Terragene®

Methods for viable spore count.

A brief comparison between USP and Terragene recomended method.

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Introduction

Spore counting is one of the main techniques for evaluating the quality of a biological indicator related to the number of reported microorganisms and in certain cases to quantitatively evaluate the reduction that a certain sterilization process can generate.

ISO11138-1:2017 and the United States Pharmacopeia (USP) provide different recommendations for the count of viable spores of biological indicators indicating the precautions to be taken into account in the extraction, cultivation and enumeration stages. Nowadays, even each manufacturer of biological indicators provide a more specific spore counting method, taking into account the characteristics of their products and the different challenges that can be generated by the selection of certain material as the spore carrier.

ISO11138-1:217 recommendations are very broad. The manufacturer of the indicator is free to select the materials and techniques to be used for the elution of the micro-organism from the test sample. ISO11138-1:2017 requires, however, that the technique used must be validated beforehand and a minimun of four test sample from lot are requested. A number of serial dilutions are recommended by this standard before plated in recommended agar plates. Colony counts between 30-300 are considered the most accurate.

The USP protocol is much more detailed. A minumum of three test samples is requested. USP provides a detailed description of each of the steps involved in sample elution, platelet, and count. In addition, the protocol is defined depending on the nature of the carrier: paper carrier, non-paper carrier and suspension. Even so, the protocol is general and does not take into account the different particularities that could result from the variability of components that determines the presence of so many brands of manufacturers of biological indicators.

Comparison between Terragene and USP protocols

Terragene provides a specific, validated and optimized spore counting protocol for each indicator presentations that the company manufactures. Terragene protocol was designed according to ISO11138-1:2017 recommendations. The main differences between Terragene and USP protocols are explaned below.

Number of test samples:
USP recommends three samples, instead Terragene recommends 4 test samples (according to the recommendations of ISO11138-1:2017). An increase in the number of samples improves the accuracy of the count, giving a better average.

Elution:
For paper carrier, USP recommends making the elution by placing the three carriers at the same time in 250 ml cup of a suitable blender with 100ml of distilled water. Terragene recommends making the elution by placing each independent carrier in 2mL tubes adaptable to a homogenizer and mixing with 2-3mm glass beads. Each tube should contain a solution with 600uL deionised distilled water and 400ul ethanol.

Doing the elution of each individual carrier helps to minimize the error that could generate a single separation process. In addition, the use of glass beads and a homogenizer makes the separation process more effective, avoiding the resistance that could generate certain material to the separation process in a blender. Moreover, Terragene prefers the use of a 40:60 ethanol:water solution instead of 100% water. Ethanol helps to slightly decrease the polarity of the medium to facilitate the elution of spores from the carriers.

Heat shock:
USP recommends two different temperature ranges for doing the thermal shock:
- 95-100°C / 15 minutes for thermophiles.
- 80-85°C / 10 minutes for mesophiles

Terragene has validated very effective results using the same temperature and time for both bacterias: 85°C / 15 minutes

Recommended medium:
USP recommends Soybean–Casein Digest Agar Medium. Terragene is much more specific and recommends a tested and validated medium. The culture medium designed for optimized counting is the LBA supplemented with calcium chloride and glucose. The composition is detailed below:
- Acid casein peptone 1.0% p/v
- Yeast extract 0.5% p/v
- Sodium chloride 0.5% p/v
- Calcium chloride 0.05% p/v
- Glucose 0.03% p/v
- Agar 1.5% p/v

Incubation:
USP recommends incubation for 48 hours of the plate at temperatures between 55 to 60°C for

Biological Indicator for Steam Sterilization, and at 30 to 35°C for Biological Indicator for Ethylene Oxide Sterilization, and for Biological Indicator for Dry-Heat Sterilization.
Terragene, because of the optimized medium recommends:
• For *Geobacillus stearothermophilus*, incubate at a temperature of (60 ± 2) ºC for 48 hours.
• For Bacillus atrophaeus, *Bacillus subtilis* or *Bacillus pumilus*, incubate at a temperature of (37 ± 2) ºC for 24 hours.

Conclusions

In case an end user wants to perform spore counting of any of Terragene indicators, we recommend to strictly follow our validated protocol. Terragene has conditioned their indicators to respond efficiently to the different processes in the market. This conditioning could make our indicators respond differently to counting methods validated by other manufacturers. Our counting method is based on the recommendations of ISO11138-1:2017 and is optimized to provide accurate and consistent values in the error range required by the standard.

References

•International Standard ISO11138-1:2017 "Sterilization of heath care products; Biological Indicators, Part 1: General requirements
•USP XXIII NF 18 Sixth Supplememnt, Chapter 55 – Biological Indicators – Resistance Performance Test
•NT04 – Terragene: Determination of viable count in Bionova Biological Indicators