Technical Report

Spore Suspensions

I. Introduction

Spore Suspensions are a calibrated preparation of bacterial spores appropriate for direct inoculation of products or preparing custom biological indicators for monitoring steam, low temperature steam, ethylene oxide, dry heat, hydrogen peroxide, radiation, and other industrial sterilization processes or performing growth promotion testing. Each vial contains 10 mL of suspension. The suspending liquid is 40% ethanol, 29% ethanol, 20% ethanol or purified water depending on species, as listed in Table 1.

Table 1 Suspending Liquid

Species	Suspending Liquid
Geobacillus stearothermophilus 7953	40% ethanol or purified water
Geobacillus stearothermophilus 12980	29% ethanol
Bacillus atrophaeus 9372	40% ethanol
Bacillus subtilis subsp. *spizizenii* 6633	40% ethanol or purified water
Bacillus subtilis "5230" 35021	40% ethanol or purified water
Bacillus pumilus 27142	40% ethanol
Bacillus smithii 51232	20% ethanol
Clostridium sporogenes 7955, 11437, 19404	40% ethanol

II. Storage

40% ethanol Spore Suspensions should be stored at -25 to -10°C.

29% ethanol, 20% ethanol and purified water Spore Suspensions should be stored at 2 to 8°C.

III. Shelf Life

40% ethanol and 20% ethanol Spore Suspensions have a 24-month shelf life from the date of manufacture, except for item 35.LPT-606. Item 35.LPT-606 has a 15-month shelf life from the date of manufacture.

Purified water Spore Suspensions have a 12-month shelf life from the date of manufacture.

29% ethanol have a 15-month shelf life from the date of manufacture.

Do not use after expiration date printed on package. Dispose of expired suspensions by autoclaving at 121°C for not less than 30 minutes or per site procedures.

MesaLabs ph: 877.746.8883 • 625 Zoot Way, Bozeman, MT 59718 • mesalabs.com

IV. Use

Product Inoculation

1. Perform inoculation operations in a clean area, remote from sterility testing area.

2. Samples to be inoculated should be representative of product being sterilized.

3. Select the spore suspension of appropriate organism and population. For most purposes, inoculation of product with approximately one million (10^6) spores provides a suitable challenge.

   NOTE: Spore suspensions are standardized based on number of spores per 0.1 mL of suspension.

4. Vortex or shake vial vigorously before each use.

5. Use a sterile pipette or syringe to accurately deliver the volume of suspension to be utilized.

   a. If syringe is used, disinfect stopper surface and pull syringe plunger halfway back. Insert needle through stopper, push plunger in, and then slowly withdraw plunger to fill syringe to desired volume.

   b. If a pipette is used, remove stopper and insert pipette. Withdraw desired volume.

6. Deposit suspension onto product. The area to be inoculated should be the one most difficult to sterilize.

7. Allow product to dry at 20 - 30°C for approximately 24 hours or until visibly dry. Some devices with small lumens may take longer to dry.

8. Package inoculated product exactly like product being sterilized and identify prominently as “Inoculated Test Samples”.

9. Distribute “Inoculated Test Samples” throughout the sterilizer load.

10. After sterilization cycle is complete, test the inoculated products as soon as possible using soybean casein digest broth and incubate at the optimal temperature for seven days.

Growth Promotion

1. Perform inoculation operations in a clean area.

2. Samples to be inoculated should be representative of product being tested.

3. Select the spore suspension of appropriate organism and population. For growth promotion purposes, inoculation of product with 10 – 100 CFUs provides a suitable challenge.

4. Vortex of shake vial vigorously before each use.

   NOTE: Spore suspensions are standardized based on number of spores per 0.1 mL of suspension.

MesaLabs ph: 877.746.8883 • 625 Zoot Way, Bozeman, MT 59718 • mesalabs.com

5. Use a sterile pipette or syringe to accurately deliver the volume of suspension to be utilized.

    a. If syringe is used, disinfect stopper surface and pull syringe plunger back halfway. Insert needle through stopper, push plunger in, and then slowly withdraw plunger to fill syringe to desired volume.

6. Inoculate product appropriately.

7. Identify the inoculated samples prominently.

8. Incubate at the optimal temperature seven days.

V. Incubation and Readout Time

The recommended incubation for items inoculated with Spore Suspensions is not less than 7 days at the appropriate temperature for the organism as listed in Table 2.

Items should be placed in the incubator immediately after culturing into growth media. Placement in an optimized growth environment which maintains the correct incubation temperature is necessary to gain accurate results.

Table 2 Incubation Temperature

Species	Incubation Temperature
Geobacillus stearothermophilus 7953, 12980	55 - 60°C
Bacillus atrophaeus 9372	30 - 35°C
Bacillus subtilis subsp. *spizizenii* 6633	30 - 35°C
Bacillus subtilis “5230” 35021	30 - 35°C
Bacillus pumilus 27142	30 - 35°C
Bacillus smithii 51232	48 - 52°C
Clostridium sporogenes 7955, 11437, 19404	35 - 39°C (anaerobic)

VI. Interpretation

Inoculated Product

The appearance of turbid medium or the formation of sediment indicates bacterial growth and a positive result. Clear medium indicates no growth and that the spores were killed in the sterilization process.

Act on a positive test as soon as it is noted. Carefully review sterilizer process records to ensure that all physical process parameters are within specifications. Always ensure that loading configuration and product and package specifications are in agreement with the sterilization validation process. Positive units may be subcultured if identification of positive growth is desired.

A positive control should be prepared periodically or at least weekly. Many users perform a positive and negative control for each cycle tested. The positive control typically turns turbid within 24 to 48 hours of incubation. As soon as the control turns positive, it should be appropriately recorded, autoclaved and discarded. The positive control is intended to confirm viable spores are present on the unexposed, inoculated carrier and the culture media will support growth of the test organism.

A positive control that has not grown is a serious problem. Fortunately, the causes are few: a grossly malfunctioning incubator; inadvertent sterilization of the positive control carrier; or inadvertent sterilization of the spore suspension vial due to improper storage.

A negative control (a tube incubated without an inoculated carrier) tests the medium for contamination. It should show no signs of growth.

Growth Promotion

The appearance of turbid medium or the formation of sediment indicates bacterial growth and a positive result or successful growth promotion. Clear medium indicates no growth and unsuccessful growth promotion.

A positive growth promotion test typically turns turbid within 24 to 48 hours of incubation. As soon as the positive result is noticed, it should be appropriately recorded, autoclaved and discarded. The positive growth promotion is intended to confirm the culture media will support growth of the test organism.

A growth promotion test that has not grown is a serious problem. Potential causes are a grossly malfunctioning incubator; inadvertent sterilization of the spore suspension vial due to improper storage or media that will not support the growth of the organism.

Many users perform a negative control (incubated, uninoculated media) with each growth promotion test. A negative control tests the medium for contamination. It should show no signs of growth.

VII. Resistance Performance Characteristics

Spore suspension resistance data (D-value) is limited to lot to lot comparison as the resistance of the spores can vary depending on the material onto which they are inoculated or the liquid product into which they are inoculated. Table 3 provides information about how the resistance data for each species is obtained.

Table 3 D-value Determination

Species	Carrier	Parameters	Method
Geobacillus stearothermophilus 7953	Paper carrier packaged in glassine	Steam 121°C ± 0.5°C	Fraction Negative
Geobacillus stearothermophilus 7953	Stainless-steel carrier packaged in Tyvek®	2.0 mg/L gaseous H_2O_2	Fraction Negative
Geobacillus stearothermophilus 12980	Stainless-steel carrier packaged in Tyvek®	2.0 mg/L gaseous H_2O_2	Fraction Negative

Bacillus atrophaeus 9372	Paper carrier packaged in glassine	Ethylene Oxide 600 mg/L ± 30 mg/L EO, 54°C and 60% ± 10% RH Dry Heat 160°C ± 2.5°C	Fraction Negative
Bacillus subtilis subsp. s*pizizenii* 6633	D-value not supplied		
Bacillus subtilis "5230" 35021	Paper carrier packaged in glassine	Steam 121°C ± 0.5°C	Fraction Negative
Bacillus pumilus 27142	Paper carrier packaged in glassine	gamma radiation (^{60}Co)	Fraction Negative
Bacillus smithii 51232	Water for Injection (WFi) in glass ampoules	Steam 121°C ± 0.5°C	Survivor Curve
Clostridium sporogenes 7955, 11437, 19404	D-value not supplied		

VIII. Population Determination

Detailed population assay instructions, TS-405 Spore Suspensions, are available on Mesa's website.

IX. Compliance

Spore Suspensions are manufactured in compliance with Mesa Laboratories' quality standards, USP, ISO 11138 guidelines and appropriate subsections.