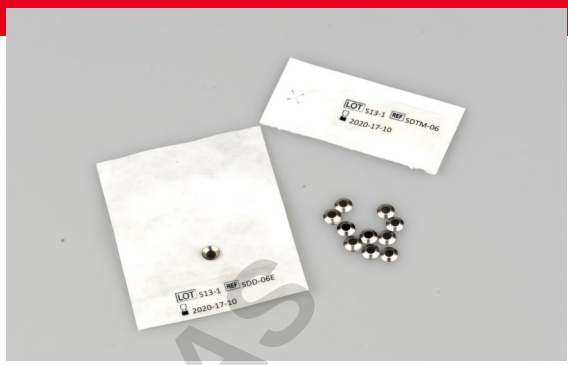


STEEL SPORE DISCS
For Monitoring Vaporized Hydrogen Peroxide (VH_2O_2) Processes

True Indicating Codes: SDTT-06
Ref. Dustcher : 633348 (10^4) / 633349 (10^5) / 633323 (10^6)

Product Description

Steel Spore Discs for monitoring VH_2O_2 processes consist of:

- An inoculated carrier, 6mm steel disc of *Geobacillus stearothermophilus* Cell Line 7953
- Primary packaging either in Tyvek®/Mylar® pouches (SDTM-06) or Tyvek® pouches (SDTT-06)

Indications for Use

The Spore discs are designed to monitor VH_2O_2 sterilization process efficacy. The Spore Discs are labeled for laboratory/industrial use only.

Process	VH_2O_2
Disc Dimensions	6 mm
Pouch Dimensions	SDTM-06: 57 mm x 70 mm SDTT-06: 28.5 mm x 63.5 mm
Packaging	100 / Pack

Physical Properties
Monitoring Frequency

For greatest control of sterilized goods it is recommended that a minimum of ten (10) Spore Discs be included with every load.

Instructions for Use

Place Spore Discs (a minimum of 10 per exposure is recommended) inside representative materials to be sterilized. Package or wrap product as usual, if applicable.

Locate the test packages or Spore Discs in areas most difficult to sterilize, as outlined in your specific sterilization validation protocol (usually four corners front, four corners rear, center-center and center-top) or according to standard operating procedure. Run the cycle.

After sterilization or exposure, remove Spore Discs or product from sterilizer

Aseptically transfer one Spore disc to 5-15 mL of Soybean Casein Digest Broth (SCDB). Conversely, modified growth medium, True Indicating Code PGM-100, may be utilized in place of the SCDB.

Transfer one Spore Disc which has not been exposed in a sterilization process as a Positive Control.

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Incubation: At least one unused tube of culture medium from the same lot should be incubated with the test series as a Negative Control. Incubate the cultured Spore Discs, the Positive Control and the Negative Control at 55°C to 65° C as outlined in the following table:

Sterilization Process	Media Type	Minimum Incubation Time
VH_2O_2	SCDB	7 Days
	PGM-100	24 Hours

Monitoring: Examine the Spore Discs daily, whenever possible during incubation. Record observations.

Interpretation:

Where SCDB (standard or unmodified) was utilized: Tubes which demonstrate turbidity with cream-colored sediment are considered positive for growth of *Geobacillus stearothermophilus*. Tubes which remain clear and without sediment are considered negative for growth.

Where modified media, True Indicating Code PGM-100, was utilized: Tubes which transition in color from Purple to Yellow and/or demonstrate turbidity are considered positive for growth. Tubes which remain Purple in color and do not demonstrate turbidity are considered negative for growth.

For unexpected positives, it is recommended that a Gram stain be performed. Gram positive rods are indicative for the indicator organism.

Positive Control: Tube(s) should demonstrate turbidity and cream-colored sediment or demonstrate a color transition from Purple to Yellow where modified media has been utilized. If the Positive Control does not result in growth, the exposure is considered invalid. Check the conditions during incubation and verify the capability of the medium to support growth.

Negative Control: Tubes of media should remain clear and Purple in where modified medium was utilized. If the Negative Control results in growth, there is a potential for false positives.

Compliance

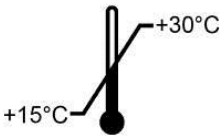
ISO 11138-1 Sterilization of health care products – Biological indicators – Part 1: General requirements

True Indicating has a validated method for Total Viable Spore Count. Please inquire for the Technical Bulletin which outlines the recommended methodology.

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Performance Characteristics

Population	≥1.0 x 10^6 per Disc
Purity	No evidence of contamination present in sufficient numbers to adversely affect the finished product.
VH_2O_2 Resistance	*D* value at 55°C ± 5°C, 2.3 mg/L ≥1.0 minutes
Post Market Criteria	Population: 50% to 300% of certified population *D* value: ± 20% of the certified *D* value

	15°C to 30°C		Keep away from sunlight
	20% to 80% relative humidity		Keep Dry
Shelf Life	36 months from the date of manufacture		Protect from heat and radioactive sources
	Short excursions outside the range of temperature and relative humidity recommended will not impact the performance of the Spore Discs. Do not use damaged Spore Discs. Do not use after the expiration date. The Spore Discs contain live cultures and should be handled with care.		

Storage and Shelf Life

Disposal

Autoclave for not less than 30 minutes at 121°C or per other validated disposal cycle prior to discard.

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This Technical Bulletin outlines the procedure recommended for use in verifying the certified population of True Indicating product configurations containing Spore Discs of filter paper or stainless steel.

1. Obtain one sterile 20 mm x 150 mm screw cap test tube (or equivalent) containing approximately 1" or 2.54 cm of sterile 3 mm glass beads for each Spore Disc to be evaluated.

   Use of a minimum of 4 Spore Discs is outlined in ISO 11138-1 Sterilization of health care products – Biological indicators – Part 1: General requirements.

2. Remove the inoculated carrier from the packaging material, if applicable, and add to the sterile test tube with glass beads. Add 10 mL of Purified Water (PW), Water For Injection (WFI) or Sterile Deionized Water (SDI).

Maceration or Dislodging of Spores

3. For filter paper carriers, macerate the carrier through agitation using either a vortex, manually shaking the test tube or a combination of the two until a homogeneous pulp is obtained. This process will take approximately 10-15 minutes; longer if the tube is only vortexed.

   For stainless steel disc carriers, ensure the disc is buried in (surrounded by on all sides) glass beads. Sonicate the tube for a minimum of 2 minutes. Vortex for a minimum of 3 minutes to dislodge the spores from the carrier. During the vortex period, frequently shake the tube to ensure that the cupped side of the disc is not stuck facing the glass tube wall, and that the disc is surrounded by the glass beads and extraction diluent. No visible evidence of the spores should remain on the steel disc. Spores will appear as a white residue on the carrier. If spores remain on the carrier, continue to vortex and manually shake until the steel disc shows no evidence of spores.

Heat Shock

4. For population levels except for 10^3/carrier, transfer 1 mL of the fluid to 9 mL of PW, WFI or SDI (this is the 10^{-2} dilution). For population levels of 10^3/carrier, retain all 10 mL in the original test tube.
5. Prepare a "blank" test tube containing 10 mL of the diluent only (PW, WFI or SDI). Place a thermometer in the "blank" test tube. For population levels of 10^3/carrier, the "blank" should also include glass beads.
6. Place the test tube(s) and the "blank" into a water bath.
7. Start timing the length of the heat shock period when the thermometer reaches the organism's minimum heat shock temperature as outlined in the table below. Continue the heat shock period for the time specified.

Organism	Heat Shock Temperature	Length of Heat Shock Period
Bacillus atrophaeus	80ºC to 85ºC	10 minutes
Bacillus pumilus		
Geobacillus stearothermophilus	95ºC to 100ºC	15 minutes

8. At the end of the heat shock period, transfer the tubes from the water bath to an ice bath and allow to cool at 0º - 4ºC.

Dilution and Plating

9. Based on the target population level of the carrier, perform 1:10 dilutions (1 mL into 9 mL diluent) or 1:100 dilutions (0.1 mL into 9.9 mL diluent) until the dilution corresponding to the theoretical population of 30 to 300 spores per mL is reached. Refer to the following table for guidance on dilution required.

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Population of Carrier	Dilution Required	Volume to Be Plated
10^3	10^{-1}	1 mL
10^4	10^{-2}	
10^5	10^{-3}	
10^6	10^{-4}	

10. Obtain 100 mm x 15 mm petri dishes. Label each petri dish with Inoculated Carrier number, the dilution factor and the plate number. A total of 3 transfers to petri dishes or three plates per carrier is recommended.

11. Transfer a 1 mL aliquot from the final dilution tube of each carrier into separate dishes as per labeled above.

12. Within 20 minutes, add approximately 20 mL of molten Soybean Casein Digest Agar (SCDA)/Tryptic Soy Agar (TSA) to each dish and mix by gently swirling. The temperature of the media is a critical factor as media which has not been properly tempered will damage and/or kill the spores thus reducing the recovery. Ensure media is approximately 45ºC when poured into the petri dishes.

13. Allow the agar to solidify.

Incubate and Enumerate

14. Invert the petri dishes and incubate for a minimum of 48 hours at the appropriate growth temperature for the organism, as outlined below:

Organism	Incubation Temperature
Bacillus atrophaeus	30ºC to 40ºC
Bacillus pumilus	
Geobacillus stearothermophilus	55ºC to 65ºC

15. After incubation, enumerate the colonies on each plate and calculate the overall mean count based on the average of the results for each carrier.

16. Based on the dilution factor, calculate the total viable spore count. See example below for guidance:

    Example: 10^{-4} Dilution

Spore Disc/Carrier No.	Plate 1	Plate 2	Plate 3	Average
1	152	140	165	152
2	180	141	191	171
3	172	193	153	173
4	144	182	196	174

Overall Mean: 167.5 = 168

Total Viable Spore Count: 1.7×10^6/disc

Acceptance Criteria

17. Per ISO 11138-1, the population should be within 50% to 300% of the certified population (manufacturer's label claim) to be considered acceptable. A Lot of Spore Discs with a certified population of 1.7×10^6/disc, would be acceptable if the verified average population was in the range of 8.5×10^5 to 5.1×10^6/disc.

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