Biological Indicators in the Pharmaceutical and the Medical Device Industry

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ABSTRACT: In the first part of this article BIs are closely examined regarding their constituents, attributes, and problems. The idea is developed that BIs are accurate measuring units when properly used but cannot be used as standards, that sterilization processes are desired to kill microorganisms, therefore, they must be validated in a way to demonstrate microbial kill, the role played by BIs. In the second part the use of BIs in the specific sterilization processes of wet-heat or steam, dry-heat, ethylene oxide, and radiation sterilization is discussed. Recommendations are made regarding the use of BIs in these processes.

A "biological indicator system," as used in the sterilization area in the pharmaceutical and medical device industry, is a system consisting of microorganisms, usually bacterial spores, plus the procedures and equipment used in preparing the biological indicator and making the measurement, that makes it possible to determine the level of the sterilization process delivered at a specific location in the product. We use the term, "biological indicator" or "BI" as though we are talking about a measuring entity, such as a mercury-in-glass thermometer. However, our casual terminology belies the complex nature of the BI measurement system. The purpose of this paper is to review the basic aspects of biological indicators (BIs) and to give some perspective on the use of BIs in the design, validation, and/or monitoring of sterilization processes.

Variability in the Living Spore

Before proceeding to discuss the BI as a measuring system, we need to have an understanding or perspective of the nature of the basic element of the BI system, the bacterial spore. First, a bacterial spore is not a fabricated, inanimate object but a living entity, and while we know a lot about living systems, what we do not know, even about a one-celled organism, exceeds what we know by many orders of magnitude! For example, the genetics and biochemistry of spore-forming microorganisms, while extensively studied (1), is far from completely understood. Even when we have a spore crop grown from one cell so all spores in, for example, an inoculum of 10^6 spores can be thought of as clones of the original parent cell, there still will be some small differences among individual spores. These small differences among clones of the parent cell may be due to one or all of the following: access to nutrients during growth will not have been identical in all spores; the growing and reproducing vegetable cells of bacterial spore formers contain parallel metabolic pathways which may lead to small morphological and biochemical differences; and mutation of the genetic material in all biological systems is continuous and occurs on a random basis among individual cells. If we expect some variability among spores in a spore crop when, as far as we can control, all spores are exposed to identical growth conditions, we do not need to stretch our imagination very far to realize that when nutrient and environmental conditions vary in a measurable way, there will be wide variability in the progeny of the same parent strain of bacterial spores. Perhaps we can get the feel for this variability if we observe the variability of individuals of the same strain of domestic animal that have been grown under different conditions. We must conclude that identification of spores by genus, species, and strain is still only a qualitative classification similar to McIntosh apples, to mention something with which we all are familiar. The variability within this strain is very wide and, in our case, the variability from spore crop to spore crop grown in different laboratories also is wide and there is almost no way this variation can be identified and classified. The consequences of using a biological entity as a measuring unit must be recognized if our measurements are to be realistic. The attributes of the system must be taken into account in specifying how we use the system.

BIs Cannot Be Used As Standards

The physical measurement of sterilization process variables is critical in the control of sterilization processes. The measurement capability of BIs has been inferred to be just as critical in the control of sterilization processes. It is possible that sterilization scientists have generated some confusion regarding what is a primary measurement and what is a secondary measurement. Therefore, we will make a special effort to clarify the relative basis of various measurements.

Since we are using BIs as a measuring device, we must examine our measurement system from a metrology

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standpoint. Accurate and reproducible measurements are based on the use of measurement standards with calibrations traceable to designated national and international standards or well-characterized reference standards based upon fundamental constants of nature. Measurements have traceability to designated standards if and only if scientifically rigorous evidence is produced on a continuing basis to show that the measurement process is producing measurement results for which the total measurement uncertainty relative to national or other designated standards is quantified.

Measurements in our individual facilities are made, employing calibrated sensors and associated instrumentation that allow measurements to be traced to the national or other accepted standards. A calibration standard must be stable, reproducible, and independent of the associated measuring equipment. Environmental conditions, other than the variable being measured, must not affect the measured value.

The BIs that use living bacterial spores as sensing units cannot be a calibration standard, no more than we can have a standard rat, guinea pig, or *Homo sapiens*. From a metrology standpoint BIs cannot be a calibration standard; BIs must be calibrated in the laboratory using systems that are based on physical standards.

The fact that bacterial spores (BIs) cannot be a calibration standard from a metrology standpoint does not diminish their value as sterilization process measuring units, but their use must always be within the limits of their capability. The BI is a reasonably accurate and reproducible measuring system when properly used. However, it cannot be used as a measurement standard itself but must be calibrated using other accepted measurement standards.

The objective of the sterilization process is to kill microorganisms in or on the product. Consequently, we must validate that the sterilization system has the specified, required microbial killing power. There must be one place in all sterilization validation programs, regardless of the sterilization system, where the microbial killing power of the sterilization process is determined. This is done by actually assessing the microbial killing power of the process by measuring its effect on *calibrated microorganisms*. Restating our point: we can only measure the microbial killing power of a sterilization process by testing the process using calibrated microorganisms. Consequently, sterilization values, based on the measurement of physical variables, are all second when compared to the basic sterilization measurement, which is the measurement of the sterilization value of the process on the basis of the destruction of *calibrated microorganisms*!

Relating Physical and Biological Sterilization Values (Integrating Sterilization Parameters)

In the development of sterilization processes it is necessary to develop and indicate the relationship and accuracy of physically-determined sterilization values compared to biologically-determined sterilization values. In general, as the sterilization system becomes more complex, physical sterilization values become less accurate in predicting microbial kill.

TABLE I. Bacterial Spores Used as Biological Indicators for Different Sterilization Conditions

Sterilization Medium	Organism as a Source of Spores	Reference
Wet heat	*Bacillus stearothermophilus*	(2)
	Bacillus subtilis, 5230	(3)
	Bacillus coagulans	(4)
	Clostridium sporogenes	(2)
Dry heat	*Bacillus subtilis*, 5230	(5)
	Bacillus subtilis var. *niger*	(6)
	Bacillus stearothermophilus	(7)
Ethylene oxide	*Bacillus subtilis* var. *niger*	(8)
Radiation	*Bacillus pumilus*	(9)

We use spores as BIs because they can integrate the sterilization effect of a lethal agent, whether it be wet heat, dry heat, a chemical such as ethylene oxide (EO), or ionizing radiation. In both dry-heat and EO sterilization the amount of water in the spore (determined by the relative humidity (RH) of the atmosphere surrounding the spore) has a major effect on the kinetics of spore destruction. Hence, if the relative humidity varies from one test condition to another, the spores will not only react differently to the dry-heat or EO condition, but will integrate the overall microbial kill as affected by—in the case of dry heat—temperature, RH, and time—and for EO—EO gas concentration, temperature, RH, and time. The bacterial spores carry out the measurement and the integration to yield the microbial kill of the process. We have instruments that will measure the physical variables of these processes; however, we are at a rudimentary level regarding integration of the several effects for both dry heat and EO.

In some sterilization processes, for example, sterilization using gamma radiation, the sterilization of surfaces using saturated steam, and the sterilization of containers of a water-based product in saturated steam or a water atmosphere, the sterilization value calculated from physical parameters correlates well with the biological sterilization values. Therefore, in these systems a great deal of confidence can be placed in the sterilization values calculated from physical parameters.

The microorganisms used in a BI system are selected to fit the specific test condition. Consequently, we find the spores of different species and strains of microorganisms used for different, specific applications (Table I). The spores may be supported on a paper or metal carrier or they may be inoculated directly on or in the product. The assay or recovery method will itself have been validated as being applicable for the specific measurement requirement or test system. The system will include calibration tests. Since measurement of sterilization conditions using microorganisms are all comparative in nature, we compare the results under the unknown or test conditions with the results obtained under the known or calibration conditions.

General Problems in the Use of BIs

Microorganisms or their spores are the basic units or sensing elements of BIs. These living entities have certain attributes and liabilities because they are alive. We use spores in BI systems because of their attributes and their sensitivity to factors that affect their death kinetics. We must use them in the ways that will minimize their liabilities.

A major problem in the use of BIs is that we have no way of obtaining an instant readout in the same way we can obtain a readout from an F_0 integrator used with a wet-heat sterilization process. Two to 7 days incubation of the BIs is required before the results are available. Attempts are being made to develop systems where this incubation time is shorter, but even if the results were available in 2 hr after the end of the sterilization cycle, it would not change the basic use of the BI results, which is an after-the-process measurement. Consequently, sterilization processes must be controlled using easily-measured physical conditions.

Microorganisms are not only sensitive to the environmental conditions associated with sterilization agents but are sensitive to a large number of environmental conditions associated with the growth and enumeration of microorganisms. These include the time interval between application of the lethal agent and spore recovery, the recovery or growth medium, the environmental conditions of temperature and RH during incubation, incubation time, plus any other environmental conditions that will affect the spores and which can vary (10–13).

It was pointed out previously that the nature and sensitivity of microorganisms to environmental variables means that we cannot use BIs as standards. The best we can do is calibrate BIs using accurate physical measurements. Calibration is a critical step in the use of BIs; measurements with BIs are not absolute but are comparative in nature. We compare the results we obtain for the unknown condition with the test results we obtained for the known condition. Comparisons are only meaningful if the known and unknown conditions are the same, except for the (single) variable we are measuring.

Presently, BIs are distributed, in general, in two forms: in a prepared state, the majority of which are spores deposited on paper strips (spore strips), and spores in a liquid solution. Spore strips are, in general, used as they are, whereas the spores that are in solution are usually either suspended in a product or inoculated directly on to the critical surface. Measurement of the characteristics of the BI in the laboratory where the spores are originally grown, titered, and calibrated will be different than the resistance measured at a second laboratory where the spores are used. There will be differences because we know environmental conditions and personnel will not be the same. We have no way of knowing the magnitude of these differences without going into the two laboratories, carrying out tests, and thereby measuring the differences. If we carry out tests to measure differences in laboratories, the results are only valid for the days on which the tests were carried out, since there is a day-to-day variation in results in almost all microbiology laboratories. It is possible that the difference in results of tests between two laboratories will be small and either result acceptable for the intended use of the BI; on the other hand, the differences may be large and unacceptable. Ideally, BIs should be calibrated in the same laboratory and by the same personnel who will test the BIs after they have been used to measure a sterilization value.

We have been discussing problems in the use of BIs; it is possible some readers will assume that these conditions are such as to make spores not usable as biological monitoring systems. This is not true in that almost any procedure has to be carried out correctly in order for it to be effective. What we have been discussing is what can happen if there is not close control in the use of BIs. When care is taken, all the way from growing the spores through to utilization of the BI, very good results can be obtained. Bacterial spores can be used in a quantitative measuring mode to produce reproducible results. What we are stressing is the necessity of having adequate control procedures when we use BIs.

The Use of BIs in the Design, Validation, and Monitoring of Sterilization Processes

In the discussion that follows we will try to point out how BIs are used in the different sterilization systems. The four most common sterilization systems that are used today to sterilize drugs and medical devices are: wet-heat or steam, radiation, dry-heat, and ethylene oxide. The objective in the use of all these sterilizing agents is to kill microorganisms in or on the product; however, each sterilization agent is used and acts in a different way. Each has its own unique attributes as a sterilant and its unique requirements regarding monitoring and control of the process. BIs can be used in the design, validation, and/or monitoring of all these sterilization processes but not necessarily in the same way. Since there are large differences in many aspects of these sterilization systems, we should expect that the requirements for the use of BIs will also vary widely among these sterilizaton systems. Consequently, the application of BIs to sterilization process validation and monitoring requires a thorough understanding, not only of the BI system but the specific sterilization operation. We must understand both the sterilization system and the measurement system if we are to meet the requirements of the FDA regulations regarding production of sterile drugs and devices. The release of product into the marketing channels is impacted by the sterilization system and requirements for monitoring. Some sterile products can be released for use on the basis of sterilization cycle parameters; in producing other products it is only the results from BI-monitoring units which can develop the necessary assurance the specified process has been delivered to that part of the product most difficult to sterilize.

There is general understanding and agreement among the scientists working on sterilization in the pharmaceutical industry and the scientists in the regulatory agencies that the design of a sterilization process for a product that will be marketed as sterile must be based on the number of microorganisms on or in the product (bioburden), their

resistance, and the final low level of microorganisms in or on the product (a probability of a nonsterile unit, PNSU, of 10^{-6} is widely used as the standard for most terminally-sterilized products). It is usually stressed by personnel in the regulatory agencies that since the objective of the sterilization process is to kill microorganisms, we must validate the microbial killing power of the sterilization process at all critical points using an appropriate challenge of calibrated microorganisms to insure that the sterilization process meets the design specification. At the same time there are varying opinions regarding the degree of biological validation required for each of the sterilization systems. There are also varying opinions regarding biological monitoring requirements. The level of biological validation and monitoring is a function of the type of product and the sterilization system. In general, the requirements for biological monitoring are a function of the accuracy with which we can estimate the actual microbial killing power of the process using physical measurements. Accuracy varies with the type of product and the sterilization process (it is a function of the number of variables in the sterilization process that affects microbial kill). We always have at least two variables that must be integrated to give microbial kill. This is illustrated below.

Radiation Sterilization: Microbial kill is a function of the radiation energy times the time of action.

Wet-Heat or Steam Sterilization: Microbial kill is a function of the temperature multiplied by the time of action.

Dry-Heat Sterilization: Microbial kill is a function of the temperature, the water content of the spore determined by its ambient relative humidity, and the time of action.

Ethylene Oxide Sterilization: Microbial kill is a function of the temperature, the ethylene oxide gas concentration, the water content of the spore determined by its ambient relative humidity, and the time of action.

In the discussion that follows we will expand on the points above to indicate the use of BIs for each of the sterilization systems.

The need and use of biological measurements is closely associated with the nature of the specific sterilization process. Consequently, we will briefly discuss each sterilization method before we discuss the use of BIs with the specific sterilization system.

Wet-Heat Sterilization: Wet-heat sterilization, often referred to as steam sterilization, is the simplest, best-understood, and most widely-used sterilization system. It is economical, as well as being easy to monitor and control. The criterion for wet-heat sterilization is that the microorganisms that are to be killed by the process must be in equilibrium with water (liquid), i.e., saturation conditions exist where the microorganisms are located, the RH is 100%, and the water activity (a_w) of the microorganisms that are to be killed by the sterilization process is 1.00. The common wet-heat sterilization conditions are: microorganisms on surfaces in contact with saturated steam and microorganisms in products of high water content heated in containers (the water activity, a_w, of the microorganisms to be killed can be considered to be 1.00).

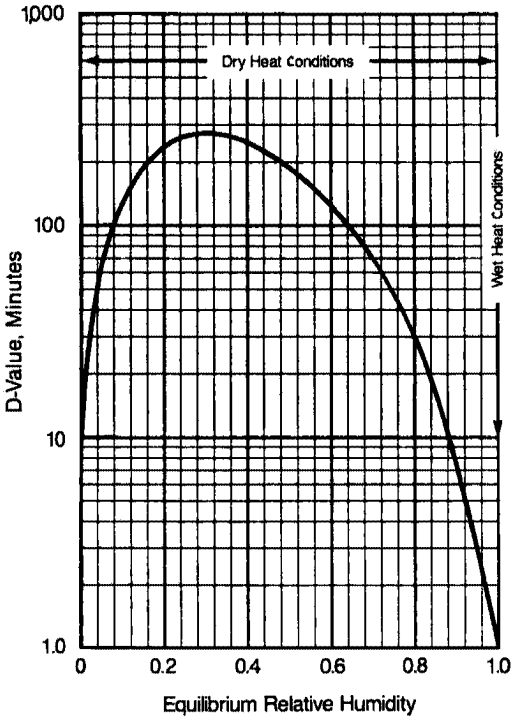

Figure 1—The effect of the equilibrium relative humidity of the environment surrounding the spores on their *D*-value; the commonly-used terms, "wet-heat conditions" and "dry-heat conditions," are identified.

The steam-heated autoclave or retort is the primary piece of wet-heat sterilization equipment in the pharmaceutical plant. When some objects, units, or systems are heated in the steam autoclave, dry-heat conditions may exist in parts of the object or system. Wet heat is one environmental condition; water, as a liquid, is present; the RH of the sterilizing environment is 100%. Dry heat includes all those conditions where the RH is less than 100%. Consequently, any occluded, mated, or enclosed part of an object, where the access of steam or water is restricted, will probably be in the dry-heat range of environmental (RH) conditions. The wet-heat/dry-heat relationship is shown in Figure 1.

In the sterilization of solutions in containers where the product, such as a large-volume parenteral (LVP) in glass or flexible containers, is heated in an autoclave or retort in steam or water, the steam or water only serves as a heat transfer medium. The microorganisms that constitute the bioburden of the product will be subject to wet-heat conditions if the product solvent is water and the water activity is 1.0. This is the situation for most parenteral solutions, irrigating solutions, and food products. However, in some products other solvents are used and in some products the composition and ionic strength is sufficiently high to lower the a_w below 1.0 to a point where we must consider that the microbial destruction is in the dry-heat area (data in Table II). (Dry-heat conditions in the wet-heat autoclave will be discussed further as follows.)

If we subject an object, for example, a piece of processing equipment, pipe, tray, tool (not wrapped or covered), to a 100% steam autoclave cycle where we accurately control the temperature of the sterilizing medium and

TABLE II. General Conditions of Water Activity During Sterilization of Different Items

Type of Item Sterilized	Water Activity (a_w) During Heat Sterilization
Solutions	
Saline, 0.99%	1.0
Glycerol, 11.25%	0.98 [a]
Glycerol, 86%	0.40 [a]
LVP Closures	<1.0 (Steam Vessel) (If water is not added prior to sterilization, a_w changes during sterilization and can approach 1.0.)
Unwrapped Goods (Tools, Etc.)	1.0 (Steam Vessel)
Empty Glass Vials	0 (Dry-Heat Ovens) 1.0 (Steam Vessel)
Wrapped equipment for aseptic processing operations	<1.0 (Vacuum Steam Cycle) (Should Reach 1.0 During the Sterilization Cycle)

[a] Reference 14.

where we control the length of the process by an accurate time-measuring device, we can integrate the time-temperature conditions and obtain a calculated sterilizing value F_o that has a high correlation with microbial kill. In other words, when we sterilize an object in saturated steam, integration of the time-temperature condition of the process will give us a measure of the microbial kill that is as accurate, or in some cases may be more accurate than, the microbial kill measured by a BI system. In the situation where an object is heated in saturated steam and we know the microorganisms that are to be killed are in contact with saturated steam where the temperature and the time of action are known, there is essentially no reason to monitor the rountine sterilization process biologically. The process will have to be biologically validated, but the purpose of the biological validation will be to establish that the physically-calculated sterilization values have the necessary equivalent microbial kill power. Usually, in the biological validation tests of the manufacturing equipment of sterile products, spores of *Bacillus stearothermophilus* are deposited directly on surfaces to be sterilized, deposited on planchettes of the same material as the objects to be sterilized, or on paper carriers. In the LVP industry *Bacillus subtilis* and *Clostridium sporogenes* are the spores that are usually used in validation of solution sterilization. In the SVP industry *Bacillus coagulans* or *Bacillus stearothermophilus* spores are usually used in validating the terminal sterilization of solutions. The spore support system must be applicable to the final sterilization process. The spores must be calibrated in the way they are used. The number of spores and their resistance must provide an adequate challenge for the specific sterilization process (15).

It is widely accepted that there are bacterial spores which have so high a wet-heat resistance [D(120 deg. C)-values of 2–10 min] that if a product contained a large number of the spores, 10^3 to 10^4 spores per product unit, the product will be severely degraded before we reach a PNSU of 10^{-6}. Fortuitously, almost all these organisms are thermophiles and are nonpathogenic to man. Our normal pharmaceutical and food products are produced so the initial product bioburden of these resistant organisms is low. The D(120 deg. C) of typical LVP microflora will be of the order of 0.2–0.5 min. The net result is that we have the possibility of using a highly-resistant spore (D, 120 deg. C of 3.0 min) as a BI to validate and monitor a wet-heat sterilization process that is based on product microflora resistance of a much lower level (D, 120 deg. C of 0.5 min). This "order of magnitude" difference between the BI resistance and the product microflora resistance allows us to biologically monitor, by count reduction procedures, processes that will produce a probability of a nonsterile unit (PNSU) of 10^{-6}.

In the development of processes for low-acid, canned foods, spores of *Clostridium sporogenes* or *Bacillus stearothermophilus* are used in validation. One type of biological validation used for canned food processes is the inoculated pack (16). Today, the processes, for food sterilized in continuous or continuously-agitating autoclaves or retorts, are usually biologically validated using the count reduction procedure (17) either by directly inoculating spores into the product (18) or using spores in a carrier, such as a plastic rod BI unit (19). In general, BIs are not used to routinely monitor food sterilization processes.

Situations Where Microorganisms on Products Heated in a Steam Autoclave are at Dry-Heat Conditions During Part of the Sterilization Process: In equipment and component sterilization, where no water is present at the start of the sterilization process, we rely on the increased diffusion rates and permeability of water at elevated temperatures to help water move to the sterilization site, thereby increasing the RH, which increases the microbial kill rate, so contaminating microorganisms are rapidly killed. For example, we may have a wrapped item, which may initially be dry or at a very low RH and contain air. During the steam sterilization process the air is removed and water (steam) moves throughout the item. Consequently, toward the end of the process the contaminating microorganism present will be subjected to an RH condition at or approaching 100%. In most cases we are moving from a dry-heat microbial destruction condition to a wet-heat microbial destruction condition. We can calculate F_o-values at the sterilization site from thermocouple time-temperature data. However, the results will not be meaningful for the varying RH conditions that exist at the sterilization site. It is usually impossible to mathematically integrate the effects of a combination of a wet- and dry-heat treatment; the only way we can do this is to integrate the sterilization effects using an appropriate BI system. In these cases a BI with a high dry-heat resistance (such as *Bacillus stearothermophilus* or *Bacillus subtilis*) can be used in a BI system to integrate the effect of time, temperature, and RH.

In the biological validation and monitoring of these complex sterilization systems we can use a BI system to establish that the design F_o-value has been delivered to the product. Using a BI system we are able to show we have

achieved the biological equivalent of the design F_o-value, which gives assurance the process will inactivate any naturally-occurring bioburden present on the equipment or component.

Microorganisms located in the mated areas of closure systems of metal and glass containers of products sterilized in an atmosphere of saturated steam may be at an RH of less than 100% during part or all of the sterilization process, therefore subject to dry-heat destruction rates. BIs containing properly-calibrated microorganisms can be used in these areas to establish that an adequate sterilization value is delivered to these areas during the process (20, 21).

Another area where there are wet-heat/dry-heat problems is the fluid path or other enclosure in a flexible container where the fluid in the container that is sterilized by wet-heat conditions does not contact the initially-dry areas. BIs placed in these areas, where the RH changes during the process, can integrate the overall sterilization effect and indicate if sterilization has been accomplished (20).

Dry-Heat Sterilization: The killing of microorganisms under dry-heat conditions falls into at least three distinct areas: (*a*) high-temperature sterilization of glassware where sterilization and depyrogenation are carried out in the same operation; (*b*) dry-heat sterilization of products; and (*c*) sterilization of dry, occluded, or mated areas of a closure system where the container is heated in a wet-heat environment and the product inside the container is sterilized by wet heat.

(*a*) *High-Temperature Sterilizing of Glassware.* In devices, such as a hot-air sterilizing tunnel, that are used for sterilization of vials and ampuls used for small-volume parenterals, the containers are both sterilized and depyrogenated in one operation that takes place at very high temperatures, perhaps as high as 300–360 deg. C. When containers are subjected to a process that is severe enough, time-temperature-wise, to destroy pyrogenic material on the surface of the containers, it is far beyond the upper limit of the survival of dry-heat microorganisms, especially those that would be used in BIs. It is therefore axiomatic that if the operation of sterilization and depyrogenation is sufficient that an endotoxin inoculum on the container will be destroyed by the process, the use of a dry-heat type of BI containing *Bacillus subtilis* var. *niger* spores will be meaningless.

(*b*) *Dry Heat Sterilization of Products.* In the dry-heat sterilization of products, such as powders and creams using dry heat, BIs can be used advantageously. In producing these BIs, *Bacillus subtilis* va. *niger* or in certain cases *Bacillus subtilis*, 5230, can be used effectively. In the dry-heat sterilization area there are three variables that must be contended with: time, temperature, and the moisture condition of the spores during heating. BI units can integrate the effect of these three variables; they are used effectively with certain types of products, or even powdered material, that are dry-heat sterilized.

(*c*) *Sterilization of Closure Systems.* This dry-heat sterilization area already has been discussed under "wet-heat sterilization." In most container closure systems there are areas that are not penetrated either by the saturated steam environment of the autoclave or by the saturated vapor inside the container during at least part of a sterilization cycle. This condition also exists where there is a fluid path or other enclosure in a flexible container where the fluid in the container is sterilized using steam or hot water. BIs put in the dry areas can integrate the sterilization value delivered to these dry-heated areas and indicate if sterilization is adequate.

Ethylene Oxide: In the ethylene oxide (EO) sterilization process there are four variables that, together, produce the microbial kill. These are time, temperature, gas concentration, and RH. It is impossible, to date, to accurately integrate by physical methods these four variables regarding the microbial kill potential of an EO process. It is possible to use a BI system to directly measure the effect of the several EO sterilization factors as they act together.

In EO sterilization we consider that the entire bioburden has sterilization resistance less than or equal to that of the microorganisms in the BI system. Consequently, we cannot measure to a probability level of 10^{-6}. In this approach the procedure is to validate fraction-cycle processes, using a BI with an N_o of 10^4–10^6 (8, 22), that provides assurance of a PNSU of 10^{-6}.

BIs are normally used to monitor each load of product sterilized using EO. Only a biological system can effectively integrate the effect of the four attributes of the process, time, temperature, EO concentration, and RH into a single-measure survival or kill of the BI.

Radiation Sterilization: In radiation sterilization it is the energy dose received by the product that provides the lethal effect on microorganisms. It is possible to integrate the lethal effect using calibrated bacterial spores (*Bacillus pumilus*) or through the use of a resistant vegetative microorganism, *Micrococcus radiodurans* (9).

Presently, it is generally assumed that a 2.5-megarad radiation dose is overkill for sterilization of medical devices. For most medical devices sterilized today it is difficult to find microorganisms that will survive this dose at a challenge level of 10^6/unit. Lower doses can be used for bioburden-based processes using one of the AAMI dose-setting methods (23).

BIs made using radiation-resistant microorganisms are used to validate radiation sterilization processes. A BI made using microorganisms with a *D*-value of 0.2 megarads and N_o of 10^6 could be used to validate a dose up to 1.2 megarads. The use of the calibrated BIs in the validation insures that the process has the desired microbial kill capability. However, for those firms which determine dose based on a bioburden-based method, BIs are generally not used in the validation program.

Tallentire (24) suggests that the validation and monitoring of radiation sterilization processes can probably be done more effectively and economically using physical dosimeters.

Conclusions

A BI, even with its inherent variability, is reasonably accurate and reproducible in measuring a sterilization

effect when properly used. The type of sterilization process must be considered in determining whether the biological indicator should be used in the initial validation of a sterilization process, in validation and routine qualifications, or in validation, qualification, and in routine monitoring.

An understanding of BI capabilities and the natue of the sterilization process is a prerequisite to their use. Faulty use of BIs can often lead to the conclusion that a sterilization process is inadequate when, in fact, it may be more than adequate for the sterility assurance desired.

A BI system, because it uses living bacterial spores as the measuring entity, cannot be a primary standard but must be calibrated using a physical measurement system traceable to recognized primary standards.

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References

1. Levinson, H. S., Sonenshein, A. L., and Tipper, D. J. (Eds.), "Sporulation and Germination," American Society for Microbiology, Washington, DC, 1981.
2. Pflug, I. J., and Smith, G. M., "Survivor Curves of Bacterial Spores Heated in Parenteral Solutions," in *Spore Research 1976*, A. N. Barker, J. Wolf, D. J. Eller, D. J. Dring, and G. W. Gould (Eds.), Vol. II, Academic Press, Incorporated, London, 1977.
3. Odlaug, T. E., Caputo, R. A., and Graham, G. S., "Heat resistance and population stability of lyophilized *Bacillus subtilis* spores," *Appl. Environ. Microbiol.*, **41**, 1374 (1981).
4. Jones, A. T., and Pflug, I. J., *"Bacillus coagulans*, FRR B666, as a potential biological indicator organism," *J. Parenter. Sci. Technol.*, **35**, 82 (1981).
5. Fox. K., and Pflug, I. J., "Effect of temperature and gas velocity on the dry heat destruction rate of bacterial spores," *Appl. Microbiol.*, **16**, 343 (1968).
6. Drummond, D. W., and Pflug, I. J., "Dry heat destruction of Bacillus subtilis spores on surfaces—effect of humidity in an open system," *Appl. Microbiol.*, **20**, 805 (1970).
7. Murrell, W. G., and Scott, W. J., "The heat resistance of bacterial spores at various water activities," *J. Gen. Microbiol.*, **43**, 411 (1966).
8. Caputo, R. A., and Odlaug, T. E., "Sterilization With Ethylene Oxide and Other Gases," Chapter 2, in *Disinfection, Sterilization, and Preservation*, S. S. Block (Ed.), 3rd ed., Lea and Febiger, Philadelphia, PA, 1983.
9. Silverman, G. J., "Sterilization by ionizing radiation," Chapter 4, in *Disinfection, Sterilization, and Preservation*, S. S. Block (Ed.) 3rd ed., Lea and Febiger, Philadelphia, PA, 1983.
10. Caputo, R. A., Rohn, K. J., and Mascoli, C. C., "Recovery of biological indicator organisms after sublethal sterilization treatment," *J. Parenter. Drug Assoc.*, **34**, 394 (1980).
11. Pflug, I. J., Smith, G. M., and Christensen, R., "Effect of soybean casein digest agar lot on number of *Bacillus stearothermophilus* spores," *Appl. Environ. Microbiol.*, **42**, 226 (1981).
12. Boris, C., and Graham, G. S., "The effect of recovery medium and test methodology on biological indicators," *Med. Device Diagn. Ind.*, 7(2), 43 (1985).
13. Busta, F. F., Foegeding, P. M., and Adams, D. M., in *Sporulation and Germination*, American Society for Microbiology, Washington, DC, 1981, p. 261.
14. Troller, J. A., and Christian, J. H. B., "Water Activity and Food," Academic Press, New York, 1978.
15. Pflug, I. J., "Textbook for an Introductory Course in the Microbiology and Engineering of Sterilization Processes," rev. 5th ed., Environmental Sterilization Laboratory, University of Minnesota; Minneapolis, MN, 1982.
16. Pflug, I. J., "Selected Papers on the Microbiology and Engineering of Sterilization," 4th ed., Environmental Sterilization Laboratory, University of Minnesota, Minneapolis, MN, 1982.
17. Pflug, I. J., and Holcomb, R. G., "The Use of Bacterial Spores as Sterilization Process Monitoring Devices: A Discussion of What They Can Do and Some of Their Limitations," Proceedings of the Third PMA Seminar Program on Validation of Sterile Manufacturing Processes, Biological Indicators, Pharmaceutical Manufacturers Association; Washington, DC, 1980.
18. Yawger, E. S., "Bacteriology evaluation for thermal process design," *Food Technol.*, **32**(6), 59 (1978).
19. Pflug, I. J., "Method and apparatus for sterility monitoring," U.S. Pat. 3,960,670 (1976).
20. Odlaug, T. E., "Microbiological validation of container closure systems," *Pharm. Manufac.*, **1**(1), 30 (1984).
21. Pflug, I. J., "The role of water in heat sterilization," *Pharm. Manufac.*, **1**(6), 16 (1984).
22. AAMI, "Guideline for Industrial Ethylene Oxide Sterilization of Medical Devices," Association for the Advancement of Medical Instrumentation, Arlington, VA 22209, 1981.
23. AAMI, "Process Control Guidelines for Gamma Sterilization of Medical Devices," Association for the Advancement of Medical Instrumentation, Arlington, VA 22209, 1984.
24. Tallentire, A., "Aspects of microbiological control of radiation sterilization," *Int. J. Radiat. Steril.*, **1**, 85 (1973).