from a single lot, were

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

purchased. Half of the laboratory-prepared indicators were stored at 22°C and 50% relative humidity (RH), and half at 4°C at less than 1% RH. All of the commercial indicators were stored at 22°C and 50% RH.

Three sets of fraction-negative tests were carried out for each combination of spore, storage conditions, and storage time. Ten spore discs or strips were tested at each heating time. Eight heating times were used for the first test for each storage time period and six heating times for the repeat tests. The initial number per unit was also determined after each storage period. The data were analyzed by Pflug and Holcomb [5] to determine the Spearman-Karber time and the D-value.

The results are summarized in Figure 5. In all three test series, the spore strips or discs showed a decrease in heat resistance during the first 16 weeks of storage. The spores stored at 22°C continued to decrease in heat resistance whereas the spores stored at 4°C did not show a loss in heat resistance after the initial 16-week sampling time.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

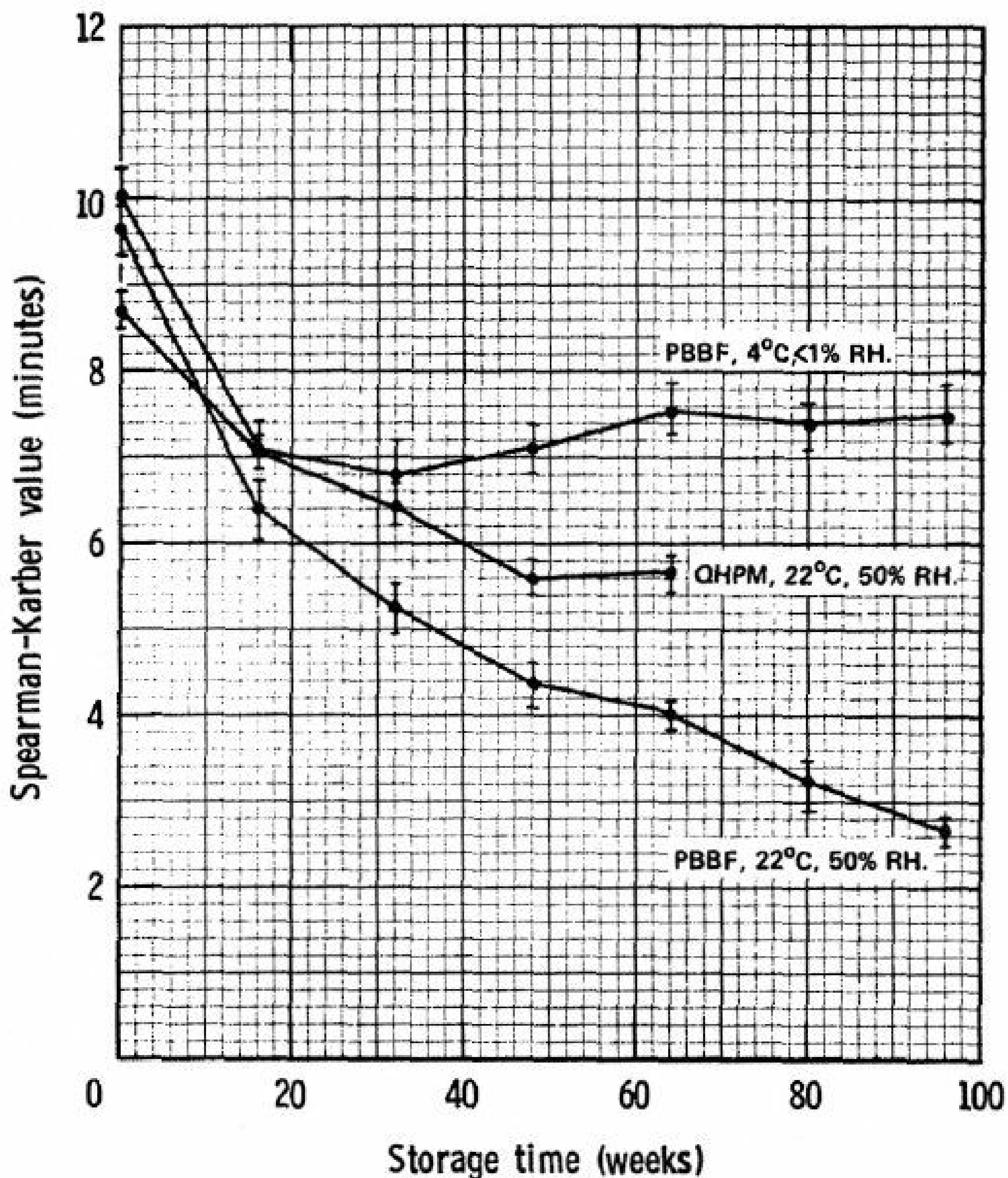


FIGURE 5. Composite graphs showing Spearman-Karber times for the three spore strips or disc storage conditions [8].

Comparing the results of the laboratory-produced PBBF spore discs, it was observed that the discs stored at 22°C showed a greater decrease in heat resistance from 0 to 16 weeks of storage than the spores stored at 4°C. Further, the spores stored at 22°C underwent a continual decrease in heat resistance during the remainder of the 96-week storage period although the rate was less than it was for the first 16-week period.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

We can observe in Figure 5 that during the first 16 weeks, spores PBBF (laboratory-prepared) stored at 22°C underwent a much greater decrease in heat resistance as measured by the Spearman-Karber time than did the spores QHPM (commercial). At the start of the experiment, the mean Spearman-Karber time for spores QHPM was 8.74 minutes, while for spores PBBF stored at 22°C, the Spearman-Karber time was 9.62 minutes. At the end of 16 weeks the spore strips QHPM showed larger Spearman-Karber times than the PBBF spore discs stored at 22°C. From the 16th to the 32nd to the 48th week, the Spearman-Karber times for both the spore strips QHPM and spore discs PBBF, both stored at 22°C, decreased at about the same rate. From 48 to 64 weeks, the decrease in the Spearman-Karber times for spore strips QHPM and spore discs PBBF stored at 22°C leveled off. No further tests were carried out on QHPM spores after 64 weeks, but for spores PBBF stored at 22°C, the Spearman-Karber time continued to decrease through 96 weeks of storage.

The effect of storage temperature on heat resistance stability is shown by observing that at 16 weeks both the spore strips QHPM stored at 22°C and the spore discs PBBF stored at 4°C yield almost identical Spearman-Karber times. However, at the end of 64 weeks of storage, the Spearman-Karber values for the spore strips QHPM stored at 22°C decrease from 7.16 minutes to 5.6 minutes (22% decrease) while the Spearman-Karber times for the spore discs PBBF stored at 4°C remain essentially constant at about 7 minutes.

In summary, both the laboratory-prepared spore discs and the commercial spore strips stored at 22°C and 50% RH, decreased in survival times with increased storage time. The laboratory-prepared spore discs, stored at 4°C and <1% RH, showed less change in numbers of spores per disc and decrease in the survival time than the discs stored at 22°C and 50% RH.

Changes in the initial number of spores per unit (N_o) did not appear to correlate directly with changes in survival time. For example, in all three series of experiments, the N_o remained stable over the 0- to 16-week storage period when the largest decrease in survival times occurred.

Effect of Spore Heating Conditions on the Performance of Spore Strips

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

A series of experiments was carried out to determine if there is

a change in biological indicator calibration when a spore strip designed for use in an envelope to be inserted in a surgical pack and sterilized in an autoclave is used to monitor the sterilization of culture media where the paper strip is removed from the envelope and placed in a bottle of media. Tests were carried out using commercial spore strip indicators coded QHSM and B. stearothermophilus spore discs PBBF, produced in our laboratory.

In all tests, heating was carried out employing a miniature retort system operated at 121°C. When spores were heated in 18 × 150 mm screw cap test tubes containing 20 ml of TSB, 2.3 min was subtracted to correct for the lag in heating and cooling.

Procedure for Testing Commercial Spore Strips

QHSM strips were aseptically removed from the glassine envelope and placed in 18 × 150 mm screw cap test tubes containing 20 ml of TSB. The strip rested on the bottom of the tube. The tubes were then heated and incubated at 55°C. In the control tests, the spore strips enclosed in the glassine envelopes were heated in the miniature retort. After heating, the strip was aseptically removed from the envelope, placed in an 18 × 150 mm test tube containing 20 ml TSB and incubated at 55°C.

Procedure for Testing the University of Minnesota Spores

Spores PBBF, produced in our laboratory, were used in experiments to compare the heat resistance of spores under three different conditions: 1) spores deposited on paper discs with the disc enclosed in a glassine envelope; 2) spores deposited on paper discs with the disc placed in test tubes containing TSB; 3) spores added directly to test tubes containing TSB. Approximately 10^5 spores were deposited on each disc or into each tube of broth.

TABLE VII

SPEARMAN-KARBER TIMES FOR SPORE STRIPS QHSM HEATED AT 121°C ($N_o = 1.9 \times 10^4$)

Test	No. of	Spearman-Karber time, min[(a)]

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

no.	units/time	Spores on strip in envelope	Spores on strip in broth
312	3	7.0	16.0
338	9	6.6	11.2
341	8	6.9	12.6
345	8	7.2	14.1
346	8	6.4	12.2

[a] Times have been corrected for any lag in heating and cooling.

TABLE VIII

SPEARMAN-KARBER TIMES FOR SPORES PBBF HEATED AT 121°C ($N_o = 2.0 \times 10^5$)

Test no.	No. of units/time	Spearman-Karber time, min[a]		
		Spores on disc in envelope	Spores on disc in broth	Spores in broth
331	3	7.8	10.4	22.7
339	9	-	17.4	25.7
348	9	7.6	13.9	23.2

[a] Times have been corrected for any lag in heating and cooling.

Results

The results of the tests using commercial spore strips QHSM are shown in Table VII. The results of spores PBBF on discs in envelopes heated in steam, on discs in broth in glass tubes, and with the spores deposited directly in the broth, are shown in Table VIII.

TABLE IX

SPEARMAN-KARBER TIMES (U) FOR SPORES ON PAPER DISCS OR STRIPS HEATED IN BROTH OR GLASSINE PAPER ENVELOPES

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Exp. No.	Spore crop	U, Broth min[a]	U, Envelope min	U_B/U_E
312	QHRM	16.0	7.0	2.29
331	PBBF	10.4	7.8	1.33
338	QHSM	11.2	6.6	1.70
341	QHSM	12.6	6.9	1.83
345	QHSM	14.1	7.2	1.96
346	QHSM	12.2	6.4	1.91
348	PBBF	13.9	7.6	1.83
Average				1.84

[a] Times have been corrected for lag in heating and cooling.

The results of all experiments were similar; spores on strips or discs in envelopes survived for a shorter period of time than when the strips or discs were placed in broth during heating. The ratio of the Spearman-Karber time for spores in broth (U_B) divided by the Spearman-Karber time for spores on strips in envelopes (U_E) has been computed and is shown in Table IX. While there is some variation, the spores on strips or discs in broth consistently survive longer. The spores on strips or discs heated in broth survive an average of 1.84 times as long as the spores on strips in envelopes.

The manufacturer's specification for spores QHSM states that at 250°F (121.1°C), all indicators are to be nonsterile at a heating time of five minutes and all indicators are to be sterile at a heating time of 13 minutes. To compare this performance specification with the result in Table VII requires that we convert the Spearman-Karber time to appropriate survival and kill times. Performance times for two survival levels, P = 0.01 and P = 0.05, and two kill levels, P = 0.95 and P = 0.99, were calculated and are tabulated in Table X. The manufacturer's specification is shown as a footnote in Table X. When the spore strips were in the envelope during heating, the performance as far as survival and kill time is concerned, was within the manufacturer's specification using either the P = 0.01 and P = 0.99 or the P = 0.05 and P = 0.95 criterion. When the strips were in broth during heating, the kill times exceeded specified kill times both for P = 0.95 and for P = 0.99. The kill time range for P = 0.95 was from

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

13.8 to 19.7 minutes and for P = 0.99, from 15.5 to 22.2 minutes. The average P = 0.99 kill time was 40% greater than the kill time suggested by the manufacturer.

When spores PBBF were inoculated directly in the broth before heating, the Spearman-Karber times were about twice as large as when the spores were deposited on paper discs and the disc placed in the broth before heating.

TABLE X

CALCULATED PROBABILITY OF STERILITY (P) FOR SPORE STRIPS IN ENVELOPES AND IN BROTH ($N_o = 1.9 \times 10^4$)

Exp. No.	Performance times in minutes				
		Survival times		Kill times	
	U_{S-K}	P=0.01	P=0.05	P=0.95	P=0.99
Spore strips in envelopes					
312	7.0	5.6	5.9	8.6	9.7
338	6.6	5.3	5.5	8.1	9.1
341	6.9	5.5	5.8	8.5	9.6
345	7.2	5.7	6.0	8.9	10.0
346	6.4	5.1	5.4	7.9	8.9
Spore strips in tubes of broth					
312	16.0	12.8	13.4	19.7	22.2
338	11.2	8.9	9.4	13.8	15.5
341	12.6	10.1	10.6	15.5	17.5
345	14.1	11.3	11.8	17.3	19.5
346	12.2	9.7	10.2	15.0	16.9

Manufacturer's calibration specifications at 250°F.
All strips positive (prob. of sterility = 0) at 5 min.
All strips negative (prob. of sterility = 1.00) at 13 min.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

The obvious conclusion drawn from these results is that, with the spore strip type of biological indicator, if the spore strip is calibrated in the envelope with the envelope placed directly in a steam atmosphere, it is essential to use it in the same way if it is to perform according to the calibration specification. The results of our experiments point out that if spore strips are used in another way, such as being placed directly in a liquid solution, the results are quite different and the manufacturer's stated performance specification is meaningless.

The experimental results in this study again point out the sensitive nature of bacterial spores to environmental conditions. Bacterial spores are capable of doing a superb job of monitoring sterilization processes if they have been calibrated to monitor the specific process. In general, a biological indicator designed and calibrated to monitor a specific sterilization process cannot be used directly to monitor a different type of sterilization condition. The results of tests carried out in this study suggest that if we are interested in having a biological indicator for monitoring sterilization processes of liquid products, then it is imperative that a specific biological indicator be developed for this application and be calibrated in the specific liquid for the specific type of sterilization condition.

SUMMARY

1) The commercial spore strips that we have evaluated meet their listed specifications if they are used in what we feel are the design conditions.

2) Variation within a commercial lot of spore strips was very small.

3) The performance of spore strips on a lot-to-lot basis varied quite widely. The specifications permit this and it probably occurs because of environmental variables during both manufacture and storage.

4) In evaluating the performance of spore strips, test system control and especially close temperature control, is required. If test conditions are not closely controlled, then we may really end up with the spore strips evaluating the test system.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

5) When we put spores on a paper strip and put the strip in a glassine envelope, we have a unique system. There are effects of depositing spores on paper strips and also apparently an envelope effect. Therefore, it is critical that spore strips be used in the same way that they are calibrated if the calibration or specification is to be meaningful.

ACKNOWLEDGMENTS

Research reported in this paper was supported in part by Contract No. FDA 72-306, Department of Health, Education and Welfare.

Scientific Journal Series Paper No. 9602, Minnesota Agricultural Experiment Station, St. Paul, Minnesota 55108.

REFERENCES

1. Pflug, I. J. (1973). Heat sterilization. In Industrial Sterilization. G. B. Phillips and W. S. Miller, Eds. Duke University Press, Durham, N. C.; pp. 239-282.

2. Pflug, I. J. and Smith, G. M. (1976). Survivor curves of bacterial spores heated in parenteral solution. In Spore Research 1976. A. N. Barker, J. Wolf, D. J. Ellar, G. J. Dring, and G. W. Gould, Eds. Academic Press, London (in press).

3. Perkins, J. J. (1969). Principles and Methods of Sterilization in Health Sciences, 2nd ed. Charles C. Thomas, Springfield, Ill.

4. NASA Standard Procedures for the Microbiological Examination of Space Hardware. (1968). NHB5340.1A.

5. Pflug, I. J. and Holcomb, R. G. (1976). Principles of thermal destruction of microorganisms. In Disinfection, Sterilization and Preservation. S. S. Block, Ed. Lea and Febiger, Philadelphia, Pa. (in press).

6. Pflug, I. J. and Schmidt, C. F. (1968). Thermal destruction of microorganisms. In Disinfection, Sterilization and Preservation, C. A. Lawrence and S. S. Block, Eds. Lea and Febiger, Philadelphia, Pa.; pp. 63-105.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

7. Pflug, I. J. (1973). Performance of biological indicators designed for monitoring wet-heat sterilization processes. In Sterile Disposable Devices: Update '73. Technical Symposium sponsored by the Health Industries Association, Washington, D. C., October 17-18; pp. 136-151.

8. Smith, G. M., Pflug, I. J., and Chapman, P. A. (1976). The effect of storage time and temperature and the variation among replicate tests (on different days) on the performance of spore discs and strips. Appl. Environ. Microbiol. 32, (in press).

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

DISCUSSION

Q. by C. Bruch:

Alan, how do you view your models and the kind of slopes that you were showing? I remember one slide you had this morning had a green line going down to the abscissa, and then it had some red lines where you had the increasing proportion of resistant cells in the group pushing that slope upward or flattening it out. I refer to the first two presentations we had this morning, where we were using the fraction-negative approach, then predicting from that what the time would be for those natural contaminants on the products to have a one in a million probability of survival, and then calculating that and what the time is to reach it. How do you see your models supporting or disagreeing with that?

A. by A. Tallentire:

The model, as it has been devised, relates the probability of contaminated items (P) and radiation dose (D). Evaluations have been made for microbial contaminants of different radiation resistance present on items in different proportions. These say that unrestricted extrapolation to low values of P cannot be applied from measurements of proportions of contaminated items taken within a restricted range (say $>10^{-3}$). That is the message of the model in its present form. What you are doing is not permissible according to it.

Comment by C. Bruch:

That is what I was trying to gather. But see, when I started this work almost three years ago, the assumption was that we could move away from the finished products sterility test if we could come up with a biological indicator system whereby its relationship relative to natural contamination was such that essentially it was six-D-values more resistant. So, when you kill the biological indicator, the probability of natural contamination surviving is one in a million. But then the trick was how to relate what your natural contamination was doing at the abscissa below log zero.

Comment by A. Tallentire:

This morning's speaker reported observing survivors that were aerobic colony forming units; that is how he defined them. I would

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

anticipate that these were mostly spores and, apart from one or two notable exceptions, the resistance of aerobic spores to radiation in their natural environment falls within narrow bounds. If one can be sure that the population of organisms on naturally contaminated items exhibits a fairly constant response to radiation, then it is probably reasonable to extrapolate from measurements of relatively high values of P obtained with samples of such items. I would add that some five years ago the members of the U. K. Panel on Gamma and Electron Irradiation collaborated on a study in which they did a similar exercise to that reported this morning using single-use syringes. They saw no hint of a tail on curves relating P and D over a wide range of values of P, suggesting again that there is a rather uniform population of organisms on naturally contaminated items with respect to their radiation resistance.

Dr. Berube did say "naturally, biological indicators irradiated at different dose rates showed different D-values". I would say that this finding is unnatural.

Comment by R. Berube:

I recorded what I found and you saw the data in that particular slide. I do not defend it one way or the other. That was what was found.

Comment by C. Artandi:

I would like to add my comments to Alan's. I raised both my eyebrows when I heard that the dose rate has such a great effect on the D-value. We have never seen this, and while I don't question the experimental information, perhaps it is worth looking into a little more.

Comment by R. Berube:

Let me interject a thought here. I did mention one specific incident, that is, in one of the slides we saw a dramatic difference between the data from the first set of exposures and those from the subsequent set of exposures. The exposure system we used for the 0.15 Mrad was different from that used for the 0.22 Mrad exposure system. And those two were different from the third. The reason why I say I do not defend, nor do I argue the comments that were made is that in chatting with the statistician we have as consultant, he said that we cannot make any statements because

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

we have got everything confounded.

Comment by C. Artandi:

Between accelerators and cobalt-60, we are talking about a 10,000 times dose rate difference, and here we are talking about perhaps a few times, so I think the statistician spoke the truth that there were some other factors involved which gave some erroneous conclusions, which are contrary to theory.

Comment by R. Berube:

We anticipate looking into that. There's a question, of course, of delegating man-hours.

Q. by W. Dierksheide:

I would like to ask Dr. Berube whether he had subjected the data to statistical analysis and whether, in fact, the differences noted were statistically different for the D-values.

A. by R. Berube:

Yes, we had the help of a biometrician. He was in on the planning of the experiment and he was a constant consultant. Where the data was amenable to analysis, it was done and where it was not, it was so stated in the final report. When there was a statistical interpretation, we used the proper statistical terminology.

Q. by W. Dierksheide:

But in the case of the D-values you noted as being different, were they statistically different?

A. by R. Berube:

No, they were not.

Q. by L. Kallings:

One of the problems, of course, when one is enumerating irradiated cells, that is damaged cells, is to be sure that you actually have the optimal growth condition. There is a big difference, as we all know, between undamaged cells and damaged cells. You could get a straight slope or a curve depending on how skillful you are in growing the bacteria. How did you try to assure yourself that you had the optimum growing conditions for

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

all these different kinds of bacteria you tested?

A. by R. Berube:

No work was done specifically on the determination as to whether we had the optimum systems. I have always had, in the back of my mind, the work done in England with the cells that were micromanipulated, separating out an individual cell and then transferring it on the stage of the microscope. If the drop of medium to which that one cell was transferred was sufficiently small, the organism might divide and then you get more and more volume and you could say you have got a viable cell. But if that cell was not put into too large a drop of medium, nothing came up. And this is one of the assumptions, unfortunately, that we always make, namely, that the test system we use is a good one.

Q. by J. Whitby:

I would like to address a question to Dr. Pflug, really just as an application of the data he showed us. Is he really advocating that all of us, the whole world, should perhaps standardize on a particular strain which is prepared in a special way? I think it is very important with respect to what we do now. I would particularly like to ask him because of the data he showed us about the different circumstances of resistance. Does this have implications in the testing of liquids and in pharmaceutical sterilization? How would he advocate this be done?

A. by I. Pflug:

In answer to your first question, I think there are too many environmental variables in growing spores to try and set up a standard biological indicator by specifying the organism and spore production procedure. Sterilization processes are specified on the basis of physical parameters; therefore, I advocate the use of wet heat biological indicators that are calibrated in terms of time and temperature on the basis of the performance of the indicator unit. If I were producing biological indicators I would grow spores with varying levels of heat resistance. The number and resistance used in a biological indicator would depend on the desired performance. The final step in production would be to calibrate the prepared biological indicators on the basis of physical parameters.

One of the points I was trying to make in my presentation is

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

that you should not try to biologically monitor the sterilization process for a liquid in a container by taking spore strips designed for use in the hospital autoclave out of their envelopes and putting them in a bottle of parenteral solution. The calibration of spore strips designed for use in envelopes in the hospital autoclave is not the same as when the spore strips are placed directly in a liquid. If you are going to monitor the sterilization process of solutions, then you must be sure that the spores are calibrated in the solution in which they will be used.

Q. by C. Bruch:

We are on the subject of parenteral solutions now. I am going to put you on the spot. When I talk about where the Bureau of Drugs is going with an F_o-value for a large volume of solutions, the number 8 comes up and they usually attribute it to you. Are you using B. stearothermophilus as the organism in calculating your F_o-value of 8?

A. by I. Pflug:

There are many ways of arriving at an F_o of 8 minutes. Analytically it is a numbers game (n × D); practically it is a judgment decision. We know there are microorganisms in nature with wet heat D(121°C)-values greater than 1 minute. If we use your 10^{-6} probability of a nonsterile and assume there is one of these resistant organisms per unit, then we must have an F_o greater than 6 minutes. Perhaps 8 minutes is a good compromise. If we are thinking about B. stearothermophilus with a D(121°C)-value of 4 minutes, then if the N_o is 10^{-4}, an F_o of 8.0 minutes will be required. If we use this approach, we are operating on the basis that the F_o we specify will always be delivered to the product. I believe that in addition to the microbiological uncertainties of our drug products, there are significant uncertainties in the delivery of the lethal agent to the product. The normal variation in the F_o actually delivered to each bottle of product will vary with the equipment that is used to carry out the sterilization process. Therefore, I believe that there is more to this problem of selecting an F_o-value than establishing the microbiological numbers; I think that the final sterilization dose or process is a judgment decision based on microbiological considerations plus the delivery variations that can be expected from the specific manufacturing procedures.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Comment by C. Bruch:

The reason I am concerned about this is because when Dr. Ruig was at the postgraduate school at Chelsea College, he was using the figure F_o of 8 from the podium. Again, I am not trying to put the Bureau of Drugs on the spot, but what they're designing for in terms of this continuous hydrostatic sterilizer is an F_o of 8. He was not sure some of the products could take that, because we are using the flexible containers. And I am just a little concerned, if that is going to be the figure, if there is going to be some flexibility. I think, in light of the remarks you've just made, there must be more flexibility. We must be concerned about the delivery of the sterilant to the product and not merely say that all products get an F_o of 8.

Comment by I. Pflug:

If we are talking about heat sterilization of drug products in containers, then the F_o-value is a logarithmic rather than an arithmetic function of the total heating time. The difference in the heating time required for the delivery of an F_o of 4 versus 8 minutes is small compared to the difference in heating time for the delivery of an F_o of 0 compared to 1 minute. If the product and package can stand a heat process delivering an F_o of 4 minutes, I do not believe there will be very much product effect if the F_o-value is increased to 8 minutes. I view an F_o-value of 8 minutes as a good starting point value, as a process that should take care of the worst case condition that can exist under good manufacturing conditions.

There are always exceptions to every rule. I believe that processes can be reduced on an individual product basis where warranted either on the basis of the manufacturing process which may produce an extremely low initial microbial contamination or the nature of the product which may produce additional bacteriological die-off during the sterilization process.

Q. by A. Bishop:

I got very confused about two minutes ago. Did I understand Dr. Pflug to seriously put forward the suggestion that the proper way to monitor the sterilization of parenteral fluids was with spore strips?

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Comment by C. Bruch:

That is not the understanding I had. The way I understood Dr. Pflug's statement is that if you take a spore strip and drop it in the fluid, the value you get there is different from that you would get if you had added those spores directly to the fluid.

Comment by A. Bishop:

I understood this, too, and it seemed interesting but highly esoteric information. But this was not the point. The question was, as I understood it: Is this a satisfactory way of monitoring the sterilization of parenteral fluids?

A. by I. Pflug:

I would say, "No". The reason we got involved is that a colleague was going to check out the autoclave and thought that a good test would be to put spore strips in the fluid material being sterilized. The autoclave cycle did not kill the spores on the spore strip. My colleague was upset. We proceeded then to find that the autoclave was performing adequately. However, an F_o of approximately 25 minutes was required to kill the spores on the spore strip when the spore strip was placed in a bottle of medium.

Comment by J. Whitby:

I have that feeling myself. I think the Joint Council on Hospital Accreditation in Canada was proposing that where water was being sterilized in the operating room, this should be tested by immersing a spore strip in the bottle. We were criticized for not following this test. I do not know whether you thought this was a good way to go either.

Comment by I. Pflug:

I think the procedure is all right if you want to use it. If you use this procedure, then you should use spores that are calibrated for use in water so you can meaningfully interpret the results.

Comment by A. Bishop:

I think this is tomfoolery of a very high order, if I may say so. I think there is not a shred of evidence that a contaminated solution, sterilized at 134°, for the sake of argument, for 3 minutes, and I mean heated, checked by physical measurement so that you know what you're talking about and you know that the

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

organisms have been exposed to this temperature for this time;

there is, I repeat, not a shred of evidence that every one of those organisms will not be killed.

Comment by C. Bruch:

We are still talking probabilities. We are still willing to say that there is a probability of a survivor of such and such a magnitude, like one in a billion or the like.

Comment by A. Bishop:

The figure, as I remember it, from work done by a man at Guys, was that we had an order of population reductions of about 10^{30} under these conditions.

Q. by C. Bruch:

That was for *B. stearothermophilus* at 3 minutes?

A. by A. Bishop:

Indeed.

Comment by J. Whitby:

But surely fluids are not sterilized at 134°C in most pharmaceutical practices. That is used for dressings. The temperature is 121°C, I think.

Comment by A. Bishop:

This is true and is a very interesting point. The questioner did say water, and water I think could be done in this way. It is the custom in my country to follow the pharmacopoeial recommendations of 115°C for half an hour. Most of us, I think, would consider that this is too low.

Comment by C. Bruch:

115°C for 30 minutes doesn't follow a very good z-function.

Comment by A. Bishop:

I would go above that, but it does not affect the issue. The issue is that you find a temperature that is satisfactory and then you stick to it and you measure it. Then, even if you accept 115°C, you still are very wise to put your trust in physical measurements.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Comment by I. Pflug:

I think that this is true in the laboratory, but I would question it in the field. I think at this point that I would have more confidence in a properly calibrated biological indicator in the field than I would in physical measurements made by a nonengineering technician in the field.

Comment by C. Bruch:

I am surprised that some of the audience has allowed some of the implications on the ethylene oxide presentation go by without discussion at length. Dr. Campbell made a very cogent comment at a recent AAMI meeting when he first saw the data that Mr. West presented on the relationship of the resistance of the spores on paper strips relative to a natural contamination on a product. Would you want to comment?

Comment by R. Campbell:

It seems to me that this morning we have pretty well ruled out the paper biological indicators with the use of ethylene oxide, and we have ruled out any product testing, and everybody tells me that we cannot calculate our D-values in a straight line. I just don't know where to go from there. But I do know that there are an awful lot of people out in the hospitals who ought to know what they must do to be saved. And we have all the gods sitting up here on the mountaintop, and it is to these gods that these people are praying for guidance. I think we really have to come up with some kind of procedure which can be used in the field and preferably one which does not require a houseful of microbiologists cooking up spores of a particular lineage and genealogy which are bred to have particular characteristics and which are fed and nurtured in a particular way so that they will behave according to their lineage. We have to have something which can be used in the hospitals without the massive laboratory backup which is necessary for the experimental work we have been meeting about today. I do not know what the answer is to any of this, but what I liked most of all was what Karl Kereluk was talking about with his graded indicator. If something along those lines could be worked out in a practical way, which could be used in the hospital, perhaps there is a light of salvation somewhere at the end of the tunnel. But right now, we are at the beginning of a very long, dark tunnel because you have taken away all the light from us.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Comment by C. Bruch:

I am a little unhappy to hear that we have taken all the light. I think in trying to elucidate here, we have been very honest. I face the problem that people challenge me constantly in Washington with the fact that the logarithmic death rate curve does not hold all the time. But, I have to take an absolute position if I am ever going to get our field people educated to go with what I call "dosimetric" or just physical parameter release as mentioned by Alex Bishop. I agree with Irv Pflug that, in terms of integrating all the parameters involved in a sterilization cycle, the biological indicator does it best. But I still would say that once you know what it is that you are sterilizing and the resistance of the contamination in that particular product to that particular sterilizing agent, you can calibrate all that and go with physical measurements alone. The particular concern I have right now is about the spore strips, the paper spore strips being used by hospitals. I think we spent $100,000 on this study with Castle. Keith only gave you the tip of all the work that went into that. But in terms of what he has shown us, it indicates that if the hospitals are putting supplies with a fair amount of contamination into the sterilizer and then relying on the kill of the paper strip in a glassine envelope to state that those supplies are sterile, there is not too much of a safety factor there. And I am now saying to the industry, "Fellows, the data is out in the open". We have got to start calibrating the resistance on those paper strip biological indicators for ethylene oxide because the hospitals cannot go to inoculated product. Industry can. In Karl Kereluk's data this morning, the 44 positives out of a 1,016,000 were inoculated product. You see how it held the theory that he was shooting for one in 10,000 survival on that biological indicator and he actually found one in 23,000, which I think is pretty close to theory. That was a beautiful piece of data to give us.

Comment by R. Campbell:

There is just one thought that I would like to drop into the discussion. I think that you and I particularly, and Alex too, have to bear in mind that the present state of legislation in all three of our countries is such that the only assurance we can give patients at the moment is the 10^{-3} probability. And what all three of us have a duty to do is to find some way of modifying our legislation so that we can give higher degrees of probability – 10^{-6} at least. And I think that what we have been looking for from

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

this discussion is some kind of basis on which we can persuade our legal people to modify the present legislation in order that we can take advantage of the technology of the latter half of the 20th century instead of being fixed in the 1920s by badly drawn regulations for drugs.

Comment by C. Bruch:

Beautifully said, I appreciate that.

Comment by L. Kallings:

In Scandinavia we are using reference preparations for ethylene oxide, but they are dried spores and that may be one of the differences. I could not follow, from the beginning, what you said, Mr. West, so I ask you now concerning the fact that the spores on the paper strips are much more sensitive than the spores on the naturally contaminated products. Do you think that was due to the different conditions of the spores? For instance, the dehydration of the natural contaminants vs wet spores on the paper strips. How were the spores prepared? What was added to the paper strips?

Comment by K. West:

The spores were prepared in the same method for both the inoculated product and the filter paper strips. The spores were placed on the filter paper strips and were carried in a water solution, placed on the filter paper strips and allowed to dry.

Comment by C. Bruch:

They were from the same preparation, the same tube of spores. So the difference is the vehicle. At least that is one of the prime differences here.

Comment by K. West:

I would like to address a comment to what you were talking about - the turmoil we are in. There are several methods of getting out of this. One is what Dr. Kereluk was talking about this morning with the graded resistance. Another would be using a protected spore of the filter paper type where the spore is protected artificially, placing the spore in a condition artificially that would be resistant to a 10^6 level, or would give you the probability of a 10^6 level. The hospitals then would have the

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

capability of utilizing the spore strip which is protected in lieu

of an inoculated product. It would be the responsibility of the manufacturer of the biological indicators to certify the resistance level of the protected spores. Then the hospitals would have some assurance.

Comment by L. Kallings:

We are using sand to protect the spores, and sodium chloride.

Comment by Dr. C. Artandi:

I feel that we are going forwards and backwards simultaneously because on the one hand we are saying: let us liberate ourselves and let some fresh air in, and let us look at new ways and recognize that sterility testing is not satisfactory and that biological indicators are just a kind of crutch to lean on. Really we are talking about process control - knowing what we are doing. Then we say that we have at least one in a million or better sterility assurance. I cannot conceive any situation really where you need better than one in a million. As a matter of fact, we should come to the point of saying that there are two or three classes of products. For some of those products, one in a thousand is perfectly adequate and for another product, one in a hundred thousand may be, while for some, perhaps, one in a million. I would like to caution against artificial tests. It is generally easy to generate good spore cultures and deposit them in some reasonably controlled way on products or other vehicles. When you start to protect them and add things to them or otherwise treat them, you introduce artificial variations in addition to the natural ones, and you will have even more spread and more difficult ways of finding a consistent condition.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

RECENT DEVELOPMENTS IN PYROGEN TESTING

Stanley Marcus and Jerry R. Nelson

University of Utah College of Medicine and
Microbiological Development and Control Corp.,
University of Utah Research Park
Salt Lake City, Utah

Although the rabbit bioassay is currently the only legally acceptable method to test for pyrogenic contamination of parenteral preparations and medical devices designed to enter the parenterum, research efforts over the past decade have made available alternative procedures to test for pyrogens which may have significant value for those responsible for quality control. Part of the reluctance to accept tests other than the rabbit bioassay may be due to the low visibility of emerging understanding of a significant theory of testing.

Much of what we think about tests and testing is apparently understood tacitly. It is well to articulate this area of tacit understanding, if for no better reason than criticism from peers; also, it is evident that many of the people with whom control personnel must deal do not have the insights of the people in control work.

Tests are designed as controls. Such a statement is redundant for those, such as personnel involved with quality or analytical testing, who never think of running a test without controls. All of our test procedures include controls which are known positively and negatively reacting materials. Unless the controls yield expected results, the results obtained with the unknowns under test are not valid. No test is valid unless controls are included.

However, we also use the term “control” for procedures which are methods that have been incorporated into production of all types to insure the quality, including performance, of the product when it is used. So, although we may understand our use of the term “control” and distinguish among different types of controls, not

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

everyone else understands this. Most critically, differences in "control procedures" and "controls in testing" may not be precisely understood by those not involved with tests and testing. It is our responsibility to make clear these distinctions, particularly to regulatory personnel.

Integral with the understanding of controls is the understanding of test theory. There exists a simple, easily comprehensible and rational theory of testing first deduced by Kahn [1] and since articulated by many others, e.g., Holland and Whitehead [2]. The theory holds that all tests are characterized by the parameters of sensitivity, specificity, reproducibility and accuracy. Professional bio-metricians and statisticians use different words and definitions, but the words we use are most simple:

1) Sensitivity is the ability of the test to give positive reactions in the presence of the material for which we test.

2) Specificity is the ability of the test to give positive reactions with only the material for which we test.

3) The reproducibility (precision or repeatability) of the test result in the same and in other laboratories must be within acceptable limits.

4) Accuracy or true determination of the substance for which we test is an ideal toward which we strive but cannot often achieve. It is necessary, therefore, to have a complete and unequivocal description of the test and the standardization method.

TABLE I

SUMMARY OF RESULTS OF REPEATED PYROGEN TESTS WITH RABBITS*

No. of rabbits	Group designation
13 (11 female, 2 male)	Consistently reliable
5 (5 female)	Consistently unreliable
10 (6 female, 4 male)	Usually reliable
3 (3 female)	Usually unreliable

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

*Total of 31 rabbits (25 female, 6 male) randomly supplied from one source, were tested 3-8 times.

RABBIT BIOASSAY

Turning to matters at hand, the rabbit bioassay employed in the United States is, of course, the procedure set forth in the United States Pharmacopeia (USP) [3]. Although not specified in the USP, we employ minimally restraining stocks and so-called "New Zealand" albino female rabbits weighing 3-4 kg. We use thermocouple probes which feed into a sensitive, but rugged galvanometer. Of course, we employ all conventional procedures for checking probes, insertion depth, screening and conditioning rabbits, and paying close attention to all phases of the rabbit test that we can control.

Table I gives some published data [4] concerning screening of rabbits to be used for pyrogen testing. It is apparent that preliminary screening is vital and that a single test with three previously untested rabbits can be erroneous. Beyond this finding, we have experienced, as have many colleagues, the trauma of a set of rabbit readings going awry; in one case, this occurred because of the appearance in the test room of a stranger (delivery man), reeking of animal odors (a vivarium employee), who stayed to chat while the animals were being injected.

The USP rabbit test for pyrogens involves a presumptive procedure with three animals. If the test material passes, it is adjudged nonpyrogenic. If the test result is positive, however, the completed test with five additional animals is to be run.

The tacit, nowhere specified assumption, is that no substance passing the presumptive test will fail the completed test. However, this tacit assumption gives rise to some unsavory practice with substances that often yield false positive reactions, for example, anticoagulant citrate solution. Some workers will resort to testing five animals and choosing the three "best", that is, lowest temperature response results. Needless to say, such practice muddies the clear interpretation of the test. Perhaps the sliding scale of the British Pharmacopoeia [5] allows a bit more reality in overall testing.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Published results [6, 7] seem to agree on the sensitivity of the

rabbit bioassay for endotoxin (ET) to be in the range of 0.001-0.01 µg/kg; for example, a "reliable" 3 kg rabbit should respond with a 0.6°C or better rise in temperature from baseline within three hours of injection of 1-10 ng of ET, without regard to the volume of material injected, provided that the volume be no more than 10 ml/kg: that is, in the case of a 3 kg rabbit, 30 ml. Current detailed analysis of the rabbit test may be found in reviews by Personeus [8] and by Nelson [9].

Table II is designed as a ready reference to the equivalent concentration values in current use. Also, it will be noted from Table II that the rabbit test may be positive or negative when each animal is injected with pyrogen-free (PF) saline, 10 ml/kg, to which has been added 1 ng of ET.

TABLE II

EQUIVALENT CONCENTRATIONS AND SMALL UNITS CONVERSIONS

	%	mg%	Ratio	µg/ml or ppm	µg/ 0.1 ml	ng/ml	ng/ 0.1 ml	pg/ ml	pg/ 0.1 ml	Rabbit test*	LAL**
10^{0}											
10^{-1}	10		1:10	10^{5}							
10^{-2}	1.0	10^{3}	1:10^{0}	10^{4}							
10^{-3}	0.1	10^{2}	1:10^{3}	10^{3}(1000)							
10^{-4}		10^{1}	1:10^{4}	10^{2}(100)							
10^{-5}		10^{0}	1:10^{5}	10^{1}(10)	10^{0}						
10^{-6}		10^{-1}	1:10^{6}	10^{0}(1)	10^{-1}						
10^{-7}		10^{-2}	1:10^{7}	10^{-1}(0.1)	10^{-2}					pos	+++
10^{-8}		10^{-3}	1:10^{8}	10^{-2}(0.01)	10^{-3}	10^{1}	1			pos	+++
10^{-9}				10^{-3}(0.001)	10^{-4}	1(10^{0})	10^{-1}	10^{3}	10^{2}	neg	+++
10^{-10}				10^{-4}	10^{-5}	0.1(10^{-1})	10^{-2}	10^{2}	10^{1}	neg	++
10^{-11}				10^{-5}		0.01(10^{-2})	10^{-3}	10^{1}	10^{0}		+
10^{-12}				10^{-6}		0.001(10^{-3})		10^{0}	10^{-1}		+
10^{-13}								10^{-1}	10^{-2}		-
10^{-14}											

*Rabbit test: 1.0 ml of material in PF-PSS, injected 10 ml/kg.

**LAL test, FDA suggested requirement for acceptable lysate in test with 0.1 ml of ET plus 0.1 ml of LAL is positive (gel) with 0.1 ng, and negative with 0.0125 ng.

Sensitivities to ET		
Rabbit	0.01 -	0.001 µg (1 ng/kg)
LAT	0.01 -	0.001 ng/ml
FDA suggested LAT	0.1 -	1.0 ng/ml
Actinomycin D	10	- 100 ng
Epinephrine rabbit	100	- 1000 ng
Chick embryo	100	- 1000 ng
Culture of fresh soln	1 live organism	
NBT	100	- 1000 ng

ET = endotoxin
LAL = Limulus amebocyte lysate
PF = pyrogen-free
LAT = Limulus amebocyte lysate test
PSS = physiological saline solution
NBT = nitroblue tetrazolium test

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

ALTERNATIVE *IN VIVO* ASSAYS

Agents that enhance the *in vivo* action of ET have been reported by many investigators. The most potent of these agents are antineoplastic drugs including mitomycin C, pactamycin, methotrexate, vincristine, cyclophosphamid, 6-mercaptopurine and actinomycin D. We know of no data comparing the relative efficacy of these agents as ET potentiators in animals, although such effort is indicated by obvious considerations, not the least of which is cost. For example, a current quote on 6-mercaptopurine is $5.00/g, while actinomycin D is $30.00 for 4 mg in twenty 200 µg vials.

Actinomycin D (dactinomycin USP or cosmagen) was shown by Berry and coworkers [10] to enhance ET lethality for mice. Pieroni, Broderick, Bundeally and Levin [11] confirmed this observation and used it to monitor removal of ET during fractionation of pertussis vaccine. Dowling and Feldman [12] reported the use of the procedure to assay freedom of ET from preparations of meningococcal polysaccharide. The sensitivity of the method is in the range 10-100 ng. The procedure may deserve the attention of those concerned with the eminently practical problem of "cleaning" vaccines that are responsible for significant side reactions due to ET contamination. No data designed to compare this procedure with the Limulus test for such considerations are available.

The procedure for the lethality enhancement test is quite simple. The enhancer is injected 48 hours before injection of the material under test. A significant error is introduced by virtue of the fact that the actinomycin itself may contribute significant lethality.

The epinephrine skin test is based on the finding that the intradermal injection of epinephrine enhances ET action. This was first reported by Dr. Lewis Thomas. The test developed by Thomas [13] has seen limited clinical use in detection of ET in the blood of patients with different severe infections. One variation of this test is performed by injecting 100 µg of epinephrine or norepinephrine into the abdominal skin of rabbits immediately prior to the intravenous injection of ET or test solution. The appearance of hemorrhage at the site of the epinephrine injection is a positive signal. The reported sensitivity of the test is from 0.1 to 1.0 µg of ET. This method deserves additional study for

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

potential use in some of the control problems we face. A mark against its use is the number of animals that must be employed.

The chick embryo lethality test [14] involves the use of ten-day-old chick embryos. Such embryos are critically susceptible to ET. The test has been quantitated and correlated with pyrogenicity in rabbits. Our results indicate sensitivity lower by a factor of 10-100 compared to rabbits. The assay involves intravenous injection of 0.1 ml volumes of test solution into ten-day-old chick embryos. The embryos are then incubated for an additional 24 hours, and then candled and recorded as dead or alive. The obvious disadvantages of the test are the need for a humidified self-turning incubator and ten-day-old chick embryos. Our experience indicates a need for practice in the injection of the embryo as well as the need for frequent observation, at least at eight-hour intervals following injection.

ALTERNATIVE *IN VITRO* ASSAYS FOR PYROGEN

Turning to some alternative *in vitro* assays, mention can be made first of the use of tissue culture cells. ET has been shown to induce cytopathogenic effects in certain cell cultures. This technique is reportedly as sensitive as the rabbit bioassay. However, no confirmatory studies are yet available.

We have experience with complement titration as a measure of endotoxic activity (unpublished). The anticomplementary action of the agent under test is determined. In our hands, the limit of test sensitivity is about 100 nanograms (0.1 µg/ml). Aside from relatively low sensitivity, this procedure, in our opinion, suffers the drawback of requiring exceptionally well trained personnel.

In 1960, we described [15, 16] the basis for and results of a bacteriologic culture method for determination of pyrogens in freshly prepared solutions of glucose, sodium chloride, or a mixture of these materials. A freshly prepared solution has been defined as one prepared with distilled water, used and sterilized within a 24-hour working period. A live count of less than 10 organisms/ml in 100 ml samples, collected on 0.45 µm membrane filters, is considered nonpyrogenic.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Although the evidence solidly supports the claims made, the

disadvantages of this test are apparent. A bacteriological laboratory and technician must be available. The culture assay must not be delayed, the samples must be tested as soon as available, results must await the 48-hour culture period, and most distressingly, for reasons considered in the publications referred to, a positive result, i. e., solutions yielding counts greater than 10/ml, are rarely positive when the sterile solutions are tested by rabbit bioassay.

As a corollary to this work, we have made a strong case for distinguishing between “nonpyrogenic” and “pyrogen-free” solutions [17]. The distinction is not semantic but exists in reality. We feel that unless a worker prepares his own bacteria-free water, sodium chloride, and saline, and can show these to be sterile before autoclaving, the preparation he employs is properly referred to (after rabbit testing) as nonpyrogenic. If the preparation is sterile before autoclaving and nonpyrogenic by test after sterilization, the preparation is properly referred to as pyrogen-free.

The significance of bacterial numbers in the area of sterility testing has been most recently explored in a series of classical experiments by Kereluk [18].

An *in vitro* tissue culture-like method that may be of value is the nitroblue tetrazolium test (NBT). We have experience with a procedure described by Park and his colleagues [19] and modified by us for ET assay. In the method employed, human granulocytes are allowed to adhere to a clean coverslip and are then exposed to ET solution. This is followed by adding NBT-containing media. ET-activated polymorphs reduce ingested NBT to blue formazan granules which constitute the positive signal. The coverslip is fixed in methanol, mounted on a slide and the percent positive cells estimated by direct count. We have employed triplicate counts of 100 cells to gather data.

Figure 1 gives results with both ET and autoclave-sterilized saline suspensions of *E. coli.* The relatively low sensitivity of the method is apparent.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

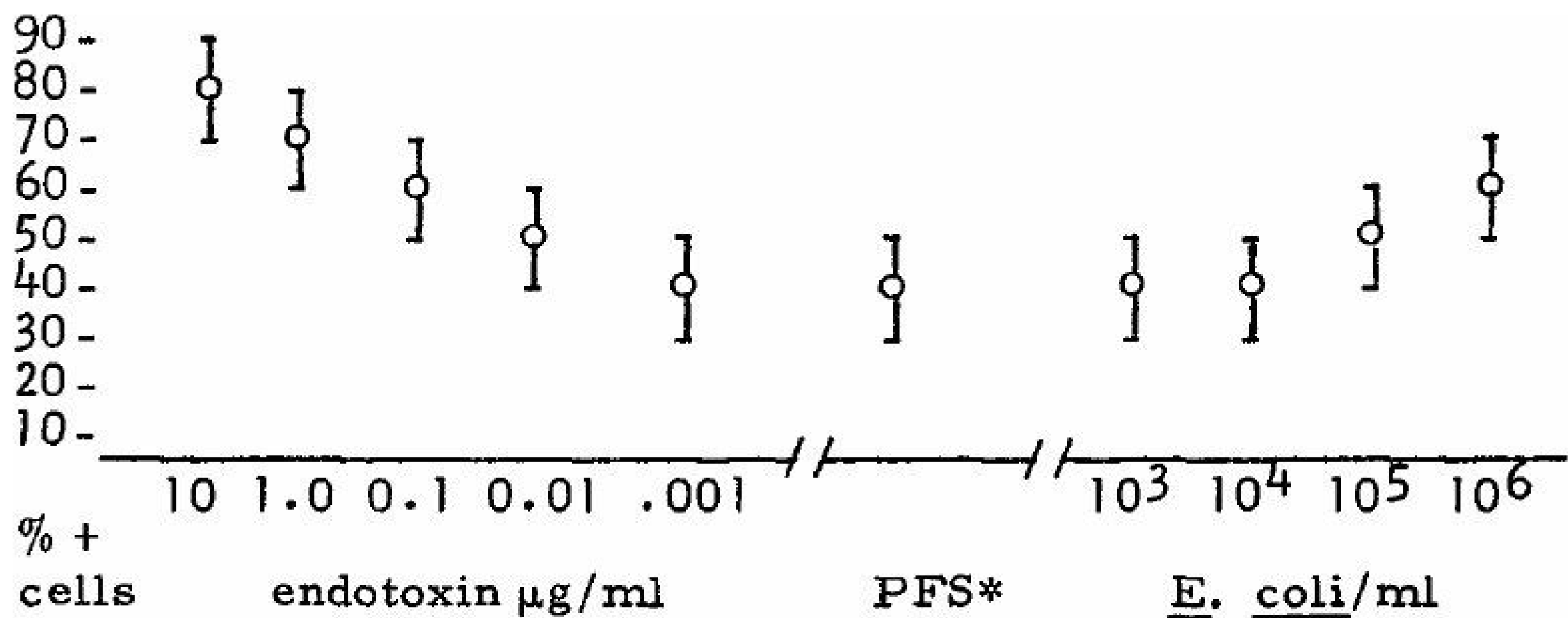


FIGURE 1. NBT-cell reaction to purified endotoxin and bacterial suspensions.

The final *in vitro* assay to consider is the Limulus amebocyte lysate test (LAT). Dr. Jack Levin, a physician at Johns Hopkins University Medical School, and involved in the study of coagulation, became interested in the coagulation process in invertebrates during time spent at the Woods Hole Marine Biology Laboratories. Serendipitously, it was discovered [20, 21] that ET activated the clotting mechanism of lobsters and crabs. These invertebrate crustacean animals responded to infection with gram-negative organisms with amebocytopenia, intravascular coagulation, and death [22]. *Limulus polyphemus*, the horseshoe crab, was chosen as the experimental animal because of availability and cost considerations.

The circulating cell of *L. polyphemus* is an amebocyte. This granule-filled cell acts both as a phagocyte and as an activator of the coagulation mechanism for the animals. When bled without use of cell antiaggregant, the amebocytes rapidly clump and degranulate. The granules lyse and clotting occurs at the site in the form of solid stringy strands or, if in a test tube, into a gel. Cellular aggregation can be inhibited analogously to inhibition of invertebrate blood clotting. However, different inhibitors are used than with vertebrate blood. A number of

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

reagents, not including the classical mammalian anticoagulants such as heparin, citrate, oxalate or EDTA, must be used. Active antiaggregants include N-ethylmaleimide or the N-ethyl ester of maleic acid (NEM), or the N-methyl ester, formaldehyde solution, 0.66% saturated ammonium sulfate, neodinium chloride and lanthanum nitrate.

In accordance with Levin's method, we have used NEM, 0.01 M at pH 7.3 (Tris buffered) in sterile PF 0.51 M (3%) sodium chloride solution. This is an excellent antiaggregant mixed with hemolymph 1:1. We have routinely used the NEM solution at 37°C. The syringe contains the NEM; the 18-gauge needle is inserted at the junction of thoracic and abdominal segments after cleansing with alcohol.

Following collection of the amebocyte-rich hemolymph, the cells are collected by centrifugation, washed by centrifugation, and then lysed. Lysing has been done with ultrasound, distilled water, freezing and thawing, and by grinding in a glass tissue homogenizer. After lysing, the suspension is cleared of debris by centrifugation, and the optically clear supernate can be kept in the refrigerator, in the freezer, or lyophilized. Tests with the Limulus amebocyte reagent are carried out in sterile, PF 8 × 75 mm to 11 × 75 mm capped test tubes. We prefer the small diameter tubes.

The volume of test material and of lysate used by us as well as by most other investigators is 0.1 ml. The substances are mixed and placed in a 37°C water bath for one hour. We have followed Levin's scheme for reading: a 3+ is a solid clot, 2+ is a visible increase in viscosity, 1+ equals flocculation or starchy granules, ± is viscous and cloudy, – equals sparkling clear. PF saline, not water, must be used for the negative control.

Different lots vary in sensitivity. The FDA currently suggested the definition of "sensitivity" as a 3+ or solid gel with 1 ng/ml, and a – (no gel) with 0.1 ng/ml employing a "standard" preparation of ET. This means that when testing 0.1 ml amounts, one must obtain a gel with 0.1 ng and no gel with 0.01 ng of the FDA standard ET.

Table III gives some results we have obtained with different lysate preparations. By the FDA criteria, we have not one

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

acceptable lot in this group; all lots were oversensitive or

undersensitive. Dilution with PF physiological saline solution (PSS) or undersensitive lots of lysate have been employed to bring oversensitive lysate to rabbit test sensitivity levels. That is, we have adjusted our amebocyte lysate material to yield a sensitivity similar to that of the rabbit test.

At this point, a few comments are offered concerning standardization of any of the alternative tests with which we may be faced.

Why standardize any pyrogen test at a specific level of sensitivity? Why not push the sensitivity level to the utmost to insure the quality of each product?

If a standard level of sensitivity is agreed upon, is the rabbit response level to ET a proper indicator or should a more sensitive indicator of pyrogenicity than the rabbit be sought?

TABLE III

COMPARATIVE REACTIVITY OF DIFFERENT LOTS OF LIMULUS LYSATE TO PURIFIED BOIVIN ENDOTOXIN (DIFCO LPS FROM E. COLI 055:B5)

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Lot #	Micrograms/ml				Nanograms/ml				PFS
	10	1	0.1	0.01	1	0.1	0.01	0.001	
T2	+++	+++	+++	+++	+++	+++	+	-	-
L2	+++	+++	+++	+++	++	++	+	-	-
R2	+++	+++	+++	+++	+++	+++	+	-	-
01	+++	+++	+++	++	+	-	-	-	-
02	+	-	-	-	-	-	-	-	-
03	++	+	+	-	-	-	-	-	-
04	+++	+++	+	+	-	-	-	-	-
05	-	-	-	-	-	-	-	-	-
S1	+++	+++	++	++	++	++	-	-	-
P2	+++	++	+	+	+	-	-	-	-
P3	+++	+++	++	+	+	+	-	-	-

LPS = lipopolysaccharide PFS = pyrogen-free saline

At present there exists a formal, scientifically and legally acceptable level of sensitivity in tests for pyrogens; it is the USP rabbit test.

With regard to ultimate level of sensitivity, it is apparent from the bacteriological tests with freshly prepared parenterals, that we can push the requirement for freedom from potential pyrogens to a practically unattainable level by demanding sterility prior to autoclaving. Accepting the argument that such a high level of sensitivity is unnecessary signifies that we have tacitly accepted a level of sensitivity less than absolute freedom from potential pyrogens. Acceptance of the rabbit level of sensitivity is based on practice as well as archival reports.

Unequivocal demonstration of instances of pyrogenic response in humans to preparations that have passed the current rabbit test

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

for pyrogens is nonexistent. This overwhelming empirical result

should satisfy the most demanding authority.

Archival reports that are apropos are few but compelling. Although Co Tui and Schrift reported in 1942 [23] that rabbits were less sensitive than humans to the effects of a typhoid pyrogen, reports since have been otherwise. Dare and Mogey [6] reported an experiment involving nine humans and over 200 rabbits in which the animals could be calculated to have 1/3 – 7 times the sensitivity to a Pseudomonas pyrogen shown by the humans. Greisman and Hornick [7] reviewed the literature, added their own experimental observations, and concluded that "on a per kg basis, rabbit and man are approximately equally reactive to threshold pyrogen quantities of endotoxin". Further, they concluded that "on a total dose basis, rabbits require smaller quantities of endotoxin to elicit threshold febrile responses…"

In the absence of data to the contrary, we must accept the current working hypothesis that the rabbit test response is an acceptable indicator of potential pyrogenicity or freedom therefrom.

But what happens if we accept the limits of sensitivity proposed by the FDA for the LAT? We increase the sensitivity level by a factor of 10-100 times. Some implications of such an increase in sensitivity are apparent: for example, some preparations that now pass the rabbit test will fail the LAT. The desirability of such an eventuality requires discussion and, more critically, actual data.

We are impartial concerning the need to increase the sensitivity level for pyrogen testing in practice. However, we contend that evidence which is not based solely on dogma, theory, or committee action is necessary to support such a move.

TABLE IV

PYROGEN TESTS IN PRACTICE

Source of sample	Rabbit (USP)	Limulus
Municipal water supply	-	+
Medical center distilled	-	+

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Redistilled (glass still)	-	-
Deionized (virus labs)	-	-
Deionized (pathology)	-	-
Deionized (ob-gyn)	-	+
Technetium-albumin	- (25)	- (10) + (15)
Catheter washings	- (>100)	- (>100)

Sullivan and Watson [24] noted the presence of a removable inhibitor in "pool" lots of lysate by extraction with chloroform and other organic solvents, and achievement of maximal sensitivity by the addition of 0.02 M Ca, Mg or Mn. All these divalent cations were equally effective. The authors noted, as we have, that PSS is a better control than PF water because the negative is optically clear with PSS but often cloudy with water.

Examples of the use of Limulus amebocyte lysate (LAL) prepared in our laboratory or in conjunction with Dr. Thomas Pistole at the University of New Hampshire are shown in Tables IV and V.

Table IV is a summary of results obtained with different samples of water available at the University of Utah Medical Center as well as with preparations of Technetium-albumin from the pharmacy. In the case of Technetium preparations, all were used on patients and in no case was an adverse reaction or fever noted. The LAL used was sensitive to less than 0.01 ng ET, which may account for the high number of positive reactions noted. With the lysate adjusted to react to 1-10 ng of ET, we have had largely negative results with the isotope albumin preparations.

We feel that the LAT with amebocyte lysate adjusted to detect 1-10 ng of ET has been a reliable indicator of negative clinical response in our small sample to date. We recognize that this observation may be entirely fortuitous.

A word about specificity of the LAT. The only thermogenic agent we have tested in the LAT is etiocholanolone, which was negative at 0.1 mg/ml. This steroid is relatively insoluble in saline and the suspension (0.1 mg/ml) injected into rabbits at 10 ml/kg induced no pyrogenic response.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

TABLE V

RESULTS OF USP AND LIMULUS TESTS ON RADIOACTIVE TECHNETIUM-ALBUMIN PREPARATIONS

Sample No.	USP	Limulus[a]	Sterility
01-72R	–	++	–
02-72R	–	–	–
03-72R	–	++	–
04-72R	–[b]	++	+
05-72R	–	+ +	–
06-72R	–[b]	++	–
07-72R	–[b]	++	–
08-72R	–[b]	++	–
09-72R	–[b]	–	–
10-72R	–	–	–
11-72R	–	++	–
12-72R	–	++	–
13-72R	–	+	–
14-72R	–	–	–
15-72R	–	–	–
16-72R	–	++	–
17-72R	–	–	–
18-72R	–	+	–
19-72R	–	–	–
20-72R	–	–	–
21-72R	–[b]	++	–
22-72R	–	++	–
23-72R	–	++	–
24-72R	–	–	–
25-72R	–	++	–

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

[a] Limulus lysate Lot No. T2

[b]Samples were positive when first tested, but were repeatedly negative when retested in new animals. Antibody against Technetium-albumin was demonstrated in at least one animal in each test group by gel diffusion.

Drugs listed by Whittet [25] to be thermogenic, for example, tetrahydro-β-naphthylamine, α-dinitrophenol, and sulfur in oil have not been tested. One suspects that these agents induce the release of endogenous pyrogen upon injection as has been suggested by Whittet.

Our initial experiments on endogenous pyrogen, prepared from rabbit peritoneal exudate leukocytes, were negative in LAT and, unfortunately, negative in rabbits. This observation begs for careful exploration since, if reproducible, it suggests that we have a reliable and simple method for distinguishing between exogenous and endogenous pyrogenic materials.

There are but few data available concerning specificity of the LAT. Wildfeuer, et al. [26] have reported that gram-positive bacteria are LAT negative but that the peptidoglycan isolated from the cell walls of these organisms does gel the lysate. It is tempting to speculate that Wildfeuer's observations are a function of either quantitation (numbers of organisms involved) or of solubilization of cell membranes, or both factors acting together.

Limulus lysate has been reported to be specific for ET or ET-containing bacteria [27, 28, 29, 30]. None of the vasoactive components in blood (serotonin, histamine, epinephrine, bradykinin) are known to induce the Limulus reaction [30]. Thrombin, log phase cultures of gram-positive bacteria (*Staphylococcus* and *Streptococcus*), streptolysin, streptokinase, and streptodornase, have all been reported as negative [29]. However, some compounds other than ET have been reported to induce positive Limulus tests. These include thrombin [31], thromboplastin [31], ribonuclease [31], poly(A). poly(U) [31], peptidoglycan [26], and fibrinogen (unpublished). Conflicting data exist concerning poly(I). poly(C). Elin [31] claims this substance induces false positive reactions whereas Niwa [32] claims it does not.

SUMMARY

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Although the rabbit bioassay is currently the only legally acceptable method to test for pyrogenic contamination of parenteral preparations and invasive medical devices, research efforts have recently made available alternative procedures to test for pyrogens which may have significant value in quality control. Part of the reluctance to accept tests other than the rabbit test may be due to the low visibility of emerging understanding of a significant theory of testing in which it is contended that the parameters of sensitivity and specificity are inversely related and that accuracy is an ideal.

The rabbit pyrogen assay detects 0.001 to 0.01 µg of enterobacteriaceae endotoxin. The Limulus test detects 0.01 to 0.1 ng/ml of endotoxin; some of the other tests approach the rabbit assay in sensitivity. Since it is current dogma that pyrogen is equivalent to endotoxin, the basis for the use of the latter to standardize pyrogen tests is rationalized. The source of endotoxin in practice is bacterial contamination; therefore, numbers of bacteria that contaminate parenteral preparations can be directly related to potential pyrogenicity. Further, viable counts of bacteria in parenteral preparations, prior to sterilization, constitute a reliable test for pyrogens.

Other tests such as nitroblue tetrazolium reduction and actinomycin-D enhancement of lethality of pyrogen for mice deserve consideration in quality control procedures. The Limulus test, the most practical of the currently available alternative tests for detection of endotoxin and therefore, of pyrogen, has application where the rabbit test cannot be used. Therefore, control personnel must learn of the availability, performance and interpretations of the Limulus test.

REFERENCES

1. Kahn, R. L. (1942). Serology in Syphilis Control. Williams & Wilkins Co., Baltimore, Md.

2. Holland, W. W. and Whitehead, T. P. (1974). Value of new laboratory tests in diagnosis. Lancet 2:391-394.

3. United States Pharmacopeia XIX. (1975). Mack Publishing Co., Easton, Pa.; p. 613.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

4. Martin, W. J. and Marcus, S. (1964). Studies on bacterial

pyrogenicity. III. Specificity of the USP rabbit pyrogen test. Appl. Microbiol. 12:483-486.

5. British Pharmacopoeia. (1968). Pharmaceutical Press, London.

6. Dare, J. G. and Mogey, G. A. (1954). Rabbit responses to human threshold doses of bacterial pyrogen. J. Pharm. Pharmacol. 6:325-332.

7. Greisman, S. D. and Hornick, R. B. (1969). Comparative pyrogenic reactivity of rabbit and man to bacterial endotoxin. Proc. Soc. Exp. Biol. Med. 131:1154-1158.

8. Personeus, G. R. (1973). Pyrogen testing of parenteral pharmaceuticals. In Quality Control in the Pharmaceutical Industry. M. Cooper, Ed. Academic Press, New York; pp. 239-268.

9. Nelson, J. R. (1973). Thesis, University of Utah, Salt Lake City, Utah.

10. Berry, J. L. (1964). Effect of endotoxins on the level of selected enzymes and metabolites. In Bacterial Endotoxins. M. Landy and W. Braun, Eds. Rutgers University Press, New Brunswick, N. J.; pp. 151-159.

11. Pieroni, R. E., Broderick, E. J., Bundeally, A., and Levine, L. (1970). A simple method for the quantitation of submicrogram amounts of bacterial endotoxin. Proc. Soc. Exp. Biol. Med. 133:790-794.

12. Dowling, J. N. and Feldman, H. A. (1970). Quantitative biological assay of bacterial endotoxins. Proc. Soc. Exp. Biol. Med. 134:861-864.

13. Thomas, L. (1956). Role of epinephrine in reactions produced by endotoxins of gram-negative bacteria. I. Hemorrhagic necrosis produced by epinephrine in skin of endotoxin-treated rabbits. J. Exp. Med. 104:856-880.

14. Milner, K. C. and Finckelstein, R. A. (1966). Bioassay of endotoxin: correlation between pyrogenicity for rabbits and lethality for chick embryos. J. Infect. Dis. 116:529-536.

15. Marcus, S., Anselmo, C., and Luke, J. (1960). Studies on bacterial pyrogenicity. II. A bacteriological test for pyrogens in parenteral solutions. J. Amer. Pharm. Assoc., Sci. Ed. 49:616-619.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

16. Marcus, S. (1964). Determination of pyrogens using membrane filtration. Bull Parenter. Drug Assoc. 18:17-24.

17. Marcus, S., Anselmo, C., and Perkins, J. J. (1958). Studies on bacterial pyrogenicity. I. Quantitative basis. Proc. Soc. Exp. Biol. Med. 99:359-362.

18. Kereluk, K. (1975). Quality control in sterilization procedures: biological indicators. In Quality Control in Microbiology. J. E. Prier, J. T. Bartola, and H. Friedman, Eds. University Park Press, Baltimore, Md.; pp. 25-39.

19. Park, B. H., Fikrig, S. M., and Smithwick, E. M. (1968). Infection and the nitroblue-tetrazolium reduction test. Lancet 2:532-537.

20. Levin, J. and Bang, F. B. (1964). A description of cellular coagulation in the Limulus. Bull. Johns Hopkins Hosp. 115:337-345.

21. Levin, J. (1967). Blood coagulation and endotoxin in invertebrates. Fed. Proc. 26:1707-1712.

22. Bang, F. B. (1956). A bacterial disease of Limulus polyphemus. Bull. Johns Hopkins Hosp. 98:325-351.

23. Co Tui, F. W. and Schrift, M. H. (1942). Production of pyrogen by some bacteria. J. Lab. Clin. Med. 27:569-575.

24. Sullivan, J. D. and Watson, S. W. (1974). Factors affecting the sensitivity of Limulus lysate. Appl. Microbiol. 28:1023-1026.

25. Whittet, T. D. (1958). Thesis, University of London, London, England.

26. Wildfeuer, A., Heymer, B., Schleifer, K. H., and Haferkamp, O. (1974). Investigations on the specificity of the Limulus test for the detection of endotoxin. Appl. Microbiol. 28:867-871.

27. Levin, J. and Bang, F. B. (1964). The role of endotoxin in the extracellular coagulation of Limulus blood. Bull. Johns Hopkins Hosp. 115:265-274.

28. Levin, J. and Bang, F. B. (1968). Clottable protein in Limulus: its localization and kinetics of its coagulation by endotoxin. Thromb. Diath. Haemorrh. 19:186-197.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

29. Reinhold, R. B. and Fine, J. (1971). A technique for quantitative measurement of endotoxin in human plasma. Proc. Soc. Exp. Biol. Med. 137:334-340.

30. Rojas-Corona, R. R., Skarnes, R., Tamakuma, S., and Fine, J. (1969). The Limulus coagulation test for endotoxin. A comparison with other assay methods. Proc. Soc. Exp. Biol. Med. 132:599-601.

31. Elin, R. J. and Wolff, S. M. (1973). Nonspecificity of the Limulus Amebocyte Lysate Test: positive reaction with polynucleotides and proteins. J. Infect. Dis. 128:349-352.

32. Niwa, M., Hiramatsu, T., and Waguri, O. (1974). The gelation reaction between horseshoe crab amebocytes and endotoxins studied by the quantitative clot protein method. Jap. J. Med. Sci. Biol. 27:108-111.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

CURRENT DEVELOPMENTS IN THE TOXICOLOGICAL ASPECTS OF ETHYLENE OXIDE STERILIZATION

Robert J. Fredericks

Ethicon, Inc.
Somerville, New Jersey 08876

INTRODUCTION

The Ethylene Oxide (EtO) Subcommittee of the Association for the Advancement of Medical Instrumentation (AAMI), formerly known as the Z-79 Committee of the American National Standards Institute (ANSI), instituted biological and chemical research in early 1973 to determine safe limits for EtO and its reaction products, 2-chloroethanol and ethylene glycol, in medical products. This research is being carried out in collaboration with the Bureau of Medical Devices and Diagnostic Products of the Food and Drug Administration (FDA). It is the purpose of this paper to report on the progress of this research. The biological research will be covered first, followed by the chemical research.

BIOLOGICAL STUDIES

In general, the biological studies have been designed to simulate, as far as possible, conditions occurring in actual medical practice. Devices or portions of devices were subjected to EtO sterilization such that different levels of EtO and its reaction products, 2-chloroethanol and ethylene glycol, were introduced. The devices were then implanted in test animals, or placed in contact with human blood or with the human body, and the effects were observed. Four biological studies were designed and are being carried out: subcutaneous implantation, hemolysis, human skin irritation and mucosal irritation. A final report on the mucosal studies conducted by John Stetson of Strong Memorial Hospital, University of Rochester, has not yet been received. The results of this study, therefore, will not be included in this presentation.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

The subcutaneous implantation and the human skin irritation studies are being carried out with funds provided by private industry; the hemolysis and mucosal studies are being funded by the FDA.

Subcutaneous Implantation Studies

The subcutaneous implantation studies are being pursued by Frederick Becker [1] of the Department of Pathology, New York University School of Medicine. Phase I of these studies has been completed.

Dr. Becker's studies involved subcutaneous implantation of polyvinyl chloride (PVC) tubing containing different amounts of EtO. Tubing containing EtO, which ranged in concentration from 140 ppm to 2,860 ppm, was implanted in 30 mice. Twenty-four or 48 hours after implantation, the animals were sacrificed and the tissue in the area of the implant was examined microscopically. Becker [1] summarizes his results as follows: "No significant tissue damage was induced in mice receiving tubing which contained from 140 ppm to 885 ppm. Between 1,226 ppm and 2,164 ppm, there was a slight increase in tissue damage with the test tubing when compared to control tissues. Detailed analysis demonstrated that only four of seventeen mice in this range demonstrated tissue damage which was greater on one side (of the mouse) than another. Three of these were associated with EtO tubing and one with a control. Clearly, this result cannot be construed as significant tissue damage by EtO tubing. Concentrations of EtO equal to or greater than 2,337 ppm produced significant tissue damage in a large number of cases when compared with control tubing.

"It is concluded from this study that concentrations of EtO in the range of 885 ppm or less produced no significant tissue damage to the subcutaneous and dermal tissues when examined at 24 and 48 hours by the modalities utilized in this study. Concentrations of EtO of 2,337 ppm or greater produced tissue damage in a significant number of instances. Concentrations in the range between 885 ppm and 2,164 ppm fall into a 'grey zone' wherein the likelihood of tissue damage is small but cannot be unequivocally ignored."

It should be noted that the concentrations of 2-chloroethanol and ethylene glycol were negligible in the PVC tubing.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Based on these results, the AAMI EtO Subcommittee made the following recommendation to the Commissioner of the FDA in November, 1974: "The Ad Hoc Committee recommends a maximum safe limit of residual ethylene oxide of 250 parts per million in implantable plastic devices. If the level is higher than this in an implantable device, then the manufacturer should demonstrate, through appropriate tests, that the product is safe."

Examples of implantable plastic devices are pacemakers, valves, sutures, and prostheses. Catheters, endotracheal tubes or renal dialysis equipment are not included in the category of implantable plastic devices.

The AAMI EtO Subcommittee considered this recommendation to the Commissioner to be conservative. The recommended maximum safe limit was some ten times less than the level at which significant tissue damage occurred and some five times less than the threshold of the "grey zone".

Phase II of the subcutaneous implantation studies is being developed. These studies will be concerned with a possible "size effect" in implants. The question which will be asked is whether a device such as a suture, weighing perhaps 100 mg and a breast implant weighing more than 100 g, each containing 250 ppm of EtO, will have the same effect on the body.

Human Skin Irritation Studies

The human skin irritation studies are being conducted by Jerome L. Shupack of the Skin and Cancer Unit of New York University Medical Center. Phase I of these studies has been completed. Four different materials are included: a thick, slow-airing patch of PVC applied with occlusive tape on the backs of two patients and semiocclusive tape on the backs of ten patients, a "worst case" situation; a thin patch of PVC applied with semiocclusive tape; brown-milled rubber; and nonwoven fabric. The materials were sterilized with EtO in such manner that different levels of this compound and its reaction products were incorporated into the materials. Analyses for EtO, 2-chloroethanol and ethylene glycol were conducted prior to the application of the patches. Twelve Caucasian volunteers were involved in the study. The patches and control samples of the four materials were applied to the backs of the volunteers for one, two, four, and eight hours. At the end of

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

each time interval, the patches were removed and the backs of the volunteers were clinically examined. When erythema and edema were observed, the experiment was terminated. The backs of the patients were examined daily until any irritation disappeared.

No positive reaction was observed in any of the volunteers with the nonwoven fabric [2]. The concentration of EtO in this material ranged from 996 to 5,104 ppm. In only one of twelve volunteers was there a positive reaction from the brown-milled rubber. The reaction was scored as a trace of irritation after one, two, and four hours of exposure and moderate erythema after eight hours of exposure. At 24 hours, the irritation had disappeared. The initial concentration of EtO was 2,343 ppm. With the thin patch of PVC, positive reactions were observed in four of the twelve volunteers. In one of the four cases, the reactions were serious enough after eight hours of exposure to terminate the experiment. The erythema and edema had disappeared five hours after removal of the patch. The initial concentration of EtO was 1,825 ppm. The other three cases ranged from severe erythema to a trace of erythema. In none of these three cases was the reaction serious enough to terminate the experiment.

In the case of the thick, slow-airing patch of PVC, positive reactions were observed in eleven out of twelve volunteers. The reactions were sufficiently severe to result in termination of the experiments in five cases after about eight hours of exposure. The backs of all but one of the volunteers had returned to normal after 24 hours. In this one case, six days were required for the volunteer’s back to return to normal. One of the volunteers experienced a delayed response in the area of his back where the thick, slow-airing patch of PVC had been placed. This occurred with two separate patches of the PVC containing initial EtO concentrations of 1,484 and 1,800 ppm. After 48 hours, the volunteer’s back, which had showed moderate erythema, had returned to normal and remained that way until the normal completion of the experiment. Two weeks after removal of the patches, the volunteer developed edema with an eczematous scale on the surface of the skin. This condition persisted for another ten days. This may be a type of sensitization to EtO.

Experiments are being developed to explore further the question of sensitivity. In addition, experiments are being developed to
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.
evaluate the effect of very high concentrations of ethylene glycol

in nonwoven fabric. In the experiments performed to date, the concentration of 2-chloroethanol and ethylene glycol was negligible in the PVC and ranged from 100 to 1,000 ppm in the brown-milled rubber and the nonwoven fabric.

Hemolysis Studies

The hemolysis studies were conducted by Alan B. Jones [3] of the Department of Pharmaceutics, School of Pharmacy, University of Mississippi. The hemolysis studies, which have been completed, consisted of two parts. The first part involved direct contact of EtO with blood and the second part, the contact of blood with natural rubber, silicone rubber, PVC tubing and cuprophane sheet, each of which had been sterilized with EtO. In both cases fresh human blood from male Caucasian donors was used. Hemolysis was determined from the degree of cell lysis.

In part one of the study, the ethylene oxide remained in contact with the blood for four hours at 37°C. The percent hemolysis was determined from a spectrophotometric examination of the blood. Hemolysis was not observed below an initial concentration of ethylene oxide of 2,000 ppm. Hemolysis occurred above an initial concentration of EtO of 2,000 ppm and increased with EtO content until a concentration of 5,000 ppm of EtO was reached. At this point, there was no increase in hemolysis with further increases in EtO concentration.

Part two of the study consisted of sterilizing the above mentioned materials with EtO and placing them, along with appropriate controls, in human blood for four hours at 37°C. Hemolysis was determined in the same manner as for part one of the experiment. In all cases, significant hemolysis, compared to control samples, was observed when the EtO concentration exceeded 2,000-3,000 ppm.

CHEMICAL STUDIES

It follows that, if safe limits for EtO in medical products are to be determined, one must be able to measure the EtO accurately and reproducibly. The AAMI EtO Subcommittee has carried out analytical chemical research, the purpose of which was to ascertain if any of the four commonly used analytical methods [4,

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

5, 6, 7] could be used as referee methods for the determination of EtO. In other words, if one had an "in-house" procedure for the determination of EtO, could another method – a reference method – be used to validate the "in-house" procedure. A round robin was conducted involving six laboratories in which the above referenced analytical methods were evaluated. The results of the round robin [8] showed that the three methods involving gas chromatography gave quite similar results and could be used as referee methods. Andersen's gravimetric procedure [7] did not correlate with the gas chromatographic method. However, Andersen has shown [9], in work pursued in her laboratory, that the gravimetric method does indeed give comparable results to the gas chromatographic procedures. Based on this, the AAMI EtO Subcommittee has recommended that if the gravimetric procedure is to be used, it first be checked against one of the gas chromatographic methods. For the determination of 2-chloroethanol and ethylene glycol, the AAMI EtO Subcommittee recommends the method of Spitz and Weinberger [5].

REFERENCES

1. Becker, F. Personal communication.

2. Shupack, J. L. Personal communication.

3. Jones, A. B. (1975). Development and Testing of Protocol for Measuring the Hemolytic Effects of Residual Ethylene Oxide in Various Medical Products. Final Report, Control No. FDA-73-190, May 28.

4. Romano, S. J., Renner, J. A. and Leitner, P. M. (1973). Gas chromatographic determination of residual ethylene oxide by head space analysis. Anal. Chem. 45:2327-2330.

5. Spitz, H. D. and Weinberger, J. (1971). Determination of ethylene oxide, ethylene chlorohydrin and ethylene glycol by gas chromatography. J. Pharm. Sci. 60:271-273.

6. Mogenhan, J. A., Whitbourne, J. E. and Ernst, R. R. (1971). Determination of ethylene oxide in surgical materials by vacuum extraction and gas chromatography. J. Pharm. Sci. 60:222-224.

7. Andersen, S. R. (1971). Ethylene oxide toxicity. J. Lab. Clin. Med. 27:346-356.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

8. Romano, S. J. and Renner, J. A. (1975). Comparison of analytical methods for residual ethylene oxide analysis. J. Pharm. Sci. 64:1412-1417.

9. Andersen, S. R. Personal communication.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

DISCUSSION

Q. by A. Bishop:

I would like to ask one question about the skin studies. Is it proposed to look at only normal human subjects, or will people with any disorders also be studied?

A. by R. Fredericks:

At this point we intend to look only at normal skin – normal skin of male and female Caucasians.

Q. by R. Campbell:

Is there any intention to do time-related studies? Your exposures are very short at the moment.

A. by R. Fredericks:

We didn't really know what we were going to encounter, and we wanted to be quite certain that we were not going to injure anybody. That really was the reason for going to the one, two, four, and eight hours. It was considered to be the first phase of our work on twelve volunteers and then we were going to take it from there. Quite frankly, we did not expect to run into this apparent problem of sensitization, so I think the point is a valid one. Another thing, of course, that we hoped to achieve in the first phase was to get a little more information about the effect of 2-chloroethanol and ethylene glycol. That did not really develop. In some cases we got up to about 600 parts/million of ethylene glycol. The 2-chloroethanol levels were very, very low. That was another thing that we had hoped to accomplish. But, we are taking it one step at a time.

Q. by C. Phillips:

In the publication which came out last November, to which Dr. Bruch referred, is that a recommended tolerable level of ethylene oxide residuals? Is this a product of your committee or did Food and Drug just do that unilaterally?

A. by C. Bruch:

It was not done unilaterally. We had an internal FDA interbureau committee which met for a year and a half looking at data of the
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.
past fifteen years, and it came out with seven or eight

recommendations. One of the recommendations was for a million dollars worth of more research, covering five or six areas. They raised the issue of mutagenicity, which we are all aware of for ethylene oxide, but people keep saying that if it is mutagenic, there must be a carcinogenicity test that we can devise to show that it is carcinogenic. The other one was a chronic IV toxicity test, as it may relate to renal dialysis, or hemodialysis. The figures which were given in the Journal of the American Hospital Association for November 16, 1975, in terms of implantable devices, is a "worst case" situation based on hemodialysis. Dr. Fredericks made the point that in terms of a suture, the level could be much higher. I agree with that wholeheartedly. Likewise, in the case of a mammary prosthesis, some that I have seen weigh much more than 100 grams. There could be a sizeable amount of ethylene oxide present, therefore, even at 250 parts/million. The tendency, therefore, has been to look at the acute response. We have to keep looking at those situations where the response might not be acute, but subacute or chronic. This is the reason for the figures in the hospital document.

Comment by S. Marcus:

Dr. Fredericks, you mentioned that one of the volunteers showed a delayed reaction, a reaction which recurred after you had carried out the test. You pointed out that this was one problem that you faced. It seems to me that part of your testing protocol in the future will have to include people who are allergic, with a known history of allergies - and ten percent of our population is said to be so - in order to determine whether you will get a lot of this recurrent reaction in that part of the population. This is a population which is sharply distinct from people who are not allergic.

Comment by R. Fredericks:

I think the point is certainly well taken. I do not know, at this point, that we are thinking about trying to determine how many people have this problem. Practical matters enter into this study. It is expensive to do this work. What we want to do in Phase 2 of this study is to definitely establish that we do, indeed, have a problem, in this case of sensitization and a delayed reaction, and get that into the medical literature so that it is available and well documented.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Comment by J. Willson:

I think this whole question of potential sensitization caused by ethylene oxide is very much up in the air at the present time. In the literature, there are reports of people indicating that this occurred in the past. However, when you study the reports, it does not really appear to be classic sensitization in the way that we all learned it. At least some of the reactions that I am familiar with are more a delayed response to a primary irritant. Whether there are new mechanisms occurring here, I do not know. I think it is premature to just label it as "sensitization" or "cutaneous delayed hypersensitivity". I think it is a little early to classify it as such. I think it demands more study before we label it as such. There is a distinct possibility that whatever response is taking place in this individual could be due to some chemical reaction that took place between the ethylene oxide and some constituent of the particular polyvinylchloride used.

Comment by R. Fredericks:

This is an excellent point and I should have mentioned it. With this particular individual, and for that matter with the other patients, we saw no reaction with the nonwoven material and the rubber. With this particular individual, the sensitization or delayed reaction occurred solely with the thick, slow-airing PVC patch. As John pointed out, it could be the ethylene oxide, although one would think that if this were the case, it should have occurred also with the rubber and the nonwoven. It could be caused by some kind of an interaction of the ethylene oxide and the PVC. This is one of the points that we will try to evaluate in Phase 2 when we attempt to thoroughly document this particular case and get it into the literature.

Q. by L. Kallings:

May I ask Dr. Fredericks if studies of the effect on the endothelium of the blood vessels has been on the program? Intravenous catheters are frequently used and irritation of the endothelium is creating a great clinical problem. This irritation is believed to be due to the drugs and infusions injected through the catheters. But, of course, irritation through the sterilization process should be ruled out. Did you discuss that?

A. by R. Fredericks:

We have not gone into that at this point. The mucosal studies

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

that Dr. Stetson is conducting employ endotracheal tubes and catheters. We have not done anything with intravenous catheters. It is one of the things that we have considered.

Q. by R. Jerussi:

Is there any regulatory body in the world that uses the lysate test as a release test?

A. by S. Marcus:

As far as I know, it is not legal anywhere in the world. We do use it, and people do use the Limulus test at the present time, particularly with the radioisotopes. Apparently, the Food and Drug Administration is going along with this. Nobody has brought any legal action against anyone that I know of for the misuse of this test at the present time. This is not to say that it may not occur tomorrow.

Comment by R. Van Essche:

I am from Europe, working for an American company there in regulatory affairs. I might comment that some of the governments over there, the Belgium government for example, accepts the Limulus test as a sublot test - not the final test. You are allowed to test in process and final test with a Limulus test, but a statistical sampling must be made, and a rabbit test made on some samples of the lot.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

REGULATORY REVIEW OF STERILIZATION CONTROL OF DRUGS IN THE UNITED STATES

Mary K. Bruch

Bureau of Drugs
U. S. Food and Drug Administration
Rockville, Maryland

The development of standards for sterilization and disinfection and review of sterilization procedures for human drug products are the responsibility of the Bureau of Drugs.

Judgment of the sterilization process and analysis of the means of testing for sterility in the Bureau occur in two ways:

1) As a review process, actually part of an Investigational New Drug Exemption (IND) or New Drug Application (NDA).

2) As a requirement of Current Good Manufacturing Practice (CGMP) (21CFR, Parts 210 and 211) by a manufacturer.

I would like to review some current thinking, problems and requirements in both of these areas.

During the review of a specific drug, the sterilization and/or disinfection procedures are reviewed for safety and effectiveness. Many times this involves simply a straight-forward steam sterilization process. However, with the evaluation of new drug materials such as bone cement, contact lens polymers, suture materials, shorter-term processing for large volume parenterals (LVP), including the specific proposed CGMPs for large volume parenterals, and the requirements for sterile ophthalmic drugs, neither the review nor the processes under review have remained simple. Let us look more closely at some of these developments.

Sterilization of some new packaging materials and drug entities such as bone cement, chymopapain, and contact lens polymers have challenged conventional cycles and procedures. For example, a new

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

lens polymer Tenite (cellulose acetate butyrate) cannot be steam or dry heat sterilized and in all likelihood would be damaged by radiation sterilization. Attempts to use ethylene oxide have revealed lens surface and moisture problems. The actual sterilization problems are complicated by the normal demands of the industry. A system allowing the processing of a few lenses at a time before shipment but without exorbitant cost or time utilization (outgassing time if ethylene oxide is used) would be ideal.

Sterilization with ethylene oxide has become one means chosen to provide sterile drug products. The Bureau of Drugs participated in the recent Agency-wide review of the safety and effectiveness of ethylene oxide sterilization [1].

The effectiveness of the process, provided that it is monitored for chemical, physical and biological parameters, is well established [2, 3, 4, 5]. Perhaps with the exception of possible entrapment of organisms or spores within a crystalline structure or the use of impenetrable or inappropriate packaging materials, a properly monitored procedure is considered effective. The reverse of this conclusion, however, is that as the variety of drugs and materials exposed to ethylene oxide increases, so does the attention devoted to residuals and their toxicity.

The results of the reports of the various participating Bureaus were published in the FDA report to the Commissioner. This report outlines the Agency's current policies concerning the safety and effectiveness of ethylene oxide sterilization. Although usage is controlled by FDA, it varies widely from Bureau-to-Bureau because of markedly different requirements under the law and the different products handled:

> "Bureau of Drugs: Controls investigational and new drugs under section 505 of the Federal Food, Drug, and Cosmetic Act. Antibiotics and insulin are controlled under section 506 and 507 of the Act. Drugs other than these are to be manufactured under Current Good Manufacturing Practice.
>
> The United States Pharmacopeia and the National Formulary in their 1975 Official Compendia and the British Pharmacopoeia (1973) have listed EtO under Methods of Sterilization. This is classified as general information and contains no standards, tests, assays nor other mandatory specifications with respect

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

to any Pharmacopeial article.

Limits are now being proposed for ophthalmic products." (FDA report to Commission on Ethylene Oxide Sterilization).

The problem of allowable residue limits is discussed and the conclusions are probably well known to most of you. Essentially, the recommended interim limits for ethylene oxide sterilization are as follows:

Ophthalmic Drug Formulations

Ethylene oxide not to exceed	50 ppm
2-Chloroethanol not to exceed	250 ppm
Ethylene glycol not to exceed	1000 ppm

Radiation sterilization has not been widely applied to the sterilization of drug materials. Some firms have made application to sterilize the drug entities with radiation. Of course, some containers for drugs (tubes and syringes) are radiation sterilized. An ophthalmic ointment has been approved utilizing radiation sterilization (2.5 Mrad). A regulation (21 CFR, 200.3, formerly 21 CFR, 3.45) in the Code of Federal Regulations addresses this subject as follows:

"There is a current interest in the utilization of newly developed sources of radiation for the sterilization of drugs. Prior to the marketing of a drug sterilized by such means, it is necessary in the interest of protecting the public health to establish by adequate investigations that the irradiation treatment does not cause the drug to become unsafe or otherwise unsuitable for use. Accordingly, all drug products, including injections, ophthalmic solutions, surgical sutures, and surgical dressings sterilized by means of irradiation are regarded as new drugs within the meaning of section 201 (p) of the Federal Food, Drug, and Cosmetic Act. An effective new drug application pursuant to section 505 of the Act is therefore a prerequisite to interstate shipment of such articles, except as provided by section 505 (i)."

Of course if radiation is to be used for a drug material which is already the subject of an NDA, effectiveness and safety are thereby reviewed as a Supplement to an already existing NDA. For
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.
drugs not already the subject of an NDA, an application is

required.

The time may be right to reassess the applicability of a blanket requirement such as this one requiring a new drug application solely because radiation sterilization is employed. Standards for assessing the effectiveness of radiation sterilization and its effect on the sterilized drug or material have been widely reviewed. I would cite White's review in Industrial Sterilization [6]. Certainly as our knowledge concerning the effect of radiation on widely used materials accumulates, these products could be cleared for use after radiation sterilization, provided there is no immediate or long-term change in them, or production or accumulation of toxic residues. New drug entities, as they are developed, of course, would still need to be investigated if radiation is proposed as the sterilization procedure.

Guidelines have recently been developed for the sterilization of intraocular lenses and new contact lenses other than the conventional lenses made of polymethylmethacrylate. These guidelines are also available from the Hearing Clerk, 5600 Fishers Lane, Rockville, Maryland 20852.

These newly revised guidelines establish a principle of microbiological evaluation of the contribution made to disinfection of each step in a multiple-step process. D-value determination for active chemical disinfectants in any proposed disinfectant procedure is a critical element. An accusation has been made in a comment on the guidelines that FDA is "obsessed with D-values". However, other scientists involved in the regulation of contact lens solutions, especially in other countries, have praised the use of these calculations and believe this is a correct and farsighted approach. The initial data from D-value determinations with chemical disinfectants are suprisingly reproducible and show conformity to expected killing data.

The guidelines also contain an important alteration in the USP preservative test to be applied to contact lens solutions and other preserved products. An organic load and a rechallenge at 14 days have been added to the standard test. This testing is critically important since these products are used daily and contamination of any product to be employed in the eye may produce severe consequences.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

The guidelines for new contact lenses concentrate on testing procedures for proposed disinfection systems for lenses whereas the guidelines for intraocular lenses deal with sterilization and verification of sterilization procedures within a given limit of probability.

Some interesting sterilization and disinfection problems have been created by the development of polymeric materials, and especially hydrophilic polymers which can be fashioned into contact lenses for either intermittent or permanent wear, and by the increased surgical insertion of intraocular lenses into the eye.

Some polymers can be easily steam sterilized (hydroxyethylmethacrylate) while other materials such as Tenite cannot be.

Ethylene oxide may be effective temporarily but it is obvious that other alternatives will have to be found. Ethylene oxide or some other chemical means will be necessary; heat appears to be too destructive to the materials, and radiation will also probably cause alterations in the material or its durability.

The requirements for the development of effective disinfection procedures for daily use by patients wearing contact lenses have meant increasing emphasis on disinfection criteria and preservative testing. There is evidence that some marketed solutions do not withstand microbial challenge testing [7]. In this study, about half the tested solutions did not fulfill the test criteria. The Guidelines and Criteria for the Microbiological Testing of New Contact Lenses (other than conventional polymethylmethacrylate) outline the testing procedures for initial sterilization of the lens, disinfection procedures for patient use, and preservative testing for multiple use solutions.

Intraocular lenses have been something of a controversial item at FDA. For those of you who may not be familiar with them, they are small lenses usually inserted in the pupillary space as a replacement lens after cataract surgery. Implantation often has dramatic effects for the patient. Development of new designs and increases in the number of implant surgical procedures performed have caused increased attention to these lenses. Their regulation as devices has been essentially self-regulation by the industry

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

and will remain so until either new device legislation is passed or they are regulated as drugs. Currently the surgeon must rely on the word of the manufacturer as to the safety and sterility of the lens material. Regulation as drugs has been proposed in the Federal Register (March 13, 1975). A disastrous incident has pinpointed the need for effective regulation. Eleven postoperative infections with five enucleations occurred after contaminated neutralizing solution was used as part of the lens sterilization procedure. The infecting organism was Paecilomyces lilacinus.

Guidelines for clinical testing, toxicity testing, and sterilization of intraocular lenses have been proposed and reviewed by the Ophthalmic Devices Advisory Committee. These intraocular lens guidelines are framed as they are because the sterilization procedure which had been utilized prior to the proposed regulatory control of these implants as drugs would now be considered uncertain by most of us here. This procedure is termed the Ridley procedure of sterilization [8]. I think this demonstrates the consequences which occur when a sterilization procedure is developed by an individual who innovates a drug or material rather than by an expert in disinfection and sterilization within a drug company, or one who is retained as consultant.

This procedure was to soak lenses in 10 percent sodium hydroxide for one hour, to transfer the lens to 0.1 percent sodium hydroxide for storage and/or shipment and subsequently, on use in the operating room, to neutralize the 0.1 percent sodium hydroxide immediately prior to insertion of the lens. I think we would all agree that this chemical sterilization procedure is difficult and questionable at best, even if the intervening, superimposed handling procedures are ignored.

The guidelines for sterilization of intraocular lenses describe an interesting trade-off approach, really something of a reward and punishment structure.

The final sterilization process is dependent on rather specially prepared spore indicators (biological indicators). These indicators are tailored to the sterilization procedure, the material, the level of probability of sterility, and the initial contaminating load on the specific product being sterilized. Thus there is a reward for good or excellent CGMP. It operates as

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

follows:

It is the basic responsibility of the manufacturer/distributor of the lens in interstate commerce to provide the following data for sterility assurance of intraocular lenses:

1) D-values for the resistance of one or more organisms as specified for the particular sterilant in this guideline.

2) A pragmatic test of the finished product that incorporates one of the two options for use of biological indicators as specified on page 712 of USP XIX (1975).

If the distributor of the lens allows resterilization of his product by the hospital, he must provide the hospital with procedures that, when measured by the two criteria cited above, will provide a sterile lens with a probability of a survivor of less than 10^{-6}. The procedures provided to hospitals for resterilization of the lenses will be subjected to verification by FDA in-house laboratories. All data on the adequacy of the sterilization procedures must be filed with the FDA prior to the undertaking of any clinical trials with the intraocular lenses.

The resistance of microorganisms comprising the natural contamination of the lens is determined by subprocess treatment, and the use of the initial level of contamination and the fraction of positive lenses after subprocess treatment are used to calculate a D-value.

These environmental contaminants may or may not be more resistant than the selected indicator [9] for the specified sterilization procedure, but in many cases both the numbers and resistance may be lower. The calculation of the process time must show that there is a probability of a survivor of less than 10^{-6}.

Subprocessing exposure to the sterilization procedure is also performed using the biological indicator organisms (not more than 85 percent nor less than 15 percent of the organisms should survive).

Twenty lenses are then finally exposed to a full sterilization cycle where the level on the biological indicator has been preselected by estimation of the number and resistance of naturally occurring contaminants. All 20 inoculated lenses must be

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

shown to be sterile by a USP XIX sterility test procedure. This concept is fascinating since here we have guidelines for a sterilization procedure which consider the natural loading on the product to be sterilized, which in turn may be the consequence of the implementation of good manufacturing procedures.

The basic concept of testing multi-items as a basis for establishing a level of probability of a survivor is applied to proposed multistep disinfection procedures for new contact lenses and is considered a practical test of an artificially contaminated series of lenses to determine if a specified procedure does, in fact, produce a disinfected lens for patient use.

Let us now move on to a discussion of CGMP and the importance of sterilization procedures in CGMP to the manufacturer and to the Bureau of Drugs.

Proposed new GMP regulations were published in the Federal Register on February 13, 1976. I would need as much time again to discuss these, but would nevertheless like to review some points concerning sterile products and verification of sterilization procedures and cycles. In recent months, I have had some rather interesting inquiries from several manufacturers concerning sterilization. The questions were something like this:

1) What is your view of placement of spore strips in an ethylene oxide sterilizer? How many? When should they be assayed?

2) What would you consider adequate verification of a sterilization cycle?

3) If processing a product which is sterilized and filled under aseptic conditions, should resistant spores (Bacillus stearothermophilus) be added to the processing vat and cycled to assure sterility?

As you might guess, these questions were stimulated in varying degrees by inspectors' questions to manufacturers or by FDA District Offices' interpretations of inspectors' reports. I would like to review with you what I consider appropriate answers to these questions.

Everyone resists change and has a tendency to remain with procedures which have been successful over the years. There are

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

changes in the proposed GMPs which will require some manufacturers to alter their current procedures. In the initial stages, this may appear to be a mixed blessing.

Biological indicators selected and manufactured with a particular resistant organism should be appropriate for the type of sterilization procedure being considered. When used in processing a sterilizer load of products, such indicators can assure the sterility of the load within determined probability limits. The USP Conference on Biological Indicators [10], quoted Dr. Pflug as saying that "he considered biological indicators as a quality control tool that comprised a calibrated system - not merely the spores themselves - by which the sterility of materials can be accepted or rejected with confidence. Measured against the design criteria of the sterilization procedure, they give a direct and reliable indication of its effectiveness, since they are influenced by all unknown as well as known factors affecting sterility." I agree with this position.

It is unnecessary to detail the theory supporting the use of biological indicators since there are numerous published articles which have done this better than I could. The basic concept which is critically important for all individuals involved in manufacturing, processing, or testing sterile products is that when there are no survivors after exposure to a sterilizing process, sterility is a probability estimation or function; thus, it can be said that depending on the number of cells (spores) constituting the indicator, there is a probability of a survivor of from 1 in 10^6 to 1 in 10^9. These limits are normally chosen both because they can reasonably be met and can also allow significant safety factors for the consumer. Steam and dry heat procedures normally meet the standard 1 in 10^9, while ethylene oxide is usually judged at a probability level of 1 in 10^6. An estimation for sterilization of product by filtration is usually around 1 in 10^3, while for radiation, the standard can be and is set at different levels up to 1 in 10^9.

The biological indicator acts as a safety precaution itself. When modern science provides gauges, valves and indicators to record the parameters of a given cycle, the biological indicator may appear unnecessary, but this biological system acts to integrate the factors necessary in a specified sterilization cycle. Thereby, assurance is provided, for instance, in an

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

ethylene oxide cycle, that moisture and gas penetration, and concentration and temperature were sufficiently controlled long enough to achieve sterilization. Both the measurement of temperature, relative humidity, and gas concentration over time verify this. When properly cultured, the biological indicator also verifies that the product can be considered sterile within the limit of probability defined by the indicator and the cycle.

Cycles for industrial use evolve after consideration of multivariate factors such as product, flow of the product, sterilization efficiency and the facilities available. In fact, a charted, integral process is required to provide sufficient sterile products demanded by a manufacturer's varying needs.

Now we turn to the question of verification that a given cycle does what it is designed to do. Constant monitoring and the use of indicators provide assurance of the production of sterile products. Many firms make highly specialized products or multiple products often requiring the interaction of several disinfection and sterilization procedures. The area everyone tends to neglect is the sterile fill and filtration processes. I think perhaps this is so because we are forced to back away from the 1 in 10^6 or 10^9 level and come down to about 1 in 10^3.

The proposed new CGMP states that sterilization cycles should be verified but does not say how. Certainly more than one procedure will be acceptable and certainly biological indicators must be a part of verification. I regard some exaggeration of the normal processing cycle challenge an adequate way to verify a cycle. A challenge of 10 to 100 times the level in the biological indicator stresses the cycle for the process for which it is designed.

As for the possibility of adding resistant spores to large volume processing tanks, I would object. I think specialized procedures such as exaggerated challenging of a pilot scale tank or system would, with final processed ingredient or product testing, serve to verify the effectiveness of such a procedure.

When a multiple-step procedure is employed to produce a sterile product, the components must be examined individually. The probability of survivors will be at a certain level, for example, for the sterilization procedure for a container, and at another for the product or specific ingredient to be filled. These

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

conditional probabilities can be calculated. The element most difficult to control and evaluate is the finished product, and essentially, one must resort to finished product sterility testing. The perplexing problems come with the uncertainty arising when positive sterility tests occur and the producer believes that each step has been controlled, assured and verified.

Further evidence of the involvement and commitment of the Bureau of Drugs to defining sterilization process, controls and verification procedures is found in the drafted new regulations on LVP products. Extensive new proposed regulations have been drafted for the production of LVP drug products (for these purposes, terminally sterilized 100 ml or greater, single dose units for human use). These new regulations have taken time and effort to develop. They include a restriction on the sterilization cycle with the requirement to show equivalence or superiority if a cycle other than a recommended one is used. In brief, these regulations, as they will probably be proposed, give a specific sterilization cycle and specific manufacturing procedures. An F_0 of eight minutes is proposed for LVP sterilization cycles. Possible alternatives and requirements for compliance are specified:

1) If an effective NDA is held and the procedures therein are less than the specified cycle, the firm must comply.

2) Changes in specifications which do not provide increased assurance of quality control may not be implemented.

3) If unique characteristics of a product or process require lower standards than specified, then an NDA or a Supplement is required.

4) If a proposed or _de facto_ process is, according to standards, equal to or greater than the CGMP, then an NDA or Supplement is required detailing the differences.

The proposed substantial revisions of Good Manufacturing Practice were published February 13, 1976. As part of this proposal, the Commissioner announced his intention to propose CGMP requirements for specific types of drug products. Since LVPs are often administered to debilitated patients and are presented to the patient parenterally, the proposed sterilization procedures are published in explicit detail "to provide assurance that the methods and facilities used and the conditions of their production

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

are adequate in design and application to preclude microbiological and pyrogenic contamination of LVP products”.

This document emphasizes, as I believe all the FDA representatives here will, that “without standardized procedures of demonstrated reliability of production and sterilization processes, assurance of quality could only be obtained through full testing of every unit”. I sincerely believe this type of statement is the reason we are all here at this meeting.

The procedures for producing LVPs are meticulously detailed in these proposed regulations. They include: responsibilities of the quality control unit, personnel responsibilities in the controlled environment areas, design and construction features in buildings and facilities, environment of sterilization areas (compressed air, water, gowning facilities), equipment design and maintenance, control of components used in the LVPs, packaging and labeling control, records and reports, and control of air and water quality. The actual sterilization process is also described and the control procedures required to test it are described in the proposed regulations.

With increasing emphasis on product liability and the continuing alertness and interest of consumers, greater emphasis on production of safe and effective products is more and more the concern of all drug producing firms. Sterilization and disinfection procedures often constitute an integral part in production of a drug product.

Corporate responsibility and commitment, even personal responsibility of management, and quality control planning and responsibility must interact if a quality product is to be produced. As perfection in product quality is approached, there is a degree of perfection, the last step, which in some instances may not be worth the trade-off in either expense, complexity or time. Or, in FDA terms, the risk/benefit equation may not be favorable. I am certain all firms have planning charts and critical path charts varying in complexity and effectiveness designed to achieve these goals. There are really two ways of achieving sterile products: building sterility in by planning for it as I have discussed, or trying to accomplish sterility as an add-on and hoping it all turns out all right. The regulatory changes influencing this required planning must be known and must be

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

communicated to the industry. Interesting new ideas, new drug entities and advances in materials, such as we are seeing with intraocular and contact lenses, must somewhere in the early design and planning stages include examination of the applicability of the materials and procedures required, if these materials are to be disinfected or sterilized.

Those of us here who go back ten years in the regulatory business would, I think, have found my discussion today of the new CGMPs and the control and verification of sterilization cycles, somewhat esoteric then. The intricacies and requirements of the current proposed GMPs will necessitate excellent planning, quality control and expert technical knowledge and will, above all, require on the part of industry, competently trained employees and impeccably objective and experienced advisers.

REFERENCES

1. Report of the FDA Ethylene Oxide Review Committee. (May 30, 1975). Available from Hearing Clerk, 5600 Fishers Lane, Rockville, Maryland 20852.

2. Bruch, C. W. and Bruch, M. K. (1970). Gaseous disinfection. In Disinfection, M. Benarde, Ed., Marcel Dekker Inc., New York; pp. 149-206.

3. Ernst, R. R. (1973). Ethylene oxide gaseous sterilization. In Industrial Sterilization. G. B. Phillips and W. S. Miller, Eds., Duke University Press, Durham, N. C.; pp. 181-208.

4. Philips, C. R. (1968). Gaseous sterilization. In Disinfection, Sterilization, and Preservation, C. A. Lawrence and S. S. Block, Eds., Lea and Febiger, Philadelphia, Pa.; pp. 669-685.

5. Bruch, C. W. (1961). Gaseous sterilization. Ann. Rev. Microbiol. 15:245-262.

6. White, J. D. M. (1972). Biological control of industrial gamma radiation sterilization. In Industrial Sterilization, G. B. Phillips and W. S. Miller, Eds., Duke University Press, Durham, N. C.; pp. 101-116.

7. Norton, D. A., Davies, D. V. G., Richardson, N. E., Meakin, B. J., and Keall, A. (1974). The antimicrobial efficiencies of contact lens solutions. J. Pharm. Pharmacol. 26:841-846.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

8. Ridley, F. (1957). Safety requirements for acrylic implants. Brit. J. Ophthalmol. 41:359-367.

9. Puleo, J. R., Favero, M. S., Oxborrow, G. S., and Herring, C. M. (1975). Method for collecting naturally occurring airborne bacterial spores for determining their thermal resistance. Appl. Microbiol. 30:786-790.

10. Abstracts of Proceedings of the USP Open Conference on Biological Indicators for Sterility Assurance. Arlington, Virginia, August 5, 1970; p. 9.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

REGULATORY ALTERNATIVES FOR ASSURING STERILITY IN MEDICAL PRODUCTS

William C. Dierksheide

Bureau of Medical Devices and Diagnostic Products
U. S. Food and Drug Administration
Silver Spring, Maryland

The Food and Drug Administration is responsible for administering the Food, Drug and Cosmetic Act. One of the characteristics most clearly to be controlled by the legislation is sterility. The importance of sterility cannot be truly compared to other concerns. Because of its applicability to most medical products and food, freedom from microbial contamination has by tradition received special attention. To determine sterility, the microbiological assay found in the U. S. Pharmacopeia (USP) has been used. In fact, this is the test currently recognized by the courts for sterility assurance [1]. However, development of sterilization technology has made it clear that we must embark on a new path to truly provide the level of sterility assurance needed to protect the public, a level capable of being delivered by the industry. This presentation will explore, from one individual's vantage point, a number of approaches by which sterility of medical devices might be regulated.

Current authority resides in the adulteration (Section 501) and misbranding (Section 502) provisions of the Act. When the Medical Device Amendments are enacted, additional regulatory options will be provided under Standards (Section 514) and Good Manufacturing Practices (Section 520 f).

In addition to the possible alternatives for legal implementation of sterility assurance requirements, the discussion will include the selection mechanism which assigns regulatory control categories to medical devices, more appropriately termed "classification", and also the Agency's struggle to define the term "sterile".

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

The adulteration section deals directly with filth of a product, which may include microbial contamination, and sanitary conditions under which a product was prepared, packed or held. It seems possible, given the intent of this section, that the Agency might develop internal guidelines for specifying levels of sterility assurance for types of devices. The Regulations [2] of the Bureau of Biologics specify certain tests which must be performed on biologicals to determine whether or not a product is sterile. The methodology established in this statement has its basis in tests found in the USP. The Bureau of Foods, on the other hand, has defined "commercial sterility" for its products [3]. This requires the sterilization cycle to be designed to render a product free from microorganisms of public health significance. Two approaches have been used here. One relies upon the finished product test of the USP; the other emphasizes process control. These two strategies are particularly important when the definition of sterility is discussed later in this presentation.

The Agency is permitted by the misbranding provision to require certain product labeling which the FDA deems necessary to protect the public health. Because various sterility levels for types of devices seem to be a rational approach, it might be useful to require labeling for probability levels to which sterility is assured by the manufacturer. This approach would set no limits of sterility for devices.

The product would be required to be labeled as sterile and the probability that the product is sterile would be required on the labeling. For example, product X, an implant, would be required to be labeled as sterile; the manufacturer would guarantee sterilization to the 99.9999% probability level. There is no precedent for taking this approach, however.

Because present law does not provide adequate control of medical devices, Congress is considering the enactment of the 1976 Medical Device Amendments to the Food, Drug and Cosmetic Act [4]. This legislation would require the Agency to control medical devices based on requirements imposed under three regulatory categories: General Controls, Performance Standards, or Premarket Approval.

All devices would be regulated by the least restrictive category, General Controls, which includes provisions for labeling and good manufacturing practices. Devices not requiring more

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

stringent controls would be regulated by the General Controls category alone. If, for certain devices, General Controls were found to be inadequate and standards of performance could be developed, Performance Standards would be the appropriate regulatory category. In the event General Controls and Performance Standards would not suffice to assure safety and efficacy, devices which present a potential unreasonable risk to the patient would be regulated by the Premarket Approval category. Under Premarket Approval, data would have to be submitted demonstrating safety and efficacy for each device before marketing could be authorized. Regulation by Performance Standards and Premarket Approval would not necessarily be mutually exclusive. In some instances both regulatory categories might be applied to the same device but to different characteristics.

All implantable devices and all devices used in supporting, sustaining or preventing impairment to health are to be automatically classified into Premarket Approval. Removal from this category is dependent upon demonstrating that a less stringent category would assure their safe and effective performance.

As previously stated, all medical devices would be regulated through General Controls with exemptions for certain devices classified in General Controls alone. Exemptions may be granted for registration, good manufacturing practices, and record keeping and reporting.

The proposed amendments would offer at least two approaches for the control of device sterility, Standards and Good Manufacturing Practices (GMP). Recently, FDA staff explored the possibility of regulating devices in the Standards category by baseline standards [5]. Embodied in this concept is the development of a number of standards which are applicable to a wide variety of devices. For example, baseline standards are currently considered for electrical safety, electromagnetic interference and environmental stress. Sections from each baseline standard would be imposed where applicable. In addition to baseline standards, labeling would be required for characteristics for which a standard could not presently be developed.

Using a hypothetical example, sections from baseline standard A might be applied to a medical device, but baseline standard B

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

would not be applicable. At some future date, a device-specific standard might be developed. In the meantime, labeling of important performance characteristics would be required.

It seems reasonable to consider the establishment of baseline sterilization standards for various types of devices. Applicable sections of a sterility standard would be applied on a case-by-case basis depending upon the probabilistic level of sterility assurance necessary to assure safe and effective performance.

Good Manufacturing Practices offer another regulatory mechanism by which sterility of medical devices can be assured. GMPs are designed to oversee the manufacturing process so that safe and effective products are produced. FDA is now developing umbrella GMP regulations. Preliminary drafts have been made available on a number of occasions, and the Bureau conducted a series of seminars explaining the proposed drafts and soliciting constructive criticism. In addition, GMPs for special groups of medical devices are anticipated.

At a recent seminar sponsored by the FDA, it was stated that prospective regulations are being considered for sterilization processes [6]. These were originally part of the general regulations but were removed for various reasons. Sterility GMP regulations will more than likely include considerations for environmental conditions, monitoring of essential parameters of sterilization cycles, design of the sterilization process, and testing.

It has become clear to the Food and Drug Administration that a definition of the term “sterile” is necessary. An informal working group within the FDA has been working for the last several months to define the term “sterile” and has recently come to a conceptual agreement on the term [7]. Limitations were placed on the scope of the definition because of published Federal Register statements on sterility for biologicals and foods. Established legal precedents required the group to work within the bounds created by these statements.

“Sterile” is an absolute state wherein freedom from contamination of viable microorganisms is maintained; but technology is not available for absolutely guaranteeing freedom from microbial contamination. There is always a probability,

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

however small, of encountering a nonsterile product. For this reason, the proposed definition should include the absolute nature of the term and yet appreciate the limits of technology. This approach reflects the conflict between finished product testing and in-process control. For some medical products, finished product testing is an appropriate way in which to assure sterility. For other products, process control, developed from microbial death curves, is a far more effective way of control.

Moreover, the definition cannot rely on theoretical considerations of process control to the exclusion of actual product analysis when the latter is clearly indicated. That is, the determination and verification of sterilization cycles for very low probabilities of insterility use a number of assumptions with respect to the kinetics of microbial death and control of the sterilization process. On occasion, direct finished product analysis may detect higher rates of insterility than theoretically calculated. When this occurs, results from direct analyses should always take precedence over theoretical values.

In summary, this discussion has included a number of options available to the Agency for controlling sterility assurance in medical devices. Under existing authority, the FDA may consider requirements under the adulteration and misbranding provisions of the Act. When medical device amendments are enacted, additional options will become available. Any one or combination of approaches discussed might be implemented. Or, control of sterility assurance may continue as an evolutionary process whereby a more simple approach is taken initially with subsequent development of more complex requirements.

REFERENCES

1. Quality Control Reports. (Sept., 1975). The “Gold Sheet”. Washington, D.C.

2. Code of Federal Regulations. (1974). Sterility. Title 21, Chapter I, Part 610.12. U. S. Government Printing Office, Washington, D.C.

3. Code of Federal Regulations. (1974). Thermally processed low-acid foods packaged in hermetically sealed containers. Title 21, Chapter I, Part 128b. U.S. Government Printing Office,

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Washington, D.C.

4. Report No. 94-853, U. S. House of Representatives. (1976). Medical device amendments of 1976: A report by the Committee on Interstate and Foreign Commerce (to accompany H. R. 11124). U. S. Government Printing Office, Washington, D. C. (Amended by the House-Senate Conference Committee, Apr. 8, 1976).

5. Wagner, J., Mueller, E. and Daley, D. (1976). Regulation by standards: An alternative view. Eleventh Annual Meeting of the Association for the Advancement of Medical Instrumentation, Atlanta, Ga., Mar. 21-25.

6. Link, D. (1976). Opening remarks. Symposium on Device Sterility Assurance. Sponsored by the Food and Drug Administration, Washington, D. C., Mar. 30.

7. Washington Drug and Device Letter. (Mar. 29, 1976). McGraw-Hill, Washington, D. C.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

COMPENDIAL STANDARDS FOR THE CONTROL OF STERILIZATION AND STERILITY TESTING — THEIR DEVELOPMENT AND APPLICATIONS

Aubrey S. Outschoorn

Drug Standards Division
United States Pharmacopeia
Rockville, Maryland

COMPENDIAL STANDARDS

Compendial standards represent a unique role to be fulfilled in quality control between manufacture and national regulation. They are published and freely available information on a variety of medical products. However, some selection must be made as to what should be included. In many countries, drug manufacture, import, control and compendial standards are all in the hands of the one agency - the Government. In the United States, as in some other advanced countries, the compendia are in the hands of an independent body, but one which has the possibility of obtaining information from many sources of expertise - industry, governmental regulation, clinical, pharmaceutical and other technical areas.

Sterility of medical products, while directed to a common end, has different implications in different areas of interest. There is a whole set of chapters in the U. S. Pharmacopeia (USP) as well as in the National Formulary (NF), all of which are concerned with the presence or absence of microorganisms in compendial items. In the latest revisions, USP XIX and NF XIV [1, 2], the chapter General Tests and Assays includes the sections, Sterility Tests, Pyrogen Test, and Microbial Limit Tests, as well as a related section, Antimicrobial Agents - Content. On the other hand, the chapter General Information, Processes, Techniques and Apparatus contains the sections Sterilization, and Microbiological Attributes of Non-Sterile Pharmaceutical Products, as well as sections on variously distributed standards, such as Pharmaceutical Dosage Forms, e.g., relating to injections. There

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

is a distinction to be made between these two kinds of chapters.

In the one case, the standards specified are mandatory; the other chapter and sections are informative and serve for guidance purposes.

STERILITY TESTS

To deal first with sterility tests, the areas of coverage could be divided into the following:

1. Specimen taking
2. Media
3. Temperature and time of incubation
4. Adventitious contamination
5. Statistical design of the test

All of these areas have been and still are controversial, some more so than others.

Specimen Taking

A “specimen” means a representative portion of one or more units, placed under conditions suitable for the development of any microorganisms which may be present. The taking of a specimen, therefore, is by its nature a compromise to considerations of feasibility and cost. Recent developments, e. g., membrane filtration, have been such as to render the procedure less of a compromise [3, 4, 5, 6].

The compendia specify minimum amounts and numbers of specimens to be taken for the conventional sterility test by direct inoculation. It must be emphasized, however, that for the purpose of the test, these are least amounts. There is no reason why a tester should not, to have greater assurance of sample adequacy and sterility of the product, use larger volumes and more specimens, or to subject these amounts, larger amounts, or even the entire product to membrane filtration. Notwithstanding the extra efficiency of the membrane filtration technique, however, the use of less than the stated minimum specimen is not permitted by the compendia.

Media

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

The standards for media specified in the compendia have had an

interesting history [7].

Sterility tests were introduced into USP XI and NF VI in June, 1936, and the medium specified was Beef Infusion Broth. The Division of Biologic Standards (National Institutes of Health) and the compendia replaced it with Fluid Thioglycollate Medium in December, 1941 and April, 1947, respectively, adding alternative Thioglycollate Medium and a Honey Medium. In November, 1950, the compendia replaced the Honey Medium with Sabourauds, which was itself replaced in September, 1970 with Soybean Casein Digest Medium [8, 9]. In 1975, the compendia and the Bureau of Biologics (FDA) dropped Alternative Thioglycollate Medium, but the First Supplement to USP XIX and NF XIV reintroduced it for limited purposes.

The World Health Organization (WHO), in relation to biological substances, specified some 16 media in 1960 [10], leaving the choice to decision making bodies, but had only Soybean Casein Digest and Fluid Thioglycollate Media in 1973 [11], identical with USP XIX and NF XIV.

The use of a medium for sterility testing requires that it does not contain viable microorganisms, so that any growth which may occur would arise from the test specimen and not from the culture medium. This necessitates certain checks on the sterility of culture media. A medium must also have demonstrable growth promoting properties for the microorganisms for which the test is being done [12]. It is impossible to test the whole range or even a very wide range of possible organisms. The compendia specify that after autoclaving, each sterilized lot of medium be tested for its growth promoting properties with not less than two species of microorganisms. The species specified are available from the American Type Culture Collection. The Bureau of Biologics has for many years distributed, on request, species of microorganisms which could be used for the checking of culture media destined for the sterility testing of biologics. They include a nonsporeforming anaerobe that can be used by laboratories not equipped to handle sporeformers [13]. The microbial species from these two sources have presumably not been compared directly with various media. There are, however, undoubted advantages in using the same battery of organisms, whether those specified in the compendia or those suggested by the Bureau of Biologics, for testing the growth promoting properties of culture media.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

The FDA/USP sponsored project, the National Coordinating Committee on Large Volume Parenterals, has been studying the results of sterility testing of large volume parenterals for a considerable time. Much work has been carried out at the Center for Disease Control in Atlanta on other media and techniques. One is the use of a concentrate of Brain Heart Infusion Broth (BHIB) [14] which is inoculated into the parenteral product. Information on these procedures, however, has not yet been introduced into the compendia.

Incubation

Prior to the Revisions USP XVIII and NF XIII (both official September 1, 1970), the inoculated media were required to be incubated at appropriate temperatures (whatever those might be) and for not less than seven days. USP XIX and NF XIV (both official July 1, 1975) specified that unless otherwise required in the monographs, the inoculated Fluid Thioglycollate Medium must be incubated at 30° to 35°C and the Soybean Casein Digest Medium at 20° to 25°C, both for 14 days. These requirements are for the conventional mandatory sterility test as specified. The informational section, Sterilization, however suggests some modifications which could be used under certain circumstances. The rationale for these suggestions depends on the role to be filled by the sterility tests.

The compendia state under Interpretation that, if growth occurs, either the article fails to meet the requirements of the sterility test or it is permitted to try to demonstrate by retesting or other means, e.g., negative controls, that the test was invalid. Such permission for retesting concedes that adventitious contamination can occur [15, 16].

Absence of growth in an uninoculated sample which is representative of a homogeneous batch of culture medium is taken to mean that there are no microorganisms in the culture medium itself. On the other hand, absence of growth in the media inoculated with the test specimen means only that there was no microbial contamination in the specimen drawn. The first supplement [17] to the compendia USP XIX and NF XIV (official July 1, 1975) carries a note clearly stating that where individual discrete units are tested for sterility, the results cannot be
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.
extrapolated with certainty to characterize the sterility status

of other units that remain untested.

A section, Biological Indicators, has existed since USP XVIII. Even in USP XIX, however, the test describes rather meagerly the nature and some of the usage of biological indicators. A table given in USP XIX is often misunderstood. Data are given for some biological indicators used under different sterilization processes. These data, however, are not standards for these biological indicators, but are merely the performance characteristics that were found when some biological indicators were used as inoculated carriers. Precise standards for various biological indicators have yet to be introduced into the compendia.

Statistical Design for Sterility Testing

Statistical design for sampling of a lot or batch is not part of the compendial sterility tests. The sterility testing of lots is of importance, however, to manufacturers and to regulatory agencies. Nevertheless, some information is given in the compendia on such matters as validation of sterilzation procedures.

Complete sterility is the absence of all microorganisms capable of multiplying. Such a state is probably scarcely achievable in pharmaceutical practice nor, if it does occur, can it be demonstrated with certainty. Sterility in practice, therefore, must be taken to mean a property within the meaning of the regulations. In the case of a lot, sterility testing refers to the ascertaining with a given degree of probability that there may be a low level of contamination. In the case of a batch of product which is terminally sterilized, i. e., in the sealed final containers, such a probability of contamination might be as low as one in one million units [18]. The probability for a lot or batch of product which is filled aseptically, e.g., biologics, has not been clearly specified. However, it has been variously suggested as high as one in one thousand units. Some compendia, not the USP, categorize pharmaceutical products into those which must have a high degree of sterility assurance, those with a moderate degree, and those with a low degree of probable sterility.

Compendial recommendations or regulations vary from country to country and differ in relation to size of container and number of containers sampled. It was pointed out by Knudsen in 1942 [19]

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

that the information on quality in a homogeneous batch is not related to the size of the batch but to the number of samples examined. The efficiency of sterility testing of a lot will rise, therefore, with an increase in the size of the sampling. In the USP, the minimum number suggested is 20. Increases thereafter are likely to be less rewarding in relation to the expenditure of effort and materials [18, 20, 21, 22].

In implementing any plan, however, one has to take into consideration that sterility testing alone cannot assure sterility of the product or lot. One of the most important developments in recent years has been the awareness that such assurance must be built into the manufacture of the product. The most important contributor to this concept has been the realization of the limitations of sterility testing procedures.

Validation of Sterility Test Results

The compendia do not specify criteria for the validation of sterility test results, but some information is given in various chapters. Evidence for validation must be obtained from a number of sources.

The final decision between failure of the product or item to pass the test and invalidity of the test procedure requires the exercise of judgment by trained personnel who are competent to make it.

MICROBIAL LIMIT TESTS

It would be an ideal situation if every drug administered to a human being could be in a sterile dosage form. This is not feasible, and sterility can be made mandatory only in the case of certain pharmaceutical forms, e. g., injectables. The best that can be done in other cases is to evaluate the viable microorganisms present so as to enable their number to be kept as low as possible, and to ensure freedom from designated pathogenic microbial species. The compendia prescribe microbial limit tests for this purpose and these tests are obligatory in a number of monographs.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

PRESERVATIVES

One of the methods of preventing microbial growth is the addition of a suitable preservative to the product. USP XIX, under General Notices, contains statements about added substances including such preservatives; they must be harmless in the concentrations used, efficacious, and not affect adversely the product or its container. They can be used to prevent further proliferation of microorganisms already present in nonsterile pharmaceutical products. Or they can prevent the proliferation of microorganisms which may accidently be introduced after the final dosage form has been made.

There are limitations to the effectiveness of antimicrobial agents for this purpose. USP XIX points out that, for ophthalmic solutions, preservatives in concentrations tolerable by the eye are not effective against some strains of Pseudomonas aeruginosa.

The compendia describe tests for the effectiveness of antimicrobial preservatives. These tests mention certain specified organisms, available from the American Type Culture Collection, which should be included. It is clearly stated, however, that the tests and standards are applicable only to the product in the original, unopened container in which it was distributed by the producer.

PYROGEN TEST

The test given in the compendia is the rabbit test. The so-called Limulus test is not yet included. It is essential, however, to have two conditions fulfilled before a new test or method can be admitted. On the one hand the test must be used regularly and found satisfactory, and on the other it must be possible to set down adequate criteria for consistency of performance between batches of reagent material, in this case the amebocyte lysate used for detecting bacterial endotoxins. Claims have been made of detectable endotoxin in picagram amounts by this test. Less information is available on variation in results with different lysate preparations.

ROLE OF COMPENDIAL TESTS

Sterility testing is mandatory at various stages of manufacture and final product testing of biologics. However, the Federal Food,

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Drug and Cosmetic Act does not make the performance of a sterility test or microbial limit test mandatory unless it is specified in a USP or NF monograph. But it does make mandatory the requirement that the USP or NF article pass the test when it is applied to any final specimen. The specified tests in the compendia are referee tests. The results, however, can provide evidence only in respect to the specimen or specimens actually tested. Further, the compendial standards should be clearly differentiated from release criteria for manufactured batches. Compendial tests, therefore, can be used to resolve an adversary situation where a dispute arises as to the sterility status of a product; some consider this in fact to be the sole role of sterility testing as described in the compendia. Sterility tests also provide an additional check on the manufacturing process for the product, i. e., they are part of good manufacturing practice.

Sterility tests have been greatly improved under a number of circumstances, by the use of membrane filtration procedures. USP XIX carries a statement that the membrane filtration procedure is particularly applicable in four cases, viz., an oil, an ointment that can be easily solubilized, a nonbacteriostatic solid not readily soluble in culture media, and a soluble powder that has antimicrobial properties. This statement has unfortunately been misunderstood: these are not the only circumstances when membrane filtration can confer benefits. There are other situations too [23].

The compendia in the General Notices state that, where a test or assay is made, compliance with standards may be shown by the use of any alternative methods chosen for convenience or other reasons. It is also stated that where a difference appears, or in the event of a dispute, only the result obtained by the procedure given in the Pharmacopeia is conclusive. This should surely be interpreted to mean that if microbial growth is evident by the use of membrane filtration but not on direct inoculation, it must be concluded that microbial contamination was present in the sample. The same conclusion must be drawn if any other reasonable procedure is used, e.g., different media, or different methods of inoculation, incubation, or examination of the inoculated media. If valid evidence of microbial contamination is obtained, such result is conclusive of failure of the product or item tested to meet the requirements for sterility.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

It is essential of course that, if an alternative method is used, it be properly validated.

INTERNATIONALIZATION OF THE COMPENDIA

It is a policy of the USP and the NF to maintain contact and exchange information with compendia in other parts of the world, as well as with corporate compendial organizations such as the European Pharmacopoeia and the World Health Organization. The USP is perhaps in a unique position to do this. For more than a century and a half, it has been one of the leading compendia. Certainly no similar compendium is as comprehensive in the information given. There is ample evidence of world interest in the USP and about a third of the Reference Standards available are distributed outside the United States. The amalgamation of the USP and NF should result in even greater advantages and wider interest.

Staff members and members of USP Expert Panels have attended meetings and been in contact with experts in other countries [11, 24].

The continued development of internationalization is highly desirable, and this First Johnson & Johnson International Kilmer Memorial Conference on Sterilization of Medical Products is an important step in the right direction.

ACKNOWLEDGEMENTS

Grateful acknowledgement is made for personal information provided by Henry D. Piersma, Margaret Pittman, Edward B. Seligmann, Jr., and Hariette D. Vera.

REFERENCES

1. U. S. Pharmacopeia, 19th Revision. (1975)*. The United States Pharmacopeial Convention, Inc., Rockville, Maryland.

2. The National Formulary, 14th Edition. (1975)*. The American Pharmaceutical Association, Washington, D. C.

3. Holdowsky, S. (1957). A new sterility test for antibiotics –

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

an application of the membrane filtration technique. Antibiot. Chemother. 2:49-54.

4. Bowman, F. W. (1966). Application of membrane filtration to antibiotic quality control sterility testing. J. Pharm. Sci. 55:818-821.

5. Bryce, D. M. (1956). Tests for the sterility of pharmaceutical preparations. J. Pharm. Pharmacol. 8:561-572.

6. Council of the Pharmaceutical Society of Great Britain: Round Table Conference on Sterility Testing. (1963). London, B1-B23.

7. Vera, H. D. (1975). Media Control with Special Reference to Sterility Testing. FDA Sterility Analysis Training Workshop. Washington, D. C.

8. Vera, H. D. – Baltimore Biological Laboratories, Inc. (ca 1960). Unpublished.

9. Bowman, F. W., White, M. and Calhoun, M. P. (1971). Collaborative study of aerobic media for sterility testing by membrane filtration. J. Pharm. Sci. 60:1087-1088.

10. General Requirements for the Sterility of Biological Substances – Report of a Study Group. (1960). World Health Organization, Technical Report Series No. 200. Geneva.

11. Twenty-Fifth Report of the Expert Committee on Biological Standardization. (1973). World Health Organization, Technical Report Series No. 530. Geneva.

12. Brewer, J. H. (1940). Clear liquid mediums for the aerobic cultivation of anaerobes. J. Amer. Med. Assoc. 115:598-600.

    Brewer, J. H. (1949). A clear medium for the “aerobic” cultivation of anaerobes. J. Bacteriol. 39:10.

13. Farber, J. F. and Seligmann, E. B. (1968). Bacteroides vulgatus, a nonsporeforming anaerobe for testing fluid thioglycollate medium. Appl. Microbiol. 16:1102-1103.

14. Mackel, D. C., Maki, D. G., Anderson, R. L., Rhame, F. S., and Bennett, J. V. (1975). Nationwide epidemic of septicemia caused by contaminated intravenous products: mechanism of intrinsic contamination. J. Clin. Microbiol. 2:486-497.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

15. Bowman, F. W. (1969). The sterility testing of pharmaceuticals. J. Pharm. Sci. 58:1301-1308.

16. Scheibel, I. and Bentzon, M. W. (1957). In Proceedings of the 3rd International Meeting of Biological Standardization. Opatija, Yugoslavia.

17. USP XIX and NF XIV. First Supplement. (1975). The United States Pharmacopeial Convention, Inc., Rockville, Maryland.

18. Bruch, C. W. (1974). Levels of sterility: Probability of survivors vs biological indicators. Bull. Parenter. Drug. Assoc. 28:105-121.

19. Knudsen, L. F. (1942). Sample size of parenteral solutions for sterility testing. J. Amer. Pharm. Assoc. 38:332-337.

Knudsen, L. F. (1949). In Statistical Analysis in Biology. K. Mather. 3rd ed. Methuen, London.

20. Berlin, H. (1975). Kabi, Stockholm (to be published).

21. Bennett, J. V., personal communication.

22. Artandi, C. (1974). Microbiological control before and after sterilization: Its effect on sterility assurance. In Experiences in Radiation Sterilization of Medical Products. International Atomic Energy Agency. Working Group Meeting, Risö, Denmark, June 5-9, 1972. IAEA, Vienna; pp. 3-14.

23. Riedel, G. W. and Todd, E. C. D. (1973). Sterility testing of large-volume aqueous pharmaceutical products (intravenous solution) by the membrane filtration technique. Can. J. Pharm. Sci. 8:23-25.

24. Regamey, R. H., Gallardo, F. P. and Hennessen, W., Eds. (1974). International Symposium on Sterility and Sterility Testing of Biological Substances. International Association of Biological Standardization, Madrid, April 9-12, 1973. Karger, Basel.

---

*Earlier revisions are not listed here.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

MEDICAL DEVICE REGULATION IN CANADA

Ronald W. Campbell

Bureau of Medical Devices
Department of National Health and Welfare
Ottawa, Canada

The Canadian legislation concerning medical devices is contained in the Food and Drugs Act. The definition of a medical device in that Act is very broad and covers almost everything used in the diagnosis or treatment of disease which is not a drug. The Act itself is enabling legislation and is very brief. There are three sections which refer to devices. One states that no device which may be hazardous when used in accordance with the manufacturer's directions may be sold in Canada. One states that no device may be advertised in a way which may be misleading or fraudulent, and one states that, where a standard exists, the device must comply with it.

These three basic sections of the Act are supplemented by regulations which are published from time to time. The regulations are passed as Orders in Council by the Canadian Cabinet and can therefore be amended and added to with much greater facility than can the original Act of Parliament. They cannot give any new powers to the Department of Health and Welfare which are not already contained in the basic Act.

There has been an organization within the Health Protection Branch of the Department of Health and Welfare dealing with devices for some four years now, and this has been a fully developed Bureau of Medical Devices since April, 1974. In September of 1975, basic regulations dealing with the labeling of devices and with the establishment of a notification system were published. The notification system requires that all manufacturers or importers of medical devices must submit certain basic information to the Bureau of Medical Devices at the time of first marketing their device in Canada. The object of this system is to establish an inventory of devices on the Canadian market and to

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

determine who is selling what. It is not in itself a control system for medical devices but is an essential prerequisite for the establishment of such a system.

Above and beyond these basic regulations, additional regulations are being prepared, and will be prepared, for special groups of devices as the need becomes apparent. Standards can be written into these regulations where they would be beneficial. Wherever possible, these will be performance standards rather than design standards.

Because of the nature of our basic legislation, with a short Act supplemented by easily amendable regulations, the characteristic of our system of control is flexibility. The system permits the development of regulations to meet an established need rather than the development of a complete system of regulations to meet all possibilities. To paraphrase one of our Canadian prime ministers in a somewhat different context, our policy is one of regulation if necessary, but not necessarily regulation.

The development of voluntary standards in Canada is controlled by the Standards Council of Canada which is a statutory body established by a separate Act of the Canadian Parliament. It has two main objectives: firstly, to ensure that voluntary national standards are developed by a true consensus process reflecting all bodies of concerned opinion; and secondly, to eliminate overlap between the multitudinous standard writing bodies which have a tendency to develop in our society. Three main bodies have been recognized by the Standards Council as standard writing bodies for medical devices. These are the Canadian Government Specifications Board, the Canadian Standards Association and the Conseil des Normes du Québec. It must be emphasized that these bodies write voluntary consensus standards. They can submit them to the Standards Council of Canada for approval as national standards for Canada but they remain voluntary standards unless they are mandated by the Federal Government or by one or all of the Provincial governments. In the case of medical devices, only the Department of Health and Welfare has the power to do this under the Food and Drugs Act which gives it exclusive jurisdiction in the field.

The flexible nature of our legislative structure makes it possible for our Bureau to adopt part of a standard or to make

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

regulations concerning the enforcement of some feature of the standard without necessarily making mandatory the whole of the standard. We can thus make regulations for those factors which require enforcement, leaving the voluntary standards or codes of procedure as guidance for all other factors. This is the approach which has been adopted in the case of our sterilization standards.

One final point should be mentioned in considering the structure of our regulatory system. We have been able to develop, over the past few years, a strong cross-fertilization between the Canadian and United States standards committees. The Sterilization Committee of the Canadian Standards Association and the Sterilization Standards Committee of the Association for the Advancement of Medical Instrumentation have exchanged representatives and exchange minutes of their meetings. By this means we hope to ensure that even if the final standards are not identical for the two countries, they will at least have been based on a common data base and will be compatible with each other.

These then are the legislative tools which are at our disposal in Canada. What are we trying to achieve by their use?

Those of us who have embarked on the journey of device regulation realized very soon after our departure that we were moving into unexplored territory and that the baggage we brought with us from the drug world was almost totally useless. When I began to ask basic questions about sterilization, I had the great good fortune to meet a food microbiologist. I was doubly fortunate in that he was transferred from Ottawa before he had told me all the answers. We may have more to learn from the food processors than from the drug processors, but they too are limited in their approach.

The U. S. space program has advanced our knowledge of sterilization techniques immensely, but leaves many problems of biocompatibility unsolved. Our own Atomic Energy Commission has contributed a great deal to the knowledge of radiation sterilization methods, but in doing so has revealed a whole new area of problems in polymer development and design.

There are thus very many areas of technology in a wide range of disciplines on which we can call. We must endeavor to maintain the

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

breadth of vision and the willingness to cross established parameters which so characterize the work of both Lister and Kilmer.

Turning now to the specific field of sterilization, I think it is fair to say that all regulatory agencies have found that the United States Pharmacopeia (USP) testing methods are inadequate for the medical device field. There are certain very obvious defects in that there are no lifetime implants in the drug world, that the USP sampling methods do not apply to short production runs of medical devices, and that the USP emphasis on product sterility testing is more a test of the tester than of the product. The greatest probability of absence of organisms one could hope to achieve is somewhere between 10^{-2} and 10^{-3}.

As many speakers in this program have already said, we must learn to think in probabilities, and in so doing, to discard all preconceived notions of what is right and proper in the drug world. It has been clearly established in our discussions both here and in the many discussions which have preceded this conference that, by proper attention to the microbiology of the plant rather than the product, by proper process control both in manufacturing and in sterilization, and by calculation of sterilizing dose on the basis of bioburden, we can achieve probabilities of 10^{-6}.

This will not be achieved without cost in limited resources and, because of this limitation, it is not possible to insist on this degree of sterility for all products, nor is it necessary that all products achieve this level of assurance. For some years now, we in Canada have been trying to develop the concept of differing levels of assurance of sterility. This is now crystallizing into the concept that, for those devices which transgress body barriers or are in contact with fluid flows, we will insist on a level of probability of 10^{-6}. For all other medical devices which are required to be sterile, we will accept a level of probability of 10^{-3}.

It should be realized that we are not saying that some are more sterile than others or that we are creating a level of supersterile. We say that all these products are sterile. In the first case the probability that we are untruthful is only 10^{-6}, while in the second case it is 10^{-3}.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

I should like to close by quoting some words of Dr. G. Briggs Phillips on a somewhat similar occasion. He said: "It is clear that a common philosophy of excellence by all concerned is needed for adequate quality of sterile medical materials. This depends in no small measure upon the development of channels of communication and understanding. It is not merely a question of the conduct and application of new knowledge gained through research or the blind application of regulations, rather, when communication and understanding exist, the common goal of better serving the health care needs of the nations is more easily accomplished."

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

CONTROL OF STERILE PRODUCTS IN THE UNITED KINGDOM

Alexander Bishop

Scientific and Technical Branch
Department of Health and Social Security
London, England

For the purpose of control, sterile products in the United Kingdom are divided into two categories:

1) Those which are defined under the Medicines Act of 1968 as medicinal products and which are subject to statutory control; these are mainly pharmaceuticals.

2) Products, mostly "devices", which are not medicinal products as defined in the Act and which are not yet subject to any legal control. There are powers under the terms of the Medicines Act to control such items but these powers have not been taken. Sterile medical devices are currently controlled on a voluntary basis which will be described in greater detail below.

STATUTORY CONTROL

The definition of a medicinal product under the Medicines Act is as follows:

1) Subject to the following provisions of this section, in this Act "medicinal product" means any substance or article (not being an instrument, apparatus or appliance) which is manufactured, sold, supplied, imported or exported for use wholly or mainly in either or both of the following ways, that is to say:

   a) use, by being administered to one or more human beings or animals for a medicinal purpose;

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

   b) use, in circumstances to which this paragraph applies, as an

ingredient in the preparation of a substance or article which is to be administered to one or more human beings or animals for a medicinal purpose.

2) In this Act, "a medicinal purpose" means any one or more of the following purposes, that is to say:

a) treating or preventing disease;

b) diagnosing disease or ascertaining the existence, degree or extent of a physiological condition;

c) contraception;

d) inducing anesthesia;

e) otherwise preventing or interfering with the normal operation of a physiological function, whether permanently or temporarily, and whether by way of terminating, reducing or postponing, or increasing or accelerating, the operation of that function or in any other way.

Sterile products covered by this definition include sterile injection and irrigation fluids, eye preparations, medicated surgical dressings, absorbable sutures, and skin preparation solutions or wipes.

Control of individual items is exercised by means of a product license and by the manufacturing license which each manufacturer and subcontractor is required to hold. For example, an irradiation plant which processes products for other companies must hold a manufacturing license if licensed products are to be irradiated in it. To operate this statutory control, a separate Division of the Department of Health and Social Security was established. The Medicines Division is staffed by administrators and by professional advisers – doctors and pharmacists. The administrative work is divided into two separate sections, one concerned with licensing, and the other with enforcement. The professional advisers are similarly divided, one section advising on products and dealing, for example, with the evidence in new drug applications; the other section advises on manufacture and wholesaling. This latter group includes the group of officers known as the Medicines Inspectorate who visit and report on firms dealing in licensed products both at home and abroad. In addition to these permanent civil servants, there are four external

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

professional advisory committees, namely, the Medicines Commission which has a general advising function and which can establish its own committees for particular problems, e.g., one such committee advises on the manufacture of sterile products; the Committee on Safety of Medicines and the Veterinary Products Committee which advise on product licenses; and the Pharmacopoeia Commission which is concerned with published standards for products.

Licenses

When applying for a "full" product license for a medicinal products, the promoter must submit an extensive dossier of information on points of safety, efficacy and quality, similar to the New Drug Applications required by the FDA. Products which were on sale before the Act came into force were permitted to be sold under a "License of Right" until such time as the product could be reviewed. A License of Right can be revoked at any time if doubt arises.

For Manufacturer's Licenses, the term "Manufacture" is subdivided in two ways. First, there is a distinction between making and packing, the latter being known as "Assembly". A contract packer, for example, concerned only with "Assembly" needs to have facilities adequate to avoid errors in filling and labeling and to have properly controlled storage conditions. The facilities for "making" products must be more comprehensive and the License normally specifies the broad categories of products which may be made, e.g., intended for injection, oral administration, and so forth. A Manufacturer's License will therefore state whether it is for assembly or for manufacture, and the category of manufacture permitted. Manufacturer's Licenses are granted to firms which have acceptable standards of competence and conditions. The standards acceptable are not defined in Regulations because of the wide variety of industries. Regulations would have to be equally fair to all and would be extremely complex if drawn up to ensure they were neither inadequate for some industries or excessive for others. Instead of Regulations, the Medicines Division has published guidelines to good manufacturing practice (known as the "Orange Guide"). These are discussed in advance with industry and are not legally binding. Each manufacturer decides how to meet the objectives described in the Guide and the Inspectorate judges whether the facilities are adequate. This leaves some room for discussion and permits

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

concessions on target dates for improvements. The Inspectorate also runs a sampling program and a defect investigation system which, in the event of an emergency, can institute immediate recalls.

Recently the legal powers of the Act have been extended to include control over the manufacture of medicinal products in hospitals. The Medicines Act gives ministers powers to direct that specified provisions of the Act shall relate to any class of articles which are not medicinal products but are manufactured and supplied wholly or partly for use for a medicinal purpose. A very wide range of sterile products, loosely and collectively described as medical devices, fall within this definition. These provisions have so far only been implemented for a few items: intrauterine contraceptive devices, contact lenses and associated solutions, and dental materials.

VOLUNTARY CONTROL

This is perhaps a misleading title since the voluntary compliance of manufacturers with agreed standards and codes should not be designated a control system, and where central control is exercised through central contracts and approval schemes, it can hardly be described as voluntary. However, the cooperative spirit with which manufacturers accept and work with my Branch in our overall surveillance of sterile devices cannot easily be defined in a simple title.

There are two factors which particularly assist us in maintaining standards without the sanction of legislative authority. Firstly, the major use of the great majority of these products is in the hospitals which, being centrally financed, provide considerable scope for central contracting and approval of products. Secondly, an effective approval scheme is in the interest of industry since in a highly competitive field, a competent and responsible manufacturer may be outbid by a firm which can supply cheaper products by economizing on essential manufacturing controls and conditions. A control scheme which ensures equivalent standards throughout the industry is therefore economically valuable. Scientific and Technical Branch is the professional arm of an administrative division of the Department of Health and Social Security; Supply Division, as the name implies, is concerned with all forms of supplies used in

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

hospitals, from furniture to radiological equipment and heart-lung machines. It works by means of central contracts, approved lists of manufacturers, specifications and evaluation schemes to provide the Health Service with good quality products at reasonable cost. Efficacy and safety are of course paramount considerations and emphasis is laid on both aspects in our surveillance of sterile products. One group within Scientific and Technical Branch staffed by pharmacists, microbiologists and experts in quality control and performance test design, is responsible for the specification, inspection and defect investigation function for all sterile products not designated as medicinal products. They provide an advisory service for both hospitals and industry covering sterilization methods and controls, packaging and labeling, environmental conditions of manufacture and clean room design, and the development of performance tests and quality assurance programs. Some of their work is issued in the form of Departmental Specifications, some as Guidance Documents and some is channeled into British Standards and International Standards.

A major part of their work is now concerned with the investigation of defective products which are reported to us by the hospital service. In a rapidly developing field such as this, new materials and designs frequently display unexpected faults not eliminated by normal testing programs. The investigation of such failures therefore not only allows the rapid recall from the hospitals of any product likely to endanger a patient or a user, but also provides valuable information which can be fed back into the industry by means of performance tests in specifications.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

LEGISLATION CONCERNING THE STERILIZATION OF DRUGS AND MEDICAL PRODUCTS IN THE FEDERAL REPUBLIC OF GERMANY

Gerhard Schwenker

Pharmazeutisch-Chemisches Institut der
Universität Heidelberg
Germany

I hope to give you a brief survey of the development of legislation concerning drugs in the Federal Republic of Germany and, as far as possible, the development of responsibilities and requirements relative to carrying out and checking sterilization.

Sterility is an extreme claim, difficult to attain in practice and difficult to prove. As the microbiological quality of nonsterile drugs began to play an increasingly important role during the past ten years, the trend was to direct our efforts toward defining suitable microbiological attributes for drugs and medical products, and developing appropriate test methods.

It was possible to do this by a new Drug Act. A draft was prepared by the appropriate board of the "Deutscher Bundestag" and is to be discussed and then passed in the near future. The bill attempts to minimize health risks to patients caused by diagnostic, prophylactic and therapeutic products. This will be accomplished by required licensing of new drugs. The license will be granted on the basis of results of tests for efficacy, quality and safety of the drug under consideration. The guarantee for a uniform quality of the licensed drug is based upon compliance with the general standards of Good Manufacturing Practices (GMP) guidelines, together with the special quality standards of the Pharmacopeia and with the specifications of the license.

The road to completion of the present draft was a long one. This draft takes into consideration all the pertinent guidelines and recommendations of both the World Health Organization (WHO) and

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

the Council of the European Communities.

Our story begins, therefore, with the approval by the "Reichsrat", in 1926, of the German Pharmacopeia VI (DAB 6) which was framed when the manufacture of drugs was largely carried out by pharmacists in accordance with the prescriptions of physicians. Thus, it was based on the technical capability of the pharmacy and avoided analytical determinations which were considered to be unnecessary if the drug was manufactured lege artis or if the method involved an overly large-scale operation for a pharmacist.

The German Pharmacopeia VI (DAB 6) stated that "to sterilize means to free an object completely of germs", but "an object may be called sterile only if it is free of all living microorganisms". The Pharmacopeia included some instructions on how to sterilize, but gave no suggestions or directions concerning sterility testing. DAB 6 was obligatory only for the pharmacist. Comprehensive legislation in the field of drugs was lacking, with the result that anyone could manufacture and deal in drugs without any inspection or control of the facility or products. Unfortunate circumstances were responsible for the fact that this Pharmacopeia remained in force without essential modifications until 1959.

Not until 1959 was the long-needed adaptation of the German Pharmacopeia to international standards accomplished. At that time, the third supplement to DAB 6 was released.

This supplement was framed in such manner that it could be adopted without essential change in a planned seventh edition of the Pharmacopeia. Above all, general instructions have been adapted to modern requirements. The third supplement presented a more satisfactory definition of the term "to sterilize", which required freeing an object of living germs. It also gave precise and detailed instructions concerning the sterilization procedure and sterility testing. In agreement with other modern pharmacopoeias, methods or apparatus other than those described as official were permissible, provided the results obtained were of equivalent accuracy.

In 1961, the "Bundestag" passed the Drug Act, which compiled, arranged and finalized legislation in the drug field in Germany for the first time. This Act, with several amendments, is still in force. The definition of the term "drug" (Paragraph 1) is not in

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

accord with the European Pharmacopeia. The definition is broader, including a number of products, e.g., sutures, dressings, catheters, prostheses, etc., but not medical instruments or other items such as gloves, which are called "medical devices". The Pharmacopeia was made obligatory for the manufacturers as well, and the Minister of the Interior was authorized to amend the Pharmacopeia (Paragraph 5).

Paragraph 6 of the Drug Act is of great importance: it establishes the liability of the manufacturer for the safety of his products, especially for medical products, which are classified as drugs in the broad sense of Paragraph 1, Section 2, if these are not specifically mentioned in the Pharmacopeia. Paragraph 6 also serves as the basis for the conversion into national laws of the guidelines of the Council of European Communities for the testing of new drugs (Paragraph 21, Section 1a) and the guidelines of WHO for GMP (Paragraphs 39 and 40).

Paragraph 7 places limitations on the use of ionizing radiation in the manufacture of drugs, the general use of ionizing radiation for this purpose being prohibited. The Minister of the Interior, however, is authorized to give permission for the use of ionizing radiation by special or general regulations. A regulation, recently in effect, restricts the energy of the ionizing radiation for the purpose of sterilization, to a lower energy limit, namely 3 MeV, and allows its application in the sterilization of sutures, dressings, and some collagen and fibrin products. The draft of a new regulation sets the upper energy limit at 8 MeV, the dose limit at 5 Mrad, and permits the application to drugs which cannot be sterilized by other means.

Although it was clear in 1965, after signing the European Pharmacopeia Convention, that a European Pharmacopeia would be obligatory in the Federal Republic of Germany, a new national Pharmacopeia (DAB 7) was adopted in 1968, in accordance with Paragraph 5 of the Drug Act. However, it comprised only about 400 monographs, while modern pharmacopoeias, in general, consist of three times that number.

The definition of "to sterilize" in German Pharmacopeia VII (DAB 7) is slightly different from that of the former edition: "to sterilize" means to render an object free from all germs "fit for breeding". Methods of sterilization with dry heat (180°C) and

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

steam (at least 120°C) are described briefly; sterilization by filtration, chemical procedures (ethylene oxide), and radiation sterilization are described inadequately or not at all. Methods of sterility testing are more advanced than those in the third supplement, but membrane filtration, although known since 1950, is not mentioned.

After the Ratification Act was passed in 1973, Volumes I (1969) and II (1971) of the European Pharmacopeia (EPI) were put in force in 1974 and 1976. By a supplement to DAB 7, the pertinent monographs of DAB 7 were at the same time rendered invalid. During the transition period during which both the German Pharmacopeia VII, together with its supplement, and the European Pharmacopeia will be in force simultaneously, there will be no concurrence of monographs, but differences in the general parts of the pharmacopoeias will cause difficulties. Thus, according to DAB 7, methods and apparatus may be used other than the official ones, while this is not so according to the EPI.

The regulations by which the volumes of the European Pharmacopeia were set in force are perplexing. From Paragraph 3 one could infer that, in the future, all products contained in the monographs of EPI must be checked by the methods given. Only by comparison with the regulation by which the second supplement of DAB 7 was set in force does it become obvious that, in fact, only the quality claims determined by the specified official analytical methods must be guaranteed.

This problem will be resolved when the new Drug Act is put in force. At that time, the German Pharmacopeia will no longer have the status of a federal regulation, but will merely be a collection of well-regarded pharmaceutical rules concerning quality, testing, storing, dispensing, and labeling of drugs.

The European Pharmacopeia is interesting in a number of respects. First, it does not define “sterile” or “sterilization”. Second, there are no instructions on how to sterilize the various drug dosage forms. And third, sterility testing is described in detail, including recommendations for the use of the membrane filter technique wherever possible. On the other hand, however, detailed descriptions of culture media are lacking.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

In conclusion, it should be mentioned that there is a great deal

of unofficial activity on the part of several boards and associations concerned with sterilization and sterility testing and, as far as I know, a very stimulating international cooperation exists among them. However, these are beyond the scope of the present report.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

REGULATION OF STERILIZATION CONTROL OF PHARMACEUTICAL AND SURGICAL PRODUCTS IN ITALY

Alfred D. Zampieri

Istituto Superiore
di Sanita Rome, Italy

Sterility testing of pharmaceutical and surgical products in Italy is performed by the Istituto Superiore di Sanita (ISS) in Rome. This Institute is the technical-scientific agency of the Public Health Service; it is a central government agency and is comparable to the federal agencies in the United States.

Before embarking on the topic assigned to me in this meeting, I would like to describe the organization of the ISS in the context of its functions within the Public Health Service.

Historically, it derives from the former Laboratories of the Chemistry and Microbiology Services of the Welfare Ministry. In 1935, with the consistent aid of the Rockefeller Foundation, it became an independent agency with four main laboratories, namely, chemistry, physics, microbiology, and parasitology, the latter being mainly devoted to the problem of epidemiology of malaria.

The extensive development of new drugs after the war required a suitable development of our Institute. The Institute invited Professor Chain to come to Rome to organize a new laboratory for research in biochemistry and antibiotics, and Professor Bovet, who was working at the Pasteur Institute in Paris at the time, to direct another laboratory of pharmacology.

During the 20 years from 1950 to 1970, our main task has been the control and survey of food and drug products. Simultaneously, a program of environmental surveillance was being initiated by the Laboratories of Physics and Engineering, in cooperation with the Chemistry, Microbiology, Veterinary, and Parasitology

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Laboratories.

Recently a new Laboratory of Epidemiology has been established to function in close cooperation with the other laboratories and with the regional and provincial Centers of Public Health as well as with International Public Health organizations. We hope that, in the near future, this new structure will assume a pivotal role in preventive medicine.

I have briefly summarized the historical evolution of our Institute during the course of these last 40 years, which has not always been a calm one. Its history may offer an explanation of why, in the meantime, we have acquired the functions, which in the United States, are shared among the Food and Drug Administration (FDA), Center for Disease Control (CDC), and the National Institutes of Health (NIH), and combined them into one organization.

As a matter of fact, we cannot satisfy the numerous requests arising from these three areas of activity.

In order to overcome the chronic manpower shortage we are faced with, our present policy is to enlist the assistance of groups of specialists on each problem; these specialists are based at our universities or various international organizations, and are called upon as the need arises.

For example, three years ago, in order to handle properly the problems presented by the occurrence of cholera, we had the assistance of the Epidemiological Department of the CDC, and I wish to take this opportunity to thank Gene Gangarosa and Bill Baine for the wonderful and invaluable collaboration we have received from them.

At present we are facing the problem of evaluating the safety of yeasts grown on normal paraffins, for use in animal nutrition. For this purpose, we are working in collaboration with an international committee of experts, which will evaluate the results of the pathogenic studies of the yeast strains to be used in industrial fermentation processes.

As I have mentioned before, ISS has the responsibility for the safety testing of pharmaceutical products as well. A new drug or

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

surgical product which is proposed for the national market by an industrial company is required by law to be approved by a special commission of the Public Health Ministry which, after having examined the pharmacotoxicology and clinical activity protocols, sends the product to our Institute for analytical, toxicological, and microbiological examination. If the product passes these tests, it is approved and may enter the market for normal therapeutic use.

About 0.1% of the different products used in hospitals or in pharmacies are taken randomly each year by the local public health officers for control purposes and are tested in the regional or provincial laboratories. If the product does not comply with the specifications, several samples of the same batch are taken in different regions and sent to our Institute for additional analytical testing. The same procedure is followed if a product shows pyrogenicity or undue toxicity after complaints by consumers or physicians.

A special control testing program for each production batch is carried out by ISS for vaccines, sera, and blood products before their distribution.

Until two months ago, surgical sutures were also submitted and subjected to such quality control testing. We have now adopted a surveillance on production to check if the company uses the correct methodology as prescribed by good manufacturing practice.

Sampling, laboratory methods, media, and interpretation of the results have been discussed at the European Pharmacopoeia meeting in Strasbourg, and are quite similar to those of the U. S. Pharmacopeia; for this reason I hope there will be, in the course of very few years, a general agreement to unify the two texts into a single one.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

REGULATORY REVIEW OF STERILIZATION CONTROL IN SCANDINAVIA

Lars Olof Kallings

National Bacteriological Laboratory
Stockholm, Sweden

SWEDEN

In Sweden, an act governing the control of industrially sterilized articles for public health and medical purposes was issued in 1975 and came into force on January 1, 1976. The statute applies to products intended for single use in public health and medical care which are sterilized in conjunction with manufacture. It covers products used in direct treatment of patients as well as products used for other purposes in public health and medical care, e. g., certain laboratory supplies. Products regarded as drugs under the Drug Decree (1962:701) are excepted.

The provisions apply to manufacture (complete or partial), including sterilization, composition, packaging, and storage, before delivery to the user. It states that, "The manufacturer shall take those precautionary and other measures which are necessary in order that the products shall be sterile when used and which can be considered reasonably called for in order to prevent or counteract their causing injury when used." This includes <u>all</u> properties such as sterility, mechanical and other properties of the material, freedom from biological risks (toxic compounds), package and fitness of the product for its purpose. Some of these properties may be affected by the sterilization procedure, e.g., discoloration and brittleness through radiation, and toxicity of residuals of ethylene oxide.

According to the principles of the statute:

1) There is no system of approval of a product or of a manufacturer. There are no fees to be paid by the manufacturer.

2) The manufacturer is responsible for his products. He or the

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

importer must inform the National Board of Health and Welfare about the products marketed and on request furnish the Board with documentation.

3) The National Board of Health and Welfare
   a) exercises the supervision of the law,
   b) may issue standards,
   c) may issue injunctions and prohibitions, and
   d) may institute public prosecution for offenses of the law.

4) An obligation of secrecy is stipulated concerning professional secrets or business conditions.

5) Violation of the law may cause sentences to fines and confiscation of the product.

Thus, the manufacturer carries the entire responsibility, but is required to notify the authorities of the product marketed. The products are not registered as approved or not approved. The idea of the regulations is simplicity: no time delay in registration, no extra costs on the products to pay for the controls, and less bureaucracy. The control system is diagrammed in Figure 1.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

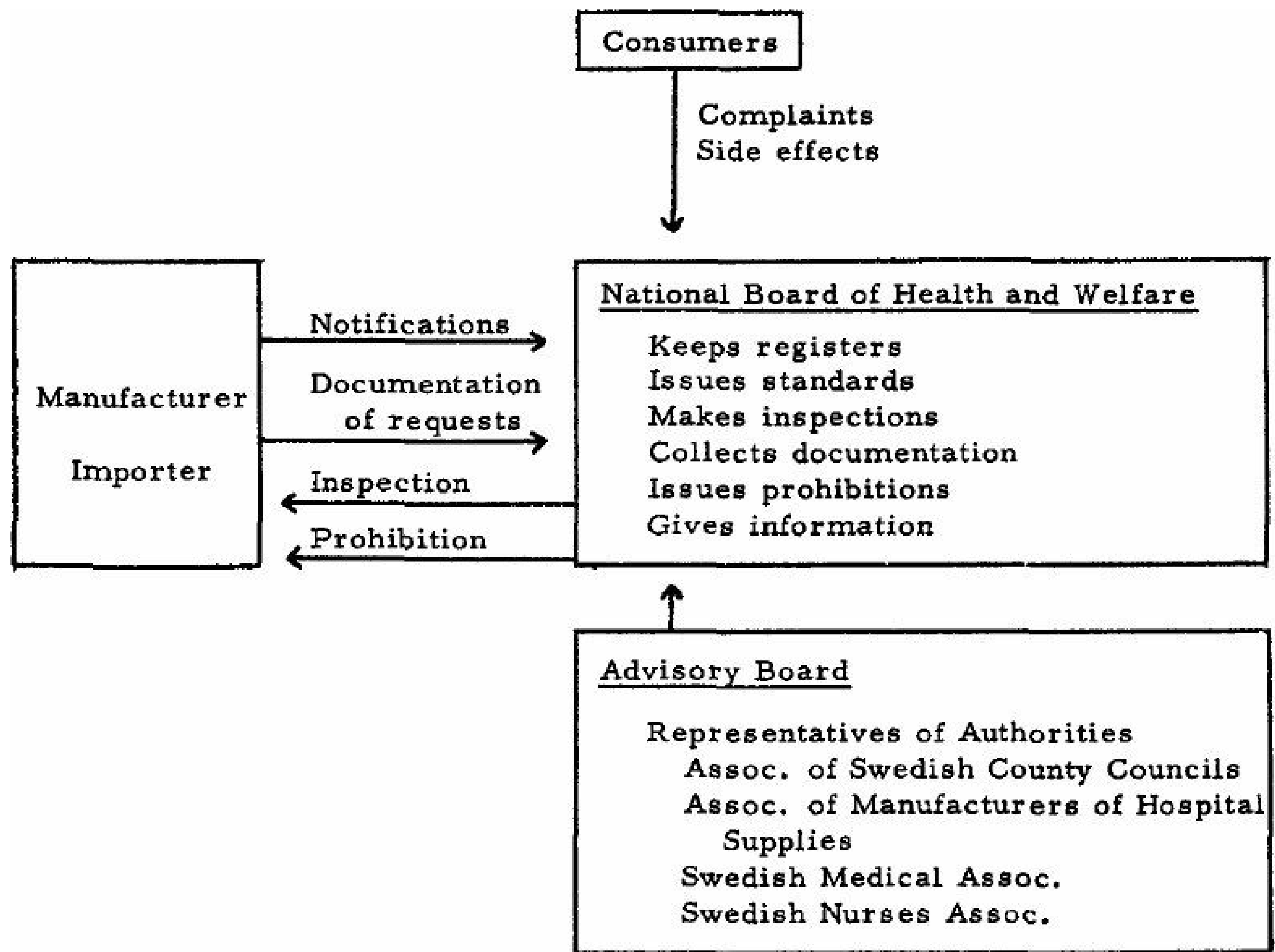


FIGURE 1. The Swedish control system of industrially sterilized articles for public health and medical purposes.

The following list may serve as an example of the notifications that must be made by the manufacturer or importer:

Product
- Type, function, size, etc.
- Manufacturers of the different parts
- Short description of manufacturing process

Particulars of materials
- Chemical name of materials
- Manufacturers of materials
- Substances added

Packaging

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

- Type

Materials
Short description of packaging procedure
Test of integrity
Type of information given on the package

Sterilization
Presterilization counts
Place of sterilization
Method of sterilization
Method of control of the sterilization process
Sterility tests of final product
Testing laboratory

The drug legislation in Sweden is different from that of sterile disposables. Drugs must be approved and registered, and a registration fee and an annual fee must be paid. Certain industrially sterilized single-use articles are considered as drugs and must be registered. These include single dose syringes used as containers for drugs and any article which is packaged or delivered with the drug, e. g., infusion sets, disposable syringes, eye drop applicators.

The quality of drugs is regulated by:

1) The Nordic Pharmacopeia.

2) The European Pharmacopeia.

3) Regulations and recommendations issued by the National Board of Health and Welfare.

4) Conventions of the European Free Trade Association.

The Nordic Pharmacopeia recognizes the following roup of pharmaceuticals: sterile, aseptically produced and others (nonsterile).

Drugs can only be labeled as sterile if they are subjected to an active sterilization process in their final containers, e.g., by saturated steam, ionizing radiation or ethylene oxide. Filtration, for example, is not considered as a sterilization method.

Drugs are categorized as follows:

A. Sterile preparations

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

1) Manufacturing procedures aiming at no more than one nonsterile unit per million.
2) Test for microbial contamination before sterilization.
3) Microbial test of sterilization procedure.
4) Should conform with the sterility test.

B. <u>Aseptically produced preparations</u>
1) Sterilized raw materials.
2) If (1) is not possible, sterility test of raw material.
3) If (1) or (2) is not possible, 100 microorganisms/gram is acceptable if the final treatment kills or removes the microorganisms.
4) Aseptic working facilities (laminar air flow, etc.).
5) Manufacturing and filling procedures should be regularly checked microbiologically.
6) The most effective method for removing and/or killing microorganisms should be used.
7) Sterility test should be carried out on each batch.

C. <u>Nonsterile preparations</u>
1) <100 microorganisms/gram.
2) If (1) is not possible, tests for pathogens must be carried out.

DENMARK

In Denmark, there is a new Drug Decree in force since January 1, 1976. A paragraph on medical utensils is included in the Decree. The paragraph gives the right to the Minister of Interior to issue statutory provisions that the law or part of it shall apply to products intended for use in the prevention, diagnosis or treatment of disease and for the diagnosis of pregnancy. An Advisory Board has been appointed, but the government has not yet issued the statutes governing the control. It is reasonable to believe that the present voluntary registration of approval will be succeeded by a compulsory registration. This compulsory registration for approval may not necessarily cover all but the most important medical utensils from the sterility point of view – at least in the beginning.

NORWAY

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

In Norway, since 1960, approval and control of special medical utensils have been required, i. e., for blood donor and transfusion sets. Provisions governing the control of other medical utensils are in preparation.

COMMON TRENDS IN SCANDINAVIA

Official biological indicators are used in Scandinavia according to the Nordic Pharmacopeia. Denmark, Norway, and Sweden utilize the same preparations of biological indicators (those for steam sterilization are prepared in Sweden, and those for ethylene oxide, in Denmark). The indicators are distributed by the National Medical Microbiological Institutes.

The indicators are based on the principle that sterilization methods, whether it be saturated steam, ethylene oxide or radiation, shall produce the same safety as to freedom from viable microorganisms on the products (that is, not more than one viable microorganism in one million units of the product).

The official biological indicators are intended for control of hospital sterilization as well as for industrial sterilization. The official indicators are intended to be reference preparations, thus not for routine use. It seems more rational for industry to design indicators that are more suitable for routine checking purposes.

The Scandinavian official biological indicator for saturated steam conforms with the USP performance characteristics. The ethylene oxide indicator complies with the USP as well, provided that the sterilization process includes a humidification procedure that rehydrates even very dehydrated microorganisms which are likely to occur in the dust of production premises. The need for rehydration may vary for different products. On some products, the water content of the microbial cells may be such that rehydration will take place rapidly and the need for rehydration will be less than calculated when preparing the spore-sand indicators. It is rational to take into account the degree of rehydration needed when data is presented that can be used as a basis to estimate how frequent extremely dry (and therefore ethylene oxide-resistant organisms) occur.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

In radiation sterilization, we do not recommend the routine use

of biological indicators. The physical or chemical dosimeters are more accurate and less expensive. We recommend that the minimum dose be related to the requested safety margin (e. g., one in one million, and to the number and resistance of the naturally contaminating microorganisms as based on examination of the products in question. In addition, we request that the antimicrobial efficiency of the dose used be compared with the efficiency of a reference plant. Therefore, we actually supply microbiological reference preparations consisting of highly radiation resistant microorganisms. These preparations can be used as biological monitors or dosimeters by assaying surviving microorganisms after exposure to different doses and by plotting the values in dose-response curves.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

DISCUSSION

Comment by A. Bishop:

It is now many years since Carl Walter, who has done more than his share in the field of sterilization, particularly in the hospital field, said that the real trouble with sterilization is the opinionated ignorance of those responsible for operating sterilizers. He would be a brave man who thought that this was no longer the case in the hospital service, but I think that it is less now the case in industry, and the breed appears to have passed to some of the regulatory agencies, if the requirements that some of our industries are now being called upon to meet in various parts of the world are anything to go by. This is the problem that we have come to discuss this morning. It was quite apparent yesterday, from what Carl Bruch was saying, that you are having problems over here, just as we are in the U. K., as to exactly what should be asked for. It is not surprising that we have even more difficulties in other countries. I chair a small committee of experts from the European Economic Community countries which meets in Brussels trying to work out some sort of guidelines for radiation sterilization. Even in a small, well understood area like this, it is quite remarkably difficult to cut across established beliefs, and unfortunately entrenched positions. Once this sort of error gets into the statutes, all the science in the world will not help you.

Q. by R. Jerussi:

I would like to ask our European visitors and our visitors from Great Britain how they handle imported sterile products, whether they be drugs or devices. And, another question would be, are these types of products handled in the Common Market? One of the things we have a problem with, and which was alluded to by the United Kingdom representative is that we do have quite a problem with importation of sterile products. We often find that they do not meet our particular standards in this area. That’s not a criticism of the standards that are used to manufacture these products but they do not happen to meet ours. They should meet ours. We are very often left in the position of using double standards. I wonder how this problem is dealt with, if it is, by the European countries and the United Kingdom.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

A. by R. Campbell:

There are, in fact, two aspects of that problem. One is the question of whether there is an adequate standard in the country of origin and whether there is some method of certification to that standard. The other question is whether the packaging of the device is such as to maintain its sterility during the transport across international boundaries. To my mind, this is a major problem - not so much the problem of the country of origin. We have provisions in our regulations for the admission in bond for resterilization in Canada.

A. by L. Kallings:

It could be added to the discussion that we accept inspection made by authorities in other countries, for example, in the United Kingdom or in the United States.

A. by A. Bishop:

I think this is an extremely important point. The answer to the question in the U. K. as far as medicinal products, that is to say, those products that are covered by the Act are concerned, there is no problem. They are licensed for sale on the market, whether they be made in Britain or anywhere else. The problem of inspecting them overseas is greatly assisted, as Professor Kallings has said, by the existence of a convention. And somebody is going to put me right here. It is a Council of Europe Convention, is it not, which predates the Common Market, i. e., an agreement between the inspectorates in these countries to accept each other's inspection? In the case of plastic disposables, which are not covered by the Act, we try to inspect the foreign manufacturer ourselves.

Comment by W. Dean:

I think the word "International" in the Kilmer Memorial Conference is very appropriate and it is to this theme of "International" that I would like to address my remarks. My appeal is to those members of international regulatory authorities who are responsible for setting sterilization standards and requirements. For example, Mr. Bishop, you will accept for steam sterilization release, against physical parameters. You like some biological monitors for ethylene oxide, specially made with special spores, of course, on aluminum foil or something like that. For irradiation, we use the dosimeter, which is fine. I can

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

live with all that – just about. But when I try to take exactly the same product with exactly the same probability of sterility to another European country, I am confronted with obstacles. I would remind you that the EEC was founded with the view to removing constraints to the movement of goods, trade, commerce, communication, science, etc., between communities. I sometimes feel it's had the converse effect. But when I try to move those self-same goods meeting U. K. standards to some other country, I find I have to put biological monitors into steam; I've got to use another form of biological indicator for ethylene oxide; and I've got to put biological indicators into irradiation. I find I have a shelf life of eight months for irradiated products, two years for ethylene oxide – apparently it's unlimited for steam. When I come to the United States, I hear that 250 ppm of ethylene oxide residue is quite safe, yet other countries are saying you must not exceed 2 ppm. I'm faced with a very difficult and complex situation. May I appeal to the men who decide, draw up and implement these regulations to talk amongst themselves, and try to reach some agreement amongst themselves and so simplify the lives of poor people like myself who are trying to reach your standards and your requirements.

Comment by A. Bishop:

I have to say I have every sympathy with this. I think we have done our little bit. I am sorry to see that the Western European country with the most obscure regulations is not represented here, or we could have asked them the basis on which they make some of their regulations. All one can say is that it does appear to me that the situation is improving and it will only improve further by meetings such as this, where everybody starts to speak the same language. What we are up against is the opinionated ignorance, of which we were speaking earlier, in some sections of government or in advisors to government, who think up these regulations and write them down. And once they are enshrined in statutes, you are stuck! And, with the best will in the world, the government officials in my sort of a position in these countries have their hands tied. So, the thing to do is to fight like our American colleagues are doing at the moment, with their new device legislation, to make sure that any regulations that are brought in are sensible.

Comment by C. Artandi:

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

I would like to make a comment and ask a couple of questions. It

was interesting to listen to Mary Bruch's comments that if all goes well, soon we are going to see a radiation sterilized drug in the form of an eye ointment. Most of you are too young to remember, but the first radiation sterilized drug in this country in 1952 or 1953 was a steroid ointment by Upjohn, which was approved at that time, if I remember correctly, but for some reason, was not kept on the market.

My questions deal mainly with Bill Dierksheide's comments on how they see the control of sterilization under the new device legislation. One particular question which I would like to get some more information on is his suggestion that we may have to label for sterility assurance. A chill went through my back when I heard that, because the poor consumer is already confused enough. He wants to have a simple word, sterile, which even if it is not absolutely true, gives him comfort. Now we are saying that no more than one in a million may be nonsterile. Another company will come along and claim no more than one in ten million. You will have a nice promotion campaign going on as to who is more sterile. I really caution very much against the labeling of sterility assurance. We should just leave things simple. Between the regulatory agency and industry, we can settle these questions; but let us not confuse the public.

The other question which I raise is that once you set these standards and put it on the labels, who is going to be liable in case of a lawsuit for sterility? Is it going to be the government or the manufacturer? You made them put it on the label and therefore, it should be right. If there is any question, that raises some other problems.

At one point you mentioned that we have to be very careful of the absolute nature of sterility and the limitations of technology. I would like to make sure that we all play to the same tune. There is no absolute sterility except in the dictionary, and we better just leave it alone in that sense. Let us call it "sterile" and let us define what we mean by this. The limitation is that if you want to be absolutely sure, you will not have anything to sell.

Comment by A. Bishop:

I would like to see the word "sterile" confined to not having
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.
babies, but this is a lost cause. Would anybody like to reply to

Dr. Artandi?

A. by W. Dierksheide:

I suppose I am rather obligated to answer. What I presented this morning were just my impressions of the alternatives that are available to the agency. I recognize that the least desirable alternative is the labeling requirement I described. My personal preference is the baseline standard approach, where probabilistic levels of sterility would be required for types of products, much as is done in Sweden and the other Scandinavian countries. But that is my personal preference. I have no way of knowing at this time where the agency is going to go.

Q. by C. Phillips:

Well over ten years ago, which probably means 15, I was invited to what I think was the Fourth International Microbiological Standardization Congress, which met at Wiesbaden, Germany. I got the impression it was more or less a voluntary association of the pharmaceutical industry and manufacturers themselves. Is that organization still going and what is it accomplishing, and are there other international efforts to get some standardization among the various countries?

A. by R. Campbell:

There are two main bodies involved. This is not specifically in the microbiological field, but in the field of international standards. The two principal bodies involved are: The International Standards Organization (ISO) and the International Electrotechnical Commission (IEC). The IEC deals with electromedical devices of all kinds. The ISO, in general terms, deals with all the other kinds of medical devices. This is not in the drug world; this is in the medical device world. Membership of ISO is made up of bodies which are representative of the governments of the member countries. In our case in Canada, the Standards Council of Canada is the national member of the International Standards Organization. Some countries are more active in participating in the development of international standards than others. In Canada we have chosen to be as active as we possibly can be in ISO and, in fact, in the field of standards for dental materials and instruments, our Canadian Dental Association made a positive decision many years ago that they would not write separate standards for Canada. They work through

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

the mechanism of ISO and they have been very active on the working groups of ISO in insuring that the international standards were written in such a way that they would be acceptable within our country. Other countries are becoming more active than they have been in the past few years, and there is a reasonable prospect that in the next five years or so we will see much more activity in ISO certainly, and I hope, in IEC as well. A lot of the European countries have, in fact, said that they will not write national standards for electromedical devices but will adopt the IEC standards as their national standards. I think that is probably the case of all the Iron Curtain countries, too. Most of the Iron Curtain countries have also said that they will adopt the IEC Standards as their national standards. In Europe there is a great deal of international standard activity going on and it is filtering through to this continent through Canada.

Comment by A. Bishop:

It is, in fact, a great grief to those of us who work with the International Standards Organization that the U. S. is not well organized for this. I am not sure whether it is that they do not have a national standard organization which is affiliated with ISO. Certainly, American delegations to the International Standards Organization that I meet always appear to be interested chaps of good will, knowledgeable, but without any sort of plenipotentiary authority. It is a very worrying thing to some of us to see international agreements arrived at, and then, particularly in this country, domestic legislation brought in or domestic standards made that run contrary to it. This makes life very difficult for industry. I do not think that this is necessarily true of the subject that we are talking about here, because, by and large, the International Standards Organization does not get involved in biology, but it does affect the standards for the actual apparatus.

Comment by R. Ernst:

In concert with what Dr. Artandi was stating, my main concern is with the consumer advocacy that is going on in the United States right now and our continued use of the phrase, "absolute sterility is unachievable". I personally disagree with that statement and disagree very much with Dr. Kelsey's statement in that now famous paper. I think that absolute sterility can be achieved. What we are trying to do is force an unnatural situation into a very practical, useful thing like survival curves and so forth that we

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

have been discussing so much at the conference. I think that what cannot be achieved is the proof of sterility. But, I think absolute sterility is probably achieved more than we would like to admit.

Comment by A. Bishop:

I am quite sure that it is achieved on the surface of the sun, but we are not talking about that sort of situation. I think this is purely a realistic acceptance of the fact that we cannot know. I was very interested in one of the slides that was shown this morning. I think it was one of Professor Schwenker's slides that made reference to organisms that could breed. Now this is one of the difficulties of our microbiological testing and always has been. We have judged the presence or absence of organisms by their capacity to multiply. And, if you think about it, we have probably been deluding ourselves for generations. But, what does the panel think about this?

Comment by R. Campbell:

If you accept the approach of viable organisms, or that organisms capable of breeding are capable of multiplying, there is a grave danger that you are going to end up in a situation where sterilization is being used as a method of cleaning up a dirty product. Sterilization does not clean up a dirty product. It gives you a lot of dead bugs on it, instead of a lot of live bugs. This raises a whole new set of problems. I think if we would word our regulations in such a way as to allow the presence of dead organisms and still call the article sterile, we are running into a completely new ball game. I think my view on the subject parallels Carl Bruch's almost entirely in that we should not be thinking of trying to test sterility into the article. What we have to do is look at the process rather than the product and clean up the process so that we do not have all these bugs lying around – alive or dead.

Comment by A. Bishop:

I am sure that this is right. I am sure that the only meaningful definition of sterility is that the article has been put through a process that informed people believe, on sound experimental data, will sterilize.

Comment by R. Ernst:

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

I think I have been misunderstood. I am not against what we are discussing. I have indicated that this is a very useful tool. What I am concerned about is alerting the outside world to the fact that we are saying it is impossible to achieve sterility. Dr. Kereluk's data, where he got 44 positives out of over a million is a very clear indication of the fact that we have sterility more often than not. I think our processing is very safe, as our luncheon speaker yesterday seemed to indicate. But, I am not for abandoning the methods for defining sterility assurance as we would in a U. S. Pharmacopeia or some other standard document. What I am against is alerting the public, especially the consumer groups, that we don't have the assurance of sterility that is implied. We should not redefine "sterility", but define what we mean by sterility assurance.

Comment by K. Kraskin:

I agree with Dr. Campbell's suggestion that if you start out with very clean raw materials, and you have a very effective Good Manufacturing Practice, you almost certainly have to come out with a much better end product. Trying to sterilize becomes more difficult if you have a higher contamination level to begin with.

Comment by A. Bishop:

In my own branch, we certainly strongly take this view. We will not accept products sterilized by radiation and ethylene oxide unless the manufacturer can demonstrate that the number of organisms on them is low. The only place where, perhaps, we would part company a bit with our Scandinavian friends would be that they are a little more logical and insist on quantifying this. This is why, in our country, we are a bit against the whole conception of people setting up contract cold sterilization facilities. We think that we can rightly say the sterilizing process begins with the cleanliness of the manufacturing plant. You cannot certify that the product is safe if you haven't had a say in the conditions under which it was made. This is particularly true of gas; it is a little less true of irradiation. We believe this very strongly.

Q. by C. Artandi:

I would like to ask the panel to express an opinion on the proposal put forward by Dr. Campbell that levels of assurance for products used for different purposes do not. necessarily have to

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

be the same. He recommended specifically that materials which go through barriers of the body or are implanted or are in a fluid path, should have a one in a million minimum, and others one in a thousand.

A. by M. Bruch:

I think the suggestion is very interesting. It has been my experience that you will have a great deal of difficulty getting people to agree on what those levels are, because it is a judgment of what safety is and for which specific devices, drugs, etc. Last year, going around that table at the contact lens meeting, I think we had about three different ideas as to what kind of a guarantee of sterility a person would like to have. As I recall, Frank Engley said that he only had one good eye so he would like a little higher guarantee than those who happened to have two good eyes. I think getting agreement is the difficulty.

A. by L. Kallings:

I would like to go back to the question of whether one could have different degrees of sterility – as one in a million and one in a thousand. We have discussed that several times and I don't think that we are the body to properly discuss it. That has to be discussed with hospital people because there are many very practical and tricky problems involved. I am afraid it requires too high a degree of sophistication.

Comment by J. Whitby:

I would like to come back to contact lenses in respect to what Mary Bruch said. I think if you only have one eye you might prefer to wear glasses, because the process of putting contact lenses into the eye is probably not a sterile technique. There is every possibility of contaminating the area when you are inserting the lens or taking it out. So, I wonder whether it is wise to seek the absolute last level of confidence in respect to the contact lens.

Comment by A. Bishop:

I could not agree with you more. Nothing is more grievous to anybody like myself with responsibility for getting things right in industry than to observe hospital practice and to see one's own general practitioner open a syringe package with his teeth. It does not give you much confidence.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Comment by C. Bruch:

I have been trying to take in the dialogue this morning on the levels of sterility. My associate, Dr. Dierksheide, is trying to get across the idea that we do want flexibility, at least in terms of medical devices. I am agreeable to this concept of levels of sterility. How we get that across to a consumer group is a problem. I know we are going to face this, if we go this way in medical devices. Right now I am faced with the situation of poured plated microbiological media being sold as sterile poured media. When we got into this situation about a year or two ago, the levels of sterility on poured plated media ranged anywhere from three plates in a hundred having organisms to as high as thirty percent of the plates showing organisms. Yet, they were going out as sterile poured plates. This is part of the problem here.

Take the situation of vacuum blood drawing tubes. These, as I understand, now are nonsterile. I would still like to see the industry do something to reduce the presence of gram-negative bacteria in some of these things, so in case the physician makes a mistake and there is backflow, we do not come up with a gram-negative septicemia.

Then you can get into a situation with a parenteral device. My wife has already mentioned that I was the chief author of the Sterilization Guidelines for Intraocular Lenses. She alerted me to the fact, as a result of her work with the soft contact lenses, that a manufacturer was thinking of a sterile assembly procedure for intraocular lenses. I made sure, when I wrote my guidelines, that I put in that we would not allow sterile assembly. You either terminally sterilize or you do not get into this business.

Let us examine the case of the large volume parenterals. I feel free to talk on this subject, since I was once with the Bureau of Drugs. Within that Bureau, there is divided opinion among several groups. There are scientific reviewing elements and inspectional elements. To try to get them to come out with a uniform opinion can be a very delicate job. In terms of large volume parenterals, I have heard the viewpoint stated that the probability of a survivor should be one in a billion (10^{-9}). My bosses in the Bureau of Devices have said to me, "Why are you accepting 10^{-6}?" I responded, "Well, parenteral devices are dry. The organisms do not grow. I can see why the Bureau of Drugs might want to go with a
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.
higher probability, since if an organism did get in, it might have

a greater chance to cause trouble if it started to grow in the solution". My point is that the body has defenses (I think Dr. Marcus has alluded to it) and not every organism which gets in is going to cause an infection. Again, the thrust of my remarks here is to make the point that I like the levels of sterility. But, how we are going to label to the satisfaction of the lawyers is really going to be a ticklish job here in the United States.

Comment by R. Jerussi:

I would like to comment on the levels of sterility, because it is something we are going to deal with eventually. I think what we have to try to do is to make the best product we can within reason and we will let the people use the product and handle it in the best way they know how. Now, perhaps, we will have to instruct them in the use of the product. We do not always have control over the product, and it would be nice to have products that were such that, if a failure occurred, the product would not even be considered as part of the failure. You would look elsewhere for the failure, not at the product. In discussing sterility, we have a difficult enough time getting together on how to declare it, how to define it, and how to achieve it. How are we ever going to get the different people who handle sterile products to get together? So I think we should not be inordinately concerned with how the doctor handles it or how the nurse handles it. If we are going to talk about a sterile product entering a ward, we must remember that the product is going into a nonsterile environment. The best we can do is to say that the material is sterile when it enters - whatever definition we accept as sterile - and then it is up to the people who handle it to handle it properly, and be trained in that use. Companies do get into the business of educating nurses and hospital personnel. This seems worthy, but I do not think we can go too far beyond that.

Comment by M. Bruch:

I want to comment for just a minute on what Dr. Jerussi said. I think the previous comment about the contact lens is interesting, because I find it unconscionable that we have reports from ophthalmologists that people who are fitting contact lenses do not resterilize them, or redisinfect them, or do anything between fittings of the patients. So I think what Dr. Jerussi said about how sterile products are handled is certainly pertinent.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Comment by A. Bishop:

I think Dr. Whitby should be allowed a reply to this.

Comment by J. Whitby:

I think I have been, as was Bob Ernst, misunderstood. I was trying to point out that nobody goes through the elaborate procedure that Dr. Greene was talking about yesterday to put a contact lens in the eye. It is quite true and has been shown that contact lenses can be a source of danger. If you pick them out of a sterilizing solution which is itself a culture, that is entirely unsatisfactory and not acceptable. But, I think that if you were using a procedure which gave you the possibility that there might be one living microorganism every thousand times that you did this procedure, you would already have a very safe product, relative to the other contaminants which might get into the eye.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

INDEX

Air, filtration sterilization, 22, 24-26, 59
effect on microbial counts, 37
HEPA filters, 24-26, 29
laminar airflow, 24-26, 29
American National Standards Institute, 264
Antimicrobial testing methods (see Sterility testing)
AOAC sporicidal test, 51
Association for Advancement of Medical Instrumentation, 267, 269, 272-273

Bacillus pumilus, 49-62, 75-76, 171, 173-178, 181-182
destruction curves, 53, 56-57
D_{Mrad}, 53, 56-57, 173, 175, 182, 189-190
dose rate, 174
dosimetric methods, 52-53
graded biological indicator, 60-65
radiation resistance, 172, 179, 182
spore stability, 174
spore yield, 173
Bacillus sphaericus, 73-75
Bacillus stearothermophilus, 44, 179, 189, 196, 216, 239, 290
spore characteristics for biological indicators, 198-201
survival rate, 200
z-values, 199, 216
Bacillus subtilis var. niger, 133, 135, 143
radiation resistance, 179
Becker, Frederick (see also Ethylene oxide toxicity), 268-270
Bioburden (see also Contamination), 17, 31-41, 59, 72, 77, 81, 90, 100, 135-141, 182, 285, 288
assay procedure, 135-141
Biological indicators (see also specific indicator), 31-36, 38, 43-66, 73-77, 91, 109, 169-192, 288-289, 291-292, 309, 346-347, 354
B. pumilus, 49-62, 73, 75-76, 171, 173-178, 181-182
B. sphaericus, 73-75
B. stearothermophilus, 47, 179, 189, 196, 198-199, 209, 215-216, 239, 290
B. subtilis var. niger, 133, 135, 143
calibration, 197-198, 239
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.
C. sporogenes, 51

death rate, 38
definition, 44, 291
D_{Mrad}, 171, 173, 175-177, 181-190
dosimetric methods, 52-53, 56
graded biological indicators, 60-65, 240
history of, 45-51
microbial radiation resistance, 73, 79-81, 169-192
minimal requirements, 48
monitoring cycles, 39, 193-229
M. radiodurans, 171-172, 178-181
rate of sterilization, 38
S. faecium, 75, 171-172, 178-179
Ser. marcescens, 84
Scandinavian standards for, 346-347
statistical sampling, 33, 35, 69-85, 195-196, 207, 211, 213-215
British Pharmacopoeia, 248

Canada, device regulation, 319-323
Bureau of Medical Devices, 320
Department of Health and Welfare, 319
Food and Drug Act, 319
manufacturing practices, 323
standards, sterility, 320-323
U.S. Pharmacopeia testing methods, 322
Carrier vehicles, biological indicators, 45, 47, 61, 76
aluminum foil, 351
filter paper, 153, 166, 242-244
intravenous catheters, 179, 181, 278
laparotomy drape, ethylene oxide sterilization of, 132-133, 136, 138-139, 156, 159, 162, 164-165
latex Foley catheter, ethylene oxide sterilization, 132, 136, 139, 153-154, 157, 160, 165
needles, 179, 181
parenteral solutions, 207-208, 237, 245, 359
plastic disposable medical products, 179
polyethylene supports, 76
spore strips, 47, 179, 193-229, 238, 241
sutures, 53-57, 93
syringes, 131, 136, 139, 153, 155, 158, 161, 163, 165, 179, 181, 183-185
2-Chloroethanol (see Ethylene oxide toxicity)
Chymopapain, 283
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.
Clostridium sporogenes, 51

Compendial standards, sterilization and sterility testing, 305-318
National Formulary, 305
role of compendial tests, 313-314
U.S. Pharmacopeia, 305-315
Contamination (see also Environmental control), 19-21, 33, 47, 78-85, 207-208, 237, 245, 294, 301, 358-361
artificial contamination, 133, 185
contact and intraocular lens, 286-287, 358-361
inoculated products, 133
manufacturing facilities, medical products, 19-21, 133, 183, 236, 294
natural contamination, 133, 182, 185, 231
survivor probability, 33, 71-85, 185, 193-196, 202-207, 209, 215, 218, 238, 242
Council of European Communities, 332-334

Destruction curves, 53, 56-57
Device manufacture, 26-28
Distribution, indicator population, 76-77
D_{Mrad}, 171-190, 233
Dose/survival curves (see Irradiation sterilization, dosimetric methods)
Drugs, parenteral preparations, 207-208, 237, 245
D-values, 34, 88-89, 92, 125-129, 140-141, 171, 198, 202-203, 219, 231-234, 240, 285, 288

Ecker, E. E., 47
Endogenous pyrogen, 261
Endotoxin (see also Pyrogen testing), 248-251, 253-254, 256, 259, 261-262
rabbit bioassay, 245-249, 251, 257, 259-260
Environmental control, manufacturing (see also Good Manufacturing Practices), 17-30, 181-183, 289
barrier technology, 17, 26-27
effectiveness, determination of, 28-29
manufacturing facility design, 22-23
Escherichia coli, 253-254, 256
Ethylene glycol (see Ethylene oxide toxicity)
Ethylene oxide sterilization, 32, 34, 40, 90, 109-168, 241, 282-296, 345-347, 351
B. subtilis var. niger, 133, 135, 143
biological indicators, 36, 51-52, 90, 109-111, 133, 135, 290, 346-347, 351
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

D-value, 125-129, 140-141, 143, 148, 240, 285
fraction-negative data, 141-148
graded biological indicators, 60-65
laparotomy drape, as carrier, 132, 136, 138-139, 156, 159, 162, 164-165
latex Foley catheters, as carrier, 132, 136, 139, 153-154, 157, 160, 165
monitors, biological, 351
overkill, 40, 90
process control, methods and materials, 111-114
product resistance relationships, 109-168
product resistance testing, 141-153, 166
residues, 267-272
shelf-life, 351
stratification of gas, 121-123, 130
stratification of humidity, 111, 113, 117-120
survivor probability, 140-141
syringes, 131, 136, 139, 153, 155, 158, 161, 163, 165
temperature stratification, 111, 114-116, 130
USP sterility testing, 109-110, 283
Ethylene oxide toxicity, 267-274
AAMI, 267, 269, 272-273
American National Standards Institute, 264
biological studies, device residue limits, 267-272
blood studies, 272
blood vessel endothelium, 278
chemical studies, analytical methods, 292-293
gas Chromatograph, 273
gravimetric method, 273
2-chloroethanol (see also Ethylene oxide toxicity, residues), 267, 270, 275
cuprophane, 272
endotracheal tubes, 278
ethylene glycol (see also Ethylene oxide toxicity, residues), 267, 270, 275
ethylene oxide, concentrations, 267-268, 270-272, 275
ethylene oxide gravimetric reference method, 273
fabrics, reaction to, 270-271, 278
hemolysis studies, 272
hospital documentation, 276
human skin irritation, 270-272, 275
implant, size effect, 270
intravenous catheters, 278
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

mammary prosthesis, 276
mutagenicity, 276
polyvinyl chloride tubing, 268-273, 277
reaction products, 267, 270
residue, safety limits, 264, 268-270, 275-276, 283, 351
residues, 90, 131, 267-270, 275-276
sterility assurance, 32
Schupack, J. L., 270
sensitization, 271, 276-277
skin coverings, occlusion dependency reactions, 270-271, 278
subcutaneous implantation studies, mice, 268-270
tissue damage, 268-270
Etiocholanolone, 259
European Economic Community, 349-351
European Pharmacopeia, 314, 334-335, 344
Exogenous pyrogen, 261

False positive frequency, 78-79, 81, 87, 92
FDA, 88-89, 91, 104, 267-268, 275-276, 283-284, 286, 288, 290, 294-295, 297-298, 301-302, 308, 327, 338
endotoxin, standardization, 256
ethylene oxide residues, safety limits, 267-268, 275-276
mutagenicity, 276
Federal Register, 101, 289
IND, 281
Limulus amebocyte lysate test, definition, 249, 256, 278-279
NDA, 281, 284, 293, 327
sterility, definition, 301
Federal Food, Drug and Cosmetic Act (see also FDA), 284, 297, 313
Federal regulations (see specific agency or country)
Filtration, airflow, 24-26, 29
Filtration, sterilization, 291-292
Food processing, 96-97, 99-100, 322
French Pharmacopoeial requirement, biological indicators, 73-75

German Pharmacopeia, 332-333
Germany, sterilization legislation, 331-334
Drug Act, 331-333
German Pharmacopeia, 332, 335
GMP, 332-335
history, regulatory procedures, 332-335
standards, regulations, 334-335
sterility, definition, 332
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

Good Manufacturing Practices, 17-31, 137, 166, 237, 281-283, 289-290, 292-295, 297, 299-301, 313, 327-330, 332-335, 357
environmental control, 17-30
Germany, 332-335
Italy, 339
Standard Operating Procedure, 21-23
United Kingdom, regulations, 325-350
Graded biological indicators, 60-65
Graded grid, 62-65
Gram-positive bacteria, Limulus reaction (see also Pyrogen testing), 261

Heat sterilization, dry, 45
Heat sterilization, wet, 45, 51, 193-229, 291, 334
B. stearothermophilus, 44, 215-216, 239
biological indicators for, 47-57, 51-52, 334
heat destruction data, analysis of, 207-229
heat resistance, 193-229, 235
monitoring, 193-229
steam sterilization, 45, 51-52
steam sterilization release, 351

Industrial sterilization, quantitative microbiological controls, 69-86, 109-168, 333-347
ethylene oxide, time/survival curves, 89
heat, time/survival curves, 69
indicator population, distribution, 77-85
irradiation, dose/survival curves, 69-79, 189-190
sterility tests, 77-85
Irradiation sterilization, 32, 34, 111, 284
B. pumilus, 49, 52-62, 171-172, 179, 181-182, 189-190
B. stearothermophilus, 179, 182
biological indicators, 291, 346-347, 351
D_{Mrad}, 171-185, 188-190
dose/survival curves, 69-77, 186
dosimetric methods, 52-53, 89, 187-188, 347
eye ointment sterilization, 284
fraction-negative response, 185-187
M. radiodurans, 171-172, 178-181
reference inactivation curve, 74
resistance to, 73, 79-81, 93, 169-179
S. faecium, 75, 171-172, 178-179
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.
shelf-life, 351

spore resistance, 38, 73, 79-81, 169-192
sterility assurance, 32, 79-85, 169, 185, 284-285
sterility test, 51-58, 77-79, 187
toxic residues, 285
Isolation equipment (see Environmental control, manufacturing)
Italy, regulation of sterilization, 337-340
Center for Disease Control, U.S., 338
FDA, U.S., 338
history, sterilization regulation, 337
Istituto Superiore di Santa, 337
NIH, U.S., 338
Public Health Service, 337

Kelsey, J. C., 43, 66, 105-106
Kilmer, Fred B., 1-16, 45, 57, 98, 106, 322, 351
Koch, Robert, 45, 51, 97

Limulus polyphemus (see also Pyrogen testing), 255
Limulus pyrogen tests (see Pyrogen testing)
Lipopolysaccharide, 257
Lister, Lord Joseph, 4-5, 322

Manufacturing facilities, contamination control (see Contamination)
Mathematical model, quality control, 70-85, 100, 125, 194-195, 231-232, 237
Medical products, contamination control, 19-21
Microbial death
confidence limits, 75
death rate curve, 33, 186, 235, 310
false positive frequency, 78-79, 81, 87, 92
microbial radiation resistance, 73, 79-81, 93, 169-192
statistical methods, 78-85
Microbial load (see Bioburden)
Microbial radiation resistance, 73, 79-81, 93, 169-192
Microbiological control, 17-30
historical perspective (see also Sterilization; Surgery, sterility in), 1-16
manufacturing practices, 17-30, 109-168
product sterility assurance, 169
Micrococcus radiodurans, 171-172, 178-181
radiation resistance, 178-179, 235
Mouse, subcutaneous implantation studies, ethylene oxide toxicity,
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

268-270
pyrogen tests, 262

NIH, 338
Nitroblue tetrazolium test, pyrogenicity, 247, 253-254, 262
Nordic Pharmacopeia, 344-346
Norway, sterility regulations, 346

Ophthalmic drugs, 282-284
Overkill, 40, 90, 105

Parenteral drugs, 207-208, 237, 245, 282, 359-361
Pasteur, Louis, 97
Polyvinyl chloride (see Ethylene oxide toxicity)
Probabilities of survivors, 31-40, 71, 140-141, 169, 182, 288-289, 322, 360
Pseudomonas aeruginosa, 312
Pseudomonas pyrogen, 257
Pyrogen testing, 245-266
British Pharmacopoeia, 248
comparative tests, 249,256, 258
controls, 245-246
accuracy, 246-247
sensitivity, 246
specificity, 246
test theory, 246
endogenous pyrogen, 261
endotoxin, 248-251, 253-254, 256, 259, 261-262
standardization, 256-257
equivalent concentrations, 249
exogenous pyrogen, 261
false positive reactions, 248
gram-positive bacteria, 261
in vitro assays, 251-256, 260
actinomycin D, 249-250, 262
bacteriologic culture method, 252
chick embryo lethality test, 249, 251
complement titration, 251
Limulus amebocyte lysate test, 249, 254-256, 258, 261, 278-279
nitroblue tetrazolium test, 249, 253-254, 262
Technetium-albumin radioactivity, 259-260
tissue culture cells, 251
in vivo assays, 250-251
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

epinephrine skin test, 249-251
rabbit bioassay, 245-249, 251, 258-260
Technetium-albumin radioactivity, 259-260
Limulus amebocyte lysate, 249, 256, 259
nonpyrogenic, definition, 252
parenteral drugs, 360
Pseudomonas pyrogen, 257
pyrogen tests, types, 248
pyrogen-free, 249, 252-253
small unit conversions, 249
test theory, 246
typhoid pyrogen, 257

Quality control, statistical, 69-86, 91-94

Rabbit peritoneal exudate leukocytes, 261
Rabbit pyrogen test (see Pyrogen testing)
Radiation (see Irradiation sterilization)
Reference standards, radiation sterilization, 49
Ridley sterilization procedure, 287

Scandinavia, reference indicator, 89-90, 242
Scandinavia, sterilization control, 341-347
definitions, 341-346
Denmark, 346
European Pharmacopeia, 344
GMP, 342-346
Sweden, 342-346
Nordic Pharmacopeia, 344-346
Norway, 346
Regulatory review, 341-347
Sweden, 341-346
Serratia marcescens, 84
Shupack, J. L. (see also Ethylene oxide toxicity, human skin irritation studies), 270
Spearman-Karber equations (see also Heat sterilization, wet; Spores, biological indicators for wet heat sterilization), 210-211, 214
Spearman-Karber times (see also Heat sterilization, wet; Spores, biological indicators for wet heat sterilization), 212, 215, 219-221, 223-225
Spores, biological indicators for wet heat sterilization, 193-229
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.
characteristics of, as biological indicators, 198-201

D-value, 219
monitoring, 193-229
shelf-life, 351
Spearman-Karber equations, 210-211, 214
Spearman-Karber times, 212-215, 219-221, 223-225
storage conditions, effect, 219-222
survival rate, 200, 212-215
temperature, effect on performance, 216-219
z-values, 199-216
Standards certification, 350
Standards, sterility, 349-350
Steam and dry sterilization (see Heat sterilization)
Sterility standards, 34, 43, 88-89, 92, 100-104, 125-129, 140-141, 243, 297-303, 320-323, 354-356, 360, 381
Bureau of Drugs, 281, 283, 289
definition of, 43, 100-104, 301, 352, 356-357
levels of sterility, 360
packaging materials, 282, 284
Sterility testing, 29, 51-53, 77-79, 87-94, 169, 252
AOAC sporicidal test, 51
B. stearothermophilus, moist heat (steam) monitors, 47, 209, 215-216, 239
bioburden assay procedure, 135-141
Canada, sterility control, 319-323
environmental control tests, 29
false positive frequency, 78-79, 81, 87, 92
filtration, 291-292
graded grid, 62-65
margin of safety, 70-85, 194-195, 202-229, 241
microbial spore challenge, 44
monitoring, wet heat, 195-229
NIH, 47
pyrogen testing, 245-266
rabbit bioassay, pyrogenicity, 245-249, 251, 258-260
radiation resistance, 177
sterility assurance, 243, 323
sterility standards, 354-356
survivor probabilities, 31-40, 71, 169, 182, 288-289, 322, 360
threshold febrile response, 257

Sterilization (see also Irradiation; Ethylene oxide; Heat; Filtration)
assurance, 281-303, 352-353
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

control, 352
Germany, legislation, 331-334
history, 1-16, 45-51, 65-66
Italy, regulation control, 337-340
monitoring cycles, 39
rate, 38
United Kingdom, sterility control, 325-330
Sterilization agents, 48
D-values, 34, 88-89, 92, 125-129, 140-141
minimal requirements, 48
testing spore strips, procedure for, 222-228
Streptococcus faecium, 75, 171-172, 178-179
Surgery, sterility in, 95-108
historical perspective, 1-16, 96-108
parenteral solutions, infectious hazards, 101
Sutures, surgical, 53-57, 93
Sweden, 341-346
Drug Decree, 341-346
National Board of Health and Welfare, 342

Technetium-albumin, radioactive (see also Pyrogen testing), 259-260
Tests, sterility (see Sterility testing)
Thermogenic drugs, 261
Time/survival curves, heat and ethylene oxide, 89
Typhoid pyrogen, 257

United Kingdom, sterility control, 325-330
devices, 325
hospitals, 328-329
licenses, 327-329
manufacturing, 327-330
medicinal product, definition, 325-327
Medicines Act, 325
statutory control, 325, 327
voluntary control, 329-330
U.S., drug sterilization, regulatory control, 281-303
U.S. Pharmacopeia, 31, 43, 47, 88-90, 93, 104, 109, 246, 283, 288-290, 297, 305-313, 346
microbial limit tests, 311-312
preservatives, 312
pyrogen tests, 247-248, 257-258, 260, 312-313
rabbit bioassay, 247-248, 257-258, 260, 312-313
Single user license provided by AAMI. Further copying, networking, and distribution prohibited.

sterility, definition, 43, 310
sterility tests, 31, 109, 236, 285, 298, 306, 340
definitions, 306
incubation, 308-310
media, 307-308
specimen taking, 306-307
statistical design, 310-311
validation of results, 311

Water purity (see also Good Manufacturing Practices), 22, 26, 258-259
WHO, 314, 332-333

z-Values, 199, 216

Single user license provided by AAMI. Further copying, networking, and distribution prohibited.