**INTENDED USE**

The Endotoxin Challenge Vial (ECV) is used in the validation of dry heat depyrogenation cycles. The ability of a particular oven cycle to destroy/inactivate endotoxin is measured by comparing the endotoxin levels in baked ECVs vs unbaked control ECVs. The United States Pharmacopeia (USP) recommends that in order for a depyrogenation process to be valid, the endotoxin content of a challenge vial must be reduced at least 1000 fold (>3 log cycle reduction). The ECVs are designed to indicate a greater than 3 log reduction in endotoxin content when tested using PYROGENT®, QCL-1000®, Kinetic-QCL®, or PYROGENT®-5000.

**EXPLANATION OF TEST**

Dry heat sterilization is used to depyrogenate glassware and other non-heat-labile materials. Following appropriate heat distribution studies, ECVs are placed in the predetermined hardest-to-heat locations ("cold spots"). After cycle completion, the log reduction in endotoxin levels can be determined by comparing the endotoxin levels in the baked vs non-baked control ECVs. The Limulus Amebocyte Lysate (LAL) assay is used to quantitate levels of endotoxin in the ECVs.

**REAGENT**

Endotoxin Challenge Vial (ECV) containing >1000 EU of E. coli 055:B5 endotoxin.

**NOTE:**
ECVs may appear to be empty due to the extremely small amount of endotoxin per vial. This "empty" appearance is normal. ECV labels are designed to withstand a cycle of 225°C for 8 hours. Temperatures higher than 225°C may render the label unreadable.

**PRELIMINARY PREPARATION**

Place the appropriate number of ECVs in the oven at the predetermined hardest-to-heat location(s). Do NOT remove crimp seal, silicone stopper or vial label. Bake vials according to user-selected cycle parameters. After the cycle is complete, remove the vials for endotoxin testing. Reconstitute each baked vial and an appropriate number of unbaked control ECVs with 1.0 ml of LAL Reagent Water. Vortex at high speed for 30 minutes. Prepare the appropriate dilutions from the unbaked samples using LAL Reagent Water (see below). Test immediately using PYROGENT, QCL-1000®, Kinetic-QCL®, or PYROGENT-5000. The user should refer to the package insert instructions included with the particular test kit used for the endotoxin assay.

**TEST PROCEDURE AND CALCULATION OF RESULTS**

Using PYROGENT, Gel-clot Lysate

Determine the concentration of endotoxin in unbaked control vials by diluting the reconstituted unbaked control vials using LAL Reagent Water. Dilutions should bracket 1/10,000. Do not dilute the baked vials.

Assay the samples using LAL with a 0.125 EU/ml lysate sensitivity. The endotoxin concentration in the reconstituted Endotoxin Challenge Vials can be calculated as follows:

Endotoxin concentration = lysate sensitivity x reconstitution volume (EU/vial) x maximum positive dilution

(Dilution refers to the denominator of the dilution fraction, e.g. for a 1/10,000 dilution, the denominator = 10,000. For undilute samples, dilution = 1)

A positive reaction indicates an endotoxin content greater than the calculated endotoxin value. A negative reaction indicates an endotoxin content less than the calculated endotoxin value.

A positive reaction in the 1/10,000 dilution of the unbaked control vials indicates an initial endotoxin concentration greater than 1250 EU/vial. Example: Endotoxin concentration = 0.125 EU/ml x 1 ml/vial x 10,000 = 1250 EU/vial

A negative reaction in the undiluted baked vials indicates a final endotoxin concentration less than 0.125 EU/vial.
Endotoxin concentration = $0.125 \text{ EU/ml} \times 1 \text{ ml/vial} \times 1$
$= 0.125 \text{ EU/vial}$

Calculate the minimum log reduction as follows:

Minimum log reduction = $\log$ endotoxin concentration of the unbaked control vials - $\log$ endotoxin concentration of the baked vials

Minimum log reduction = $\log 1250 \text{ EU/vial} - \log 0.125 \text{ EU/vial} - 3.097 - (-0.903)$
$= 4$

**NOTE:** To limit the log reduction calculation to 3 logs, assay a 1/10 dilution of the reconstituted baked vials.

Using QCL-1000, Chromogenic Lysate

- Dilute the reconstituted unbaked control vials 1/10,000 using LAL Reagent Water. Do not dilute the baked vials.
- The endotoxin concentration in the reconstituted ECVs can be calculated as follows:
  
  Endotoxin concentration = Endotoxin concentration of test sample (EU/vial) x reconstituted volume x dilution factor
  (For undilute samples, dilution factor = 1)

  The resulting Mean $\Delta$Absorbance value from a diluted control ECV sample that falls on the standard curve can be used to calculate a corresponding endotoxin value. Using the above formula, the endotoxin concentration of the ECV can be calculated.

**Example:**

Endotoxin concentration = $0.21 \text{ EU/ml} \times 1 \text{ ml/vial} \times 1$
$= 0.21 \text{ EU/vial}$

The log reduction in the above example would be calculated as follows:

Log reduction = $\log$ endotoxin concentration of the unbaked control ECV - $\log$ endotoxin concentration of the baked ECV

Log reduction = $\log 3700 \text{ EU/vial} - \log 0.21$
$= 3.568 - (-0.678)$
$= 4.25$

**REFERENCES**


7. PN187-6

8. 02/07

Lonza Walkersville, Inc.
www.lonza.com