Pharma&Biotech

Lonza

Endotoxin Challenge Vials™

Endotoxin Indicator for Depyrogenation

Translated versions available at **www.lonza.com**

Content

Important: Read Entire Brochure Before Performing Test
Caution: Contents Are Pyrogenic

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 level(s) in baked ECVs vs unbaked control ECVs. The United States Pharmacopeia (USP) states that the endotoxin indicators used in depyrogenation process challenges allow for accurate indication of at least a 3-log reduction in USP Endotoxin Units[1,2]. The ECVs are designed to indicate a minimum 3-log reduction in endotoxin content when tested using PYROGENT™, QCL-1000™, Kinetic-QCL™, or PYROGENT™-5000 Limulus Amebocyte Lysate (LAL) Assays.

Explanation of Test

Dry-heat sterilization is used to depyrogenate glassware and other non heat-labile materials[3]. 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 LAL assay is used to quantitate levels of endotoxin in the ECVs.

The United States Pharmacopeia[1], the Parenteral Drug Association's Technical Reports numbers 3[4] and 7[5], and the LAL Users' Group[6] describe the use of the LAL assay to validate such dry heat processes.

Reagents Supplied and Storage Conditions

Reagent (00555447) Red-Labeled Vial

Endotoxin Challenge Vial™ containing >1,000 EU of purified *E. coli* 055:B5 endotoxin. Store at 2–8°C in original package. For disposal, depyrogenate at 250°C for 120 minutes.

Note: This product does not contain fillers or stabilizers. ECVs may appear to be empty due to the extremely small amount of endotoxin per vial. This "empty" appearance is normal. ECV labels and stoppers are designed to withstand a cycle of 250°C for 30 minutes. Temperatures higher than 250°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, 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 (or equivalent). Vortex at high speed for 30 minutes. Prepare the appropriate dilutions from the unbaked samples using LAL Reagent Water (see page 4). Test immediately using a PYROGENT™, QCL-1000™, Kinetic-QCL™, or PYROGENT™-5000 LAL Assay. 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 LAL

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

The following example demonstrates calculations using LAL with a 0.125 EU/ml lysate sensitivity. The endotoxin concentration in the reconstituted ECVs can be calculated as follows:

Endotoxin concentration (EU/vial) = lysate sensitivity × reconstitution volume × 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 endpoint 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 endpoint 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 × 1 ml/vial × 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 EU/ml × 1 ml/vial × 1

= <0.125 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 EU/vial – log 0.125 EU/vial

= 3.097 – (-0.903)

= 4

Using QCL-1000™ Endpoint Chromogenic LAL

Dilute the reconstituted unbaked control vials 1/10,000 using LAL Reagent Water or equivalent. **Do not** dilute the baked vials.

The endotoxin concentration in the reconstituted ECVs can be calculated as follows:

Endotoxin concentration (EU/vial) = Endotoxin concentration of test sample (from standard curve) × reconstitution volume × dilution factor

(For undilute samples, dilution factor = 1)

The resulting Mean Δ 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 = 0.37 EU/ml (from standard curve) × 1 ml/vial × 10,000 = 3700 EU/vial

The resulting Mean Δ Absorbance value from an undiluted baked ECV sample that falls on the standard curve can be used to calculate the endotoxin concentration for that sample. Using the formula above, the endotoxin concentration of the baked ECV can be calculated. Example:

Endotoxin concentration = 0.21 EU/ml (from standard curve) × 1 ml/vial × 1 = 0.21 EU/vial

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

Log reduction = log endotoxin concentration of the unbaked control vials – log endotoxin concentration of the baked vials

Log reduction = log 3700 EU/vial – log 0.21 EU/vial

= 3.568 – (-0.678)

= 4.25

If the resulting Mean Δ Absorbance value from an undiluted baked ECV is less than the Mean Δ Absorbance value of the lowest standard, use the value of the lowest standard as the endotoxin concentration of the sample. The calculated log reduction will be the minimum log reduction.

Using Kinetic-QCL™ Chromogenic or PYROGENT™-5000 Turbidimetric LAL

Dilute the reconstituted unbaked control vials 1/10,000 using LAL Reagent Water or equivalent. **Do not** dilute the baked vials.

After completion of the assay, the WinKQCL™ Software will automatically calculate the endotoxin concentrations for both the control and baked ECVs.

Calculate the log reduction in endotoxin levels as in this example:

Log reduction = log endotoxin concentration of the unbaked control ECV – log endotoxin concentration of the baked ECV

= log 3700 – log 0.21

= 3.568 – (-0.678)

= 4.25

References

1. Chapter <1211> Sterilization and Sterility Assurance of Compendial Articles. Rockville, MD: *United States Pharmacopeia.*

2. Endotoxin Indicator For Depyrogenation. Rockville, MD: *United States Pharmacopeia.*

3. Weary, M., and F. Pearson. A manufacturer's guide to depyrogenation. *Biopharm.* 1, 4 (1988).

4. Parenteral Drug Association. Validation of dry heat processes used for sterilization and depyrogenation. *Technical Report* No. 3 (1981).

5. Parenteral Drug Association. Depyrogenation by dry heat. *Technical Report* No. 7 (1985).

6. LAL Users' Group. Preparation and use of endotoxin indicators for depyrogenation process studies. J. Parenter. *Sci. Technol.* 43, 3 (1989).

Notes

www.lonza.com/pharmabiotech

Certificate of Analysis: www.lonza.com/coa

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