Technical Report

EZTest® H_2O_2

I. Introduction

EZTest H_2O_2 is a self-contained biological indicator (SCBI) for use in monitoring the efficacy of H_2O_2 plasma sterilization cycles. EZTest H_2O_2 is easy to use; no sophisticated laboratory testing or analysis is required. EZTest H_2O_2 units consist of 10^5 or 10^6 *Geobacillus stearothermophilus* strain 7953 spores inoculated onto a 7.16mm stainless-steel disc 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 a hole is set over the top which allows sterilant penetration into the EZTest. The filter paper under the cap also serves as a chemical indicator, allowing for visual indication of whether or not the unit has been exposed to hydrogen peroxide vapor.

II. Storage

EZTest H_2O_2 should be stored at room temperature. The indicators should not be stored near sterilants or other chemicals. Do not desiccate.

III. Shelf Life

EZTest H_2O_2 has an 18-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 disc. 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.

V. Use

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

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2. Place EZTest SCBIs in a horizontal position with representative materials to be sterilized and in the “worst case” (least lethal) location in the load.

3. The EZTest H_2O_2 SCBI is available in a 10^5 population for use in lumens or process challenge devices (PCD), and in a 10^6 population for standalone testing.

4. Process the load as usual.

5. Retrieve the EZTest SCBIs from the sterilizer load.

6. The chemical indicator on the cap filter changes from pink to blue when exposed to hydrogen peroxide. The purpose of the chemical indicator is to distinguish exposed from unexposed units. A blue color does not indicate acceptable sterilization.

7. To culture the disc in an EZTest SCBI, place the indicator in an upright position and compress the plastic vial with a crushing device to break the glass ampoule. This will allow the growth medium to come in contact with the spore disc. Ensure that the spore disc 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 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 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 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 disc appearing wet.

   - Missing or damaged components including cap, cap filter, spore disc, medium ampoule and plastic vial.

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

VI. Incubation and Readout Time

The recommended incubation for EZTest H_2O_2 is not less than 24 hours at 55° – 60°C.

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.

Mesa Labs’ I1410 dry bath incubator is a small, convenient, tabletop incubator capable of maintaining the correct incubation temperature for all EZTest SCBIs.

The incubation time of EZTest H_2O_2 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). The CDRH reduced incubation time (RIT) protocol for validation of RIT may or may not meet each user's requirements for regulatory compliance. Users should therefore confirm regulatory requirements for reduced incubation time or incubate for 7 days.

NOTE: If incubation will be longer than 24 hours, the growth medium must be protected from evaporation by sealing the hole in the cap. Mesa's Clear Sealing Caps (part #758001) serve this purpose.

VII. Interpretation

The appearance of a yellow color indicates bacterial growth. No color change indicates the spores were killed in the sterilization process.

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'. Carefully review sterilizer process records to ensure that all physical process parameters are within specification. Always ensure that loading configuration and product and package specifications are in agreement with the sterilization validation process. Always retest the sterilizer with several EZTest indicators throughout the test load. EZTest indicators can be subcultured if identification of positive growth is desired.

A positive control should be run for each cycle tested, or at least once per week. As soon as a control turns yellow, it should be appropriately recorded and then autoclaved and discarded. The control is intended to assure you that viable spores are present on the SCBI lot prior to testing the sterilizer. Positive controls are not intended to be a 'color standard' for comparing test results. It is not recommended to incubate these positive controls more than 24 hours.

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.

VIII. Resistance Performance Characteristics

Hydrogen peroxide resistance assessment testing is performed by exposing EZTest H_2O_2 SCBIs in a hydrogen peroxide resistometer conforming to ANSI/AAMI/ISO 18472:2018. Exposure conditions are 2.3 mg/L at 50°C. D-value is determined using the Fraction Negative method.

IX. Population Determination

Detailed population assay instructions, TS-406 EZTest H_2O_2 and Stainless-Steel Discs, are available on Mesa's website: https://biologicalindicators.mesalabs.com/documents-manuals/

X. Compliance

EZTest H_2O_2 is manufactured in compliance with Mesa Laboratories' quality standards, USP and ISO 11138-1:2017 guidelines.

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