Effect of Storage Conditions upon the Resistance (D-value) of Spore Strip Biological Indicators (BIs) used in EtO Sterilization Monitoring

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Abstract

Spore strip storage recommendations vary among manufacturers. The manufacturers' recommended storage conditions range from freezing (-15ºC), to ambient (22ºC), to refrigeration (5ºC). This study investigated the various storage conditions and their effect upon EtO D-value resistance. Using commercially prepared *Bacillus subtilis* spore strips (ATCC #9372) prepared according to US Pharmacopeia XXIII performance parameters, spore strips from three different production lots were stored at the various recommended temperatures. Periodically, samples from each storage condition were removed and tested in a Joslyn/Steris EtO Biological Indicator Evaluator Resistometer (BIER) vessel to determine D-value using the Spearman-Karber Fraction Negative method. Population assays were performed according to USP XXIII, supplement 5 'viable spore count' to determine population stability. The resulting D-values were plotted and recorded. Resulting D-value changes were compared over time, and D-value change trends were graphically presented

Introduction

Ethylene oxide sterilization combines the lethal effects of the sterilent, exposure time, relative humidity and temperature on a microbial population. BIs are commonly used to monitor the efficacy of ethylene oxide sterilization cycles. This study focuses on the use of spore strips. To function as effective monitors, BIs should maintain a stable level of resistance

while being stored over a specific period of shelf-life time.

If it is so important to maintain the resistance level in spore strips and other BIs, how and where should they be stored? This question is the primary focus of this experiment wherein three lots of spore strips inoculated with *B. subtilis* spores were stored and studied over a period of nine months. After inoculation of approximately 3,000 spore strips (1,000 per subtilis lot), they were assayed to find the population per strip, packed into glassine envelopes, and stored under differing conditions.

Since manufacturers recommend the strips be stored in conditions which range in temperature from freezing (-15°C), to refrigeration (5°C) to ambient (22°C). Our study was to compare these three storage conditions in order to determine the possible effects upon population and resistance for the three lots of BIs. In our study, the storage temperatures were -8°C, 5°C, and 27°C.

Materials and Methods

Materials

1. One thousand of each lot of W-135GB, W-136GB and W-137GB spore strips in glassine envelopes.
2. Joslyn ethylene oxide BIER vessel.
3. Sterile Tryptic Soy Broth/Phenol Red (TSB/PR) prepared according to manufacturer's instructions.

4. Tryptic Soy Agar for population assays.
5. Incubator with temperature set at 35ºC.
6. Laminar flow hood.
7. Standard laboratory materials.

Methods

Raven Biological Laboratories manufactured three lots of spore strips containing one thousand biological indicators per lot. On July 10, 1998, population assays were done on the three lots of spore strips according to U.S. Pharmacopeia XXIII guidelines. Upon completion of the population assays, ethylene oxide exposures were run using a Joslyn Ethylene Oxide BIER vessel. The data was collected to determine an all-kill and all-survive time with three dichotomous points in between. The Spearman-Karber (Fraction Negative) method was used to compute the D-values. The BIs within each lot were then divided into three sublots, and stored in a refrigerator (ref), freezer (fr), and on a shelf at room temperature (rt) for a total of nine months.

Population assays were done again after nine months of storage in the strips' respective environments. Change in spore count was noted and various EtO exposure times were run on each sublot of the three lots of spore test strips after nine months. The Spearman-Karber D-value was determined by exposing ten of each sublot of BIs to ethylene oxide at selected exposure times. Exposures were run in increments of one, two, or four

minutes. After the EtO exposures, the individual BIs were aseptically transferred from their glassine envelopes into 10-ml tubes of TSB/PR. The cultured samples were then incubated at 30°-35°C for seven days and observed for signs of growth daily.

Positive growth was determined by a color change of the media from red to yellow and by its turbidity. The growth results for each exposure time were recorded. This data, combined with the results of the population assays, was used to determine the EtO D-value using the Spearman-Karber fraction negative formula listed below. A table created through the use of Microsoft Excel was also used to calculate the D-values. The data obtained from the assays and EtO exposures were then compared to the original results and graphed.

Spearman-Karber (fraction negative) formula:

$D=U/(\log N_0+.\ 2507)$

$U=T-d/2-(d/10 \times \Sigma f_{k-1} \text{ to } f_1)$

D=D-value.

U=mean time until sterility.

N_0=Population of sample.

T=Time to achieve f_k.

f_k=All samples sterile and adjacent to a dichotomous result.

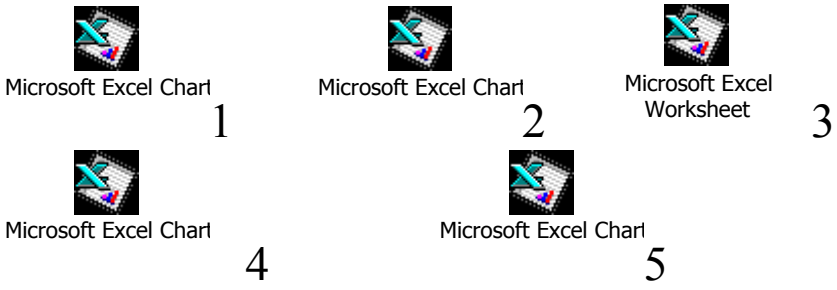
d=Time interval between runs in

minutes.

Results

In the majority of the lots tested, populations dropped over the nine-month storage regardless of the storage conditions. All remained within an allowable population range to serve as a suitable EtO cycle challenge in relation to population (by USP standards).

When compared to spore strips stored at room temperature, all of the three lots tested that were stored under freezer conditions showed a decrease in D-value. The average decrease was .3 minutes. Two of the three lots stored under refrigeration had a slight D-value or resistance increase over the nine months of storage.



Discussion

The challenge or resistance offered by a BI for a particular sterilization cycle is the important characteristic that needs to be maintained during the storage of the BIs. A BI stored under certain conditions that best maintains the population but reduces resistance or D-value is deleterious to the effectiveness, or the BI challenge. 'Resistance stability' is of prime importance when evaluating storage conditions.

Conclusion

Bacillus subtilis spore strips stored under the conditions of refrigeration and at room temperature seem to show the least overall change in resistance characteristics while strips stored under freezing conditions uniformly indicated that the resistance will decrease over extended storage. Decreased resistance will produce a ‘less challenging’ BI.

This study was only done with three lots of spore strips over a nine-month period of storage time. Extended studies covering a full twelve to twenty-four month range need to be done to confirm the indicated trends in resistance modification due to storage conditions.