特约专稿

The factors of biological indicator resistance

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ABSTRACT: Biological indicator (BI) is the gold standard in verifying the effectiveness of the sterilization processes. The resistance of biological indicators generally means the population of viable organism and D value. The population and the D value are all not intrinsic, which will vary according to the lot, the manufacturer. Spores, carrier, recovery medium and the packaging compose the self-contained biological indicators. And all of the component will affect BI resistance. Different kinds of carrier spore inoculated on exhibit different manners to attach different D-values. Another factor that affects the resistance of BI is spore production methods. The sporulation conditions including temperature, pH, humidity, nutrient and ions content, largely influence the spore resistance to heat. Furthermore, resistometer performance and the different test method are also the major source of the D value variation. All the factors affect the resistance of biological in an comprehensive manner, and when a resistance variation occur, all the factors above should be taken into consideration.

KEY WORDS: biological indicator; resistance; viable spore count; D value

1 Introduction

Biological indicator (BI) is used to verify the effectiveness of the sterilization processes, which is routinely used by medical device manufacturers, healthcare, the pharmaceutical, and food industries. A biological indicator is contained of a kind of microorganism who has the highest resistant to the sterilizing agent, like dry heat, moist heat, ethylene oxide, hydrogen peroxide, peracetic acid and so on. In ISO11138, the biological indicator is defined as a test system containing viable microorganisms providing a defined resistance to a specified sterilization process [1].

Chemical indicators (CI) monitor the efficacy of a sterilization procedure via the color change. Compared with chemical indicator (CI), a biological indicator provides a direct evidence to monitor whether the sterilization process conditions are sufficient to kill spores. The use of high number and high resistant spores within BI can assure the fewer and less resistant microorganisms existing on medical devices have also been killed. More importantly, biological indicator is able to detect even marginal sterilization failures that result from inability to reach temperature, inadequate air removal or superheated steam. Hence, BI is the "gold standard" in sterilization monitoring. And BI is recommended to monitor the steam sterilization once a week in China. D value, the decimal reduction time, means the required time or dose to inactivate 90 % of a population of the test microorganism under stated dose conditions[1]. Resistance characteristics of biological indicators mean the viable count population of a BI and the D value of a specified BI. While the resistance of each batch of biological indicators varied according to the manufacturer and the lot.

2 Biological indicator

The test organisms contained in BI should be a defined strain, which has the highest resistance to the sterilizing agent. The test organism shouldn't be pathogenic bacterium. And when handling or transporting, it

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should not need specific procedures. During storage and transporting, the resistance characteristics are able to maintain the same. Traditionally, the test organisms of biological indicators are spores. But if the organism can be certified with appropriate resistance, it's also suitable. For moist heat, the widely used test organism is the spore of Geobacillus stearothermophilus.

The key factors that affect a biological indicator are purity, population and D value. The microorganism in a BI must be verified for purity by phenotypic identification, gene sequence alignment or other approaches[2]. The population of biological indicators is typically $>1\times10^5$. When performing the BI population according to USP or ISO11138, the results should be not less than 50% and not more than 300% of manufacturers label claims[1,3].

D value is the most important characteristic of biological indicators, which is a value that can evaluate the BI resistance to a particular sterilization process. When exposed to sterilizing agent, the rate of spore death is linear. And D value is the concept of above logarithmic decrease. The D value will fluctuate according to the different experimental conditions. The higher the D-value, the more resistant the microorganism is to destruction[4]. A self-contained biological indicator is composed of test organisms, carrier, primary packaging, secondary packaging and growth medium. Test organisms composed the basic biological indicator. The suspension and the inoculated carrier are two different forms to carry test organisms. Self-contained biological indicator is easy to use with no need for aseptic technique.

3 Factors that affect BI resistance

The resistance of biological indicators generally means the population of viable organism and D value. The population and the D value are all not intrinsic, which will vary according to the lot, the manufacturer. And what the resistance measured is about the BI system but not simply the spores. In General, the factors impacting the resistance of BI conclude the list below. First of all, the components of BI: carrier material, primary packaging, recovery media. Then, the spore preparation process will also affect the resistance. And the test method, the resistometer used are all make a difference to the result.

3.1 Carrier material

Spores are inoculated on various carrier platforms which concluded solid carrier like paper strip, tyvek disc, glass fiber, stainless steel disc and plastic and liquid carrier like water, ethanol and so on. And the most widely used are paper strip and stainless steel disc[5-6]. Spores exhibit different manners to attach to the carrier and exhibit different D-values with the same lot of spores. Spores interact with the surfaces differently depends on the surface morphology, surface hydrophilicity, the surface chemical property. When using different carrier to inoculate spores, the prepared BIs may show remarkable difference in the spore distribution and D value.

Volker Sigwarth studied the effect of different carrier materials on the resistance of spores of Bacillus stearothermophilus to H_2O_2[7] They evaluated 20 different materials. And the selection process was based on the material surface quality after decontamination and the risk of contamination. In their study, the D value can range between 1.0min to 33.7min. Due to the physical and chemical properties of the carrier, the BI resistance ranged remarkably .

The carrier caused resistance variations is easily to be demonstrated experimentally, we can get the D value of each material accurately. More difficultly, the homogeneity of mounting spores to carrier is inconsistence. Because of the spores are easy to gather inhomogeneous, which is not always being in monolayers on carrier surfaces. And the gather can protect spores from effective contact with the sterilant.

3.2 Sporulation conditions

Another factor that infects the resistance of BI is spore production methods. The sporulation conditions including temperature, pH, humidity, nutrient and ions content, largely influence the spore resistance to

heat[8-10]. Studies found that although the heat resistance is the intrinsic property of the spore species, it is strongly relevant to the mineral and moisture content of the media[11-13] Researchers studied the relationship of pH of the sporulation medium and the heat resistance of two different bacterial spore[14]. KOICHI SASAKI studied the relationship between the D value and the calcium concentration in the assay medium. The bacterial strain they used is Bacillus stearothermophilus ATCC 7953. And they found that ATCC7953 spore strips have a higher D value with the higher calcium concentrations in assay media. To minimize the variation of D value from as far as possible, the calcium concentrations in assay media is a main factor[15]. To produce spore crops of B. stearothermophilus, different temperature used in the microorganism culture showed different heat resistance of the spore. And many studies found that Metal ion concentration can regulate the heat resistance of spore[16-17]. Interestingly, about the effect of nutrient concentrations to spores D value, there are opposite result. Plackett-Burman discovered that the lower the nutrient concentrations, the greater the heat resistance of the spores. But Penna et al. found that the higher concentrations of yeast extract and peptone in the medium, the greater the heat resistance of the spores.

3.3 Resistometers

Resistometer is the test equipment designed to create defined reference combinations of the physical and/or chemical variables of a sterilization process, which is able to precisely control the critical parameters of the sterilization process[1]. It is used to characterize the performance of biological indicators. D value often varied between different laboratories, and the variation is greater than 20%. The major source of the above variation is the resistometer performance.

In ANSI/AAMI ST44:2002, the time required to hit the target temperature set point of a resistometer should be within 10 seconds, and it should be able to maintain the temperature within a deviation of 0.5 °C. At the end of the sterilization cycle, the post-vacuum time to reach atmospheric pressure must be within 10 seconds or less. The chamber of resistometer is fairly small when compared to sterilizer chamber. The design of small chamber is for the precisely controlling of the parameters. The small chamber can allow for an extremely fast steam charge and rapid increase in chamber temperature. The depth of vacuum a resistometer can achieve is another source of the result variation. In the standard of ISO11138, the vacuum depth for steam and EO cycle is 4.5 kPa and 10 kPa, respectively. The depth of vacuum is to assure BI exposed completely to the sterilizing agent.

3.4 Test method

The resistance test methods include the total viable spore count and the determination of D value. For BI manufacturer and BI user, the total viable spore count is a common test. The using of different procedure may have a result varied. Generally, there are three test methods to physically elute the spore from a spore trip: stomacher lab blender, blender cups, and glass beads and vortex. And each method has its own advantages and disadvantages. One who needs to calculate the population of spore should confirm his test method. And the acceptable result is not less than 50% and not more than 300%. A D-value is the time to reduce the microorganism population to 1-log or 90%. And the most common methods to determine D value are survivor curve method, the fraction negative method and over kill approach. In survivor curve method, the test samples are subjected to defined exposure conditions. Then the test samples are treated with a viable count assay. Using the above data, plot a survivor curve. And D value is the negative reciprocal of the slope. The fraction negative method establishes the number of surviving test organisms by indirect calculation based on the recoverable number of microorganisms as determined by visual observation of growth in fluid growth medium. The over kill method determine the minimum time when there are no survivors.

3.5 Other factors

Many other factors may also affect the BI resistance. The storage relative humidity can affect biological in-

dicator behavior. The recovery media have different ability to promote the injured spore growth, including the different brand and even different lots.

4 Conclusion

To sum up, spores, carrier, recovery medium and the packaging compose the self-contained biological indicators. Keep it in mind, the resistance verification can be very difficult to accomplish and be within acceptable variation. And all of the component will affect BI resistance. Different kinds of carrier spore inoculated on exhibit different manners to attach different D-values. Another factor that affects the resistance of BI is spore production methods. The sporulation conditions including temperature, pH, humidity, nutrient and ions content, largely influence the spore resistance to heat. And, resistometer performance and the different test method are also the major source of the D value variation. All the factors affect the resistance of biological in an comprehensive manner, and when a resistance variation occur, all the factors above should be taken into consideration.

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