Smart-Read™
EZ TEST®
BIOLOGICAL INDICATOR MONITORING SYSTEM

Geobacillus stearothermophilus

TECHNICAL REPORT

Complies with:
USP
ISO 11138
and all appropriate subsections.

Technical Data and Use of Smart-Read EZTest Steam

Rev.2
TR-015

INTRODUCTION

Smart-Read EZTest Steam is a self-contained biological indicator for use in monitoring the efficacy of 121°C, 132°C, 134°C, and 135°C steam sterilization cycles. Smart-Read EZTest is easy to use; no sophisticated laboratory testing or analysis is required. Smart-Read units consist of bacterial spores *Geobacillus stearothermophilus* (7953[1]) inoculated onto a paper carrier, which is placed into a thermoplastic vial that serves as a culture tube. A small glass ampoule containing sterile culture medium and color indicator is also contained in the vial.

US HEALTHCARE INTENDED USE

Smart-Read EZTest is a Self-contained Biological Indicator (SCBI) intended for use in determining the efficacy of steam sterilization processes. The SCBI may be used in the following steam sterilization cycles:

Cycle Type	Cycle Temperature	Cycle Exposure Time
Gravity	121°C	30 minutes
Gravity	132°C	10 minutes
IUSS	132°C	3 minutes*
Pre-Vac	132°C	4 minutes
Pre-Vac	135°C	3 minutes

*Unwrapped nonporous devices only.

Smart-Read EZTest has a validated reduced incubation time of 10 hours.

STORAGE

Smart-Read EZTest indicators should be stored at room temperature. The indicators should not be stored near sterilants or other chemicals. Smart-Read biological indicators have a 24-month shelf-life. Do not desiccate.

MEDIUM

The culture medium, consisting of a proprietary formulated soybean casein digest base, is filled into glass ampoules and flame sealed. Following manufacture, the ampoules are exposed to a steam processing cycle to render them sterile. The sealed ampoules are of a convenient size to be placed into the plastic body with the spore paper. The ampoule is an "onion skin" glass that allows it to be easily crushed when the plastic body is compressed. This provides the spores with a nutrient medium for growth.

The culture medium has a pH indicator (bromocresol purple) which appears purple. After activation (when the plastic body is compressed) if the medium changes to yellow it indicates viable spores were present, grew and produced acid. If the medium remains purple, the spores did not grow, indicating they were killed in the sterilization process. Therefore, if the sterilization process was not effective, the spores will grow and turn the medium yellow. If any ampoules show signs of a visual color change or turbidity prior to use, they should be autoclaved and discarded.

(1) Culture is traceable to a recognized culture collection identified in USP and ISO 11138.

USE

EXPOSURE:

1. Remove an appropriate number of Smart-Read EZTest units from the box.
2. Identify the indicators by labeling pertinent process information.
3. Place the Smart-Read indicator in a suitable test pack which is representative of the load (e.g., a linen pack for load of linen, a tray of instruments for metal goods).
4. Place this test pack in the most challenging areas of the sterilizer, generally on the bottom shelf near the door over the drain.

   NOTE: If a test pack is not being used, the Smart-Read EZTest unit should be oriented in a horizontal position during load processing.
5. Process the load as usual.
6. Remove from the sterilizer and allow the pack and biological indicator to cool for a sufficient time, at least 10 minutes.
7. Retrieve the Smart-Read biological indicator from the test load.
8. The chemical indicator on the label changes from blue to a green/gray color when exposed to steam. Extended exposure will result in further change to black color. This distinguishes exposed from unexposed units.

   NOTE: A black color does not indicate acceptable sterilization.
9. To activate the media, place the indicator in the crusher on the Smart-Well® incubator and pull forward. This will fracture the glass ampoule and allow the growth media to come in contact with the spore strip.

INCUBATION IN SMART-WELL INCUBATOR

Smart-Read EZTest biological indicators are designed to be used with the Smart-Well incubator which is calibrated to maintain a temperature of 60°±2°C. The units must be activated prior to incubation. Activate the Smart-Read biological indicator using the crushing chamber located on the Smart-Well incubator. Place the indicator in an upright position in the crushing chamber and slowly pull forward to break the glass ampoule and release the media. This will fracture the ampoule and allow the growth medium to come in contact with the spore strip. Ensure that the spore strip is completely saturated with the culture medium. Gently flick the Smart-Read EZTest BI to remove any air bubbles within the plastic tube. Do not allow the culture medium to come in contact with the filter in the cap at any time. Immediately place the exposed activated Smart-Read BI in the Smart-Well incubator, cells 1-10.

The Smart-Well incubator continuously monitors the BI and alerts the user to a positive BI test (color change to yellow) or a negative BI test (color remains purple) at the completion of the incubation period.

INTERPRETATION OF SMART-READ RESULTS

LEDs located in front of each cell of the Smart-Well incubator display the status of the test cell: **amber** = testing; **red** = detection of yellow change in color; **green** = BI purple at end of incubation cycle. The appearance of a yellow color indicates bacterial growth. No color change indicates the spores were killed in the sterilization process. For detailed instructions for the Smart-Well 1710 refer to the company website www.mesalabs.com.

The Smart-Read EZTest steam will indicate a failed sterilization cycle in as little as three to five hours. The Smart-Well incubator will announce the detection of a positive Smart-Read EZTest BI. The announcement consists of an audible alarm, a change of the incubation cell status LED from amber to red and a printed record of the exact time the positive test was detected. The confirmation of a positive test can be performed immediately by the user and is 100% visually verifiable.

Act on a positive test (a color change to yellow) as soon as the color change is noted. Color change is to be interpreted as "inadequate sterilization". Follow your institution's guidelines when a failed sterilizer process occurs. Always investigate sterilizer failures. Check the sterilizer for leaks or malfunctioning steam traps, check valves, etc. and retest the sterilizer with several Smart-Read biological indicators throughout the test load after any repairs to critical components. Smart-Read indicators can be subcultured if identification of positive growth is desired.

The response of the Smart-Read EZTest BI is 100% biological based on the growth of spores that have survived the sterilization process. Both positive and negative tests are 100% visually verifiable by the user. A purple color is a negative test indicating all spores were killed in the sterilization cycle. The recommended incubation time of 10 hours meets the CDRH FDA Reduced Incubation Time protocol.

A positive control should be run at least once per day. As soon as a control turns yellow, appropriately verify and then autoclave and discard. The control is intended to ensure that viable spores are present on the BI lot prior to testing the sterilizer. It is recommended to incubate the positive controls a maximum of five hours. If the control has not turned yellow in five hours the test is considered negative. A true negative or no growth in a positive control is a serious problem. Fortunately, the causes are few: a grossly malfunctioning incubator; inadvertent sterilization of the control vial; or inadvertent sterilization of the box of indicators - due to improper storage.

INCUBATION READ-OUT TIME

The 10-hour incubation time was validated according to the CDRH Guidance for Industry and FDA Staff: Biological Indicator (BI) Premarket Notification [510(k)] Submissions, issued October 4, 2007. The CDRH RIT protocol for validation of reduced incubation time (RIT) may or may not meet each user's requirements for regulatory compliance. Users should therefore confirm regulatory requirements for RIT, or incubate for 7 days.

The incubation time of Mesa Labs' Smart-Read EZTest product was validated according to the Center for Devices and Radiological Health, FDA protocol entitled "Guide for Validation of Biological Indicator Incubation Time". Three crops used in the manufacturing of Smart-Read EZTest were prepared according to Mesa Labs' Standard Operating Procedures. For each crop, 100 biological indicators were exposed to a

steam BIER cycle for the times indicated in Table 1. Exposure conditions were 121°C ± 0.5°C. The exposed biological indicators were incubated at 58°-62°C for seven days. The results of the test that were valid according to the FDA protocol (30%-80% of the tubes positive for microbial growth) are shown in Table 1.

Table 1: Results of the Reduced Incubation Time Study at 121°C

Biological Indicator Crop Number	Exposure Time (Minutes)	# Positive 48 Hours	# Positive 7 Days	Percent Positive [1]
GST-071514	9.5	85	85	100%
GST-022916	12.0	72	72	100%
GST-050216	13.0	65	65	100%

[1] Acceptable protocol results require greater than 97% of the base number of biological indicators to test positive. This % is calculated by using the number of positive biological indicators on day 7 as the base number (denominator data) and using the number of positive biological indicators at ten (10) hours as the numerator.

This data shows that the 10-hour incubation time claim was valid (ratio of positives at 10 hours vs. 7 days greater than 97%). A 10-hour incubation time provides users with a rapid release of sterilized product. It should be emphasized that incubator performance is critical to achieve these incubation times.

RESISTANCE PERFORMANCE TESTING

D-value determination was performed by fraction negative analysis. Smart-Read EZTest Steam biological indicators were exposed in a steam BIER vessel that meets the ANSI/AAMI/ISO 18472 Resistometer standard and cycle exposure conditions in accordance with ISO 11138-3. Exposure conditions were at 121°C ± 0.5°C, 132°C ± 0.5°C, 134°C ± 0.5°C and 135°C ± 0.5°C in saturated steam using a pre-vacuum cycle. Following exposure, samples were activated and incubated at 60±2°C for 10 hours. Performance data is presented in Table 2.

Table 2
Smart-Read Performance Data

121°C

Crop/Dilution Number	Number Negatives Out of 25 — Exposure Times (in minutes)												Population/ Unit	D-value[1] (Minutes)
	7	8	9	10	11	12	13	14	15	16	17	18		
GST-071514/E4-1	NA	0	0	4	11	19	24	25	25	NA	N/A	N/A	1.6×10^5	2.1
GST-022916/E6-1	NA	NA	0	0	1	3	11	22	25	23	25	25	1.9×10^6	2.0
GST-050216/E4-1	0	0	1	7	16	24	24	25	25	NA	NA	NA	1.9×10^5	1.9

[1] Calculated according to USP methods.

132°C

Crop/Dilution Number	Number Negatives Out of 20 — Exposure Times (in minutes)										Population/ Unit	D-value[1] (Minutes)
	1	1.5	2	2.5	3	3.5	4	4.5	5.0	5.5		
GST-071514/E4-1	0	0	2	7	8	13	13	15	20	20	1.6×10^5	0.6
GST-022916/E6-1	NA	NA	0	0	2	5	13	17	20	20	1.9×10^6	0.6
GST-050216/E4-1	0	0	1	2	4	11	15	18	20	20	1.9×10^5	0.6

[1] Calculated according to USP methods.

134° C

Crop/Dilution Number	Number Negative Out of 20 — Exposure Times (in minutes)											Population/ Unit	D-value[1] (Minutes)
	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5.0	5.5		
GST-071514/E4-1	NA	0	0	3	7	8	15	17	18	20	20	1.6×10^5	0.6
GST-022916/E6-1	0	0	2	3	4	6	16	17	20	20	NA	1.9×10^6	0.5
GST-050216/E4-1	0	0	2	1	3	11	13	15	20	20	NA	1.9×10^5	0.6

[1] Calculated according to USP methods.

135° C

Crop/Dilution Number	Number Negative Out of 20 — Exposure Times (in minutes)										Population/ Unit	D-value[1] (Minutes)
	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5.0		
GST-071514/E4-1	0	0	3	4	6	12	16	18	20	20	1.6×10^5	0.5
GST-022916/E6-1	NA	NA	0	0	4	10	13	18	20	20	1.9×10^6	0.5
GST-050216/E4-1	NA	0	0	3	5	5	13	19	20	20	1.9×10^5	0.6

[1] Calculated according to USP methods.

POPULATION DETERMINATION

Detailed population assay instructions are available in PDF format on the Mesa Labs website. Log onto the www.http.://biologicalindicators.mesalabs.com and select Documents & Resources; then select Documents & Manuals; then select Procedures (scroll down to Population Assay –EZTest Steam, Smart-Read EZTest & EZTest Gas).

CERTIFICATE

Units are manufactured in compliance with Mesa Labs, Bozeman Manufacturing Facility's quality standards, USP, and ISO 11138 guidelines and all appropriate subsections. Sample Certificate of Analysis can be found in Figure 1.

Figure 1: Sample Certificate of Analysis

Smart-Read™ EZTEST®
BIOLOGICAL INDICATOR MONITORING SYSTEM
SELF-CONTAINED
BIOLOGICAL INDICATOR

CERTIFICATE OF ANALYSIS

Reorder No: SEZS/5
SEZS/525

Geobacillus stearothermophilus 7953(1)
Biological Indicator for: Steam Sterilization.

Culture: **EZTest** Media, 58-62°C. The supplied bacteriological medium will meet requirements for growth promoting ability.

Purity: No evidence of contaminants using standard plate count techniques.
Lot No: **SR-000** Manufacture Date: YEAR MONTH DAY
Expiration Date: YEAR MONTH DAY
Heat Shocked Population: 0.0 x 10^5 Spores / Unit
Carrier size: ¼" x ¾" (6 mm x 19 mm)
Assayed Resistance:

	D-Value (2)	Survival	Kill	
Steam 121°C	0.0	00.0 (3)	00.0 (3)	min
Steam 132°C	0.0	0.0 (4)	0.0 (4)	min
Steam 134°C	0.0	0.0 (4)	0.0 (4)	min
Steam 135°C	0.0	0.0 (4)	0.0 (4)	min

Z-value: 00.0°C

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

(1) Culture is traceable to a recognized culture collection identified in USP and ISO 11138.
(2) Resistance was determined in an AAMI BIER vessel and calculated using the Fraction Negative method. The D-value is reproducible only when exposed and cultured under the exact conditions used to obtain results reported here.
(3) Survival/Kill values are calculated according to a formula in USP and ISO 11138. A D-value rounded to four decimal places is used in this calculation.
(4) Empirically derived data.

Certified by: _____ _____
Quality Representative Date

MesaLabs
Bozeman Manufacturing Facility
10 Evergreen Drive
Bozeman, MT 59715
T: 303/987-8000 ♦ F: 406/585-9219
www.mesalabs.com