Technical Report

Smart-Read™ EZTest®

I. Introduction

Smart-Read EZTest is a self-contained biological indicator (SCBI) for use in monitoring the efficacy of 121°C, 132°C, 134°C and 135°C steam sterilization cycles. See table below for United States Healthcare intended use. Smart-Read EZTest is easy to use; no sophisticated laboratory testing or analysis is required. Smart-Read EZTest units consist of 10^5 or 10^6 *Geobacillus stearothermophilus* strain 7953 spores 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 pH indicator is also contained in the vial. Filter paper is placed over the top of the culture tube to prevent contamination, and a cap with holes is set over the top which allows sterilant penetration into the Smart-Read EZTest. A label on the outside of the vial includes a chemical indicator that serves as a visual indicator of whether or not the unit has been exposed to steam.

United States Healthcare Intended Use

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.

II. Storage

Smart-Read EZTest should be stored at room temperature. The indicators should not be stored near sterilants or other chemicals. Do not desiccate.

III. Shelf Life

Smart-Read EZTest has a 24-month shelf life from the date of manufacture when stored at recommended conditions.

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

IV. 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 autoclaved to render them sterile and growth promotion is performed using less than 100 spores of *Geobacillus stearothermophilus* 7953. 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) added to it, which appears purple. After activation (when the plastic body is compressed) and an appropriate incubation period, if the spores grow the medium changes to yellow which means viable spores were present and acid is being produced. 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 cloudy and yellow. If any ampoules show signs of a visual color change, or turbidity, prior to use, they should be autoclaved and discarded.

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V. Use

1. Remove an appropriate number of Smart-Read EZTest units from the box and identify the indicators by labeling with pertinent process information.

2. Place a Smart-Read EZTest indicator in a suitable test pack which is representative of the load.

3. Place this test pack in the most challenging area of the sterilizer, generally on the bottom shelf near the door.

   NOTE: If a test pack is not being used, the Smart-Read EZTest unit should be oriented in a horizontal position during load processing.

4. Process the load as usual.

5. Remove from the sterilizer and allow the pack and biological indicator to cool for a sufficient time, at least 10 minutes.

6. Retrieve the Smart-Read EZTest SCBI from the test load.

7. The chemical indicator on the unit label changes from blue to a green/gray color when exposed to steam. Extended exposure will result in further change to a black color. The purpose of the chemical indicator is to distinguish exposed from unexposed units. A black color does not indicate acceptable sterilization.

8. To culture the strip in a Smart-Read EZTest SCBI, place the indicator in an upright position and compress the plastic vial with a crushing device or in the crushing cavity of the Smart-Well incubator to break the glass ampoule. This will allow the growth medium to come in contact with the spore strip. Ensure that the spore strip is completely saturated with the culture medium. Do not allow the culture medium to come into contact with the filter in the cap at any time

   NOTE: The medium ampoule contained in Smart-Read EZTest is made from thin walled glass that is designed to break easily during culturing/activation. For this reason, the ampoule can be damaged in shipping or in handling (placement in a load, product or process challenge device, or removal from a load, product or process challenge device). Inspection of Smart-Read EZTest units both prior to use in a sterilization process and after the process is critical because damaged units may produce inaccurate results.

   Inspect each Smart-Read EZTest unit for:

   - Indication of a damaged ampoule including low medium fill volume, wet or dried medium inside vial, cap filter appearing wet or discolored or spore strip appearing wet or discolored.
   - Missing or damaged components including cap, cap filter, spore strip, medium ampoule and plastic vial.

   Dispose of any damaged or questionable units per site procedure. Results obtained from damaged units should be considered suspect.

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VI. Incubation and Readout Time

The recommended incubation for Smart-Read EZTest is not less than 10 hours at 58° – 62°C.

Smart-Read EZTest should be placed in the incubator immediately after activation. Placement in an optimized growth environment which maintains the correct incubation temperature is necessary to gain accurate results.

The incubation time of EZTest Steam was validated according to the guidelines set forth in Attachment II of the *Guidance for Industry and FDA Staff: Biological Indicator (BI) Premarket Notification [510(k)] Submissions*, issued October 4, 2007 by the Food and Drug Administration (FDA) Center of Devices and Radiological Health (CDRH). 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 were valid according to the FDA protocol (30% to 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 10 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.

VII. 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.

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VIII. Interpretation of Smart-Read EZTest 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, refer to the Smart-Well 1710 incubator manual on Mesa's website.

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.

IX. Resistance Performance Characteristics

Steam resistance assessment testing is performed by exposing Smart-Read EZTest Steam SCBIs in a steam resistometer conforming to ANSI/AAMI/ISO 18472:2018. Exposure conditions are at 121°C ± 0.5°C, 132°C ± 0.5°C, 134°C ± 0.5C and 135°C ± 0.5°C in saturated steam using a pre-vacuum cycle. Additional D-value assessment at 124°C ± 0.5°C and 127°C ± 0.5°C are performed for calculation of z-value. D-value is determined using the Fraction Negative method.

Sample performance data is presented in the Table 2.

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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.

X. Population Determination

Detailed population assay instructions, TS-403 Paper, Quartz, & Cotton Thread Carrier Products, are available on Mesa's website.

XI. Compliance

Smart-Read EZTest Steam is manufactured in compliance with Mesa Laboratories' quality standards, USP, ISO 11138-1:2017 and ISO 11138-3:2017 guidelines.

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