

Technical Report

DriAmp® Biological Indicator Culturing Set

I. Introduction

DriAmp Biological Indicator Culturing Set is used in monitoring the efficacy of Dry Heat sterilization processes. The set consists of DriAmp ampoule biological indicators (BIs) and tubes of Mesa Labs' Releasat® culture medium.

DriAmp consist of 1 mL scored snap-top glass ampoules containing silica sand inoculated with 10^6 *Bacillus atrophaeus* strain 9372 spores.

Mesa's Releasat medium is specially formulated for rapid outgrowth of *Bacillus atrophaeus* spores that may have survived the Dry Heat process. The 16mm x 100mm culture tubes are filled with 3.6 ± 0.2 mL of Releasat medium.

II. Storage

DriAmp Biological Indicator Culturing Set should be stored at room temperature. Do not desiccate.

III. Shelf Life

DriAmp Biological Indicator Culturing Set has a 12-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 incineration or by exposure to a dry heat cycle that is two times the labeled kill time.

IV. Medium

The Releasat culture medium, consisting of a proprietary formulated soybean casein digest base, provides the spores with a nutrient medium for growth. Following manufacture, the tubes of medium are autoclaved to render them sterile and growth promotion is performed using less than 100 spores of *Bacillus atrophaeus* 9372.

The culture medium has a pH indicator added to it, which appears red-orange color. If viable spores are added, the medium changes to yellow as the acidic metabolic products of the growing bacteria accumulate. If the medium remains red-orange and clear after culturing of the sand and incubation, no microbial growth occurred, indicating that the spores were killed in the sterilization process. If the sterilization process was not effective, the spores will grow, and the medium will turn yellow and/or turbid. If a media tube shows signs of a visual color change or turbidity prior to use, it should be autoclaved and discarded.

V. Use

1. Identify the DriAmp ampoules by labeling with pertinent process or load location information. Place inside the product or product package and place in the most difficult location to sterilize. Refer to the manufacturer's operating manual for guidelines.
2. Place a sufficient number of ampoules throughout the load to be sterilized.

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3. Expose the load to the validated sterilization cycle.
4. Following the exposure, remove the ampoules and transfer them to the laboratory for culturing.
5. In the laboratory, the ampoules should be handled using strict aseptic technique and working within confines of a laminar flow hood
6. Open each ampoule to culture the sand by holding the top of the ampoule with one hand and the body of the ampoule with the other hand and positioning thumb tips spread away from scored line of the ampoule.
 - 6.1 Safety goggles should be worn as a precaution.
 - 6.2 The first knuckle of each hand should touch to form a fulcrum with the knuckles acting as a hinge.
 - 6.3 Apply pressure at the scored glass line.

NOTE: Thumbs placed tip-to-tip can result in laceration.
7. The sand contained in the ampoule should be poured into individual Releasat medium tubes without touching the ampoule to the tube.
8. Dispose of empty glass ampoules.
9. The tubes should be placed in the incubator immediately after the sand is cultured.

VI. Incubation and Readout Time

The recommended incubation for the DriAmp BI Culturing Set is not less than 72 hours at 36 - 38°C. Placement in an optimized growth environment which maintains the correct incubation temperature is necessary to gain accurate results.

The incubation time of the DriAmp BI Culturing Set 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.

VII. Interpretation

The appearance of a yellow color and/or turbidity indicates microbial growth. No color change indicates 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 the 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.

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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 yellow within 24 to 48 hours of incubation. As soon as the control turns positive, it should be appropriately recorded, sterilized and discarded. The positive control is intended to confirm viable spores are present on the sand inside the glass ampoule 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 sand ampoule; or inadvertent sterilization of the entire box of indicators due to improper storage.

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

VIII. Performance Characteristics

Dry Heat resistance assessment testing is performed by exposing DriAmp ampoules in a Dry Heat resistometer conforming to ANSI/AAMI/ISO 18472:2018. Exposure conditions are $150^{\circ}\text{C} \pm 2.5^{\circ}\text{C}$, $160^{\circ}\text{C} \pm 2.5^{\circ}\text{C}$ and $170^{\circ}\text{C} \pm 2.5^{\circ}\text{C}$. D-value is determined using the DriAmp ampoules, cultured in Releasat medium, and calculated using the Fraction Negative method.

Dry Heat z-value is calculated using 150°C , 160°C and 170°C D-values.

Survival and Kill times at 160°C are calculated per the equations in ISO 11138-1, Annex E, using a population value and a D-value rounded to four decimal places.

IX. Population Determination

Detailed population assay instructions, TS-402 DriAmp, are available on Mesa's website.

X. Compliance

DriAmp Biological Indicator Culturing Set for Dry Heat is manufactured in compliance with Mesa Laboratories' quality standards, USP, ISO 11138-1:2017 and ISO 11138-4:2017 guidelines.