Pharma&Biotech

Lonza

Limulus Amebocyte Lysate (LAL) Kinetic-QCL™

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Content

Important: Read Entire Brochure Before Performing Test

Intended Use

This product is intended as an *in vitro* end-product endotoxin test for human and animal parenteral drugs, biological products, and medical devices. This product is not intended for the detection of endotoxin in clinical samples or as an aid in the diagnosis of human disease. This test utilizes a preparation of Limulus Amebocyte Lysate (LAL), in combination with an incubating microplate reader and appropriate software, to detect endotoxin photometrically.

The Pharmacopeia outlines procedures that are considered necessary for:

1. Establishing endotoxin limits for pharmaceuticals and medical devices
2. Validating the use of LAL as an end-product endotoxin test
3. Developing a routine testing protocol[8]

The procedures described herein are based on the Pharmacopeial guidelines.

Warning

For *In Vitro* Diagnostic Use Only. The Kinetic-QCL™ Assay is not intended to detect endotoxemia in man. The LAL Test may be substituted for the USP Rabbit Pyrogen Test when used according to the Pharmacopeial guidelines for end-product testing of human and animal parenteral drugs, biological products, and medical devices[8].

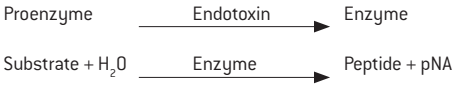
Explanation of Test

Kinetic-QCL™ is a quantitative, kinetic assay for the detection of Gram-negative bacterial endotoxin. A sample is mixed with the LAL/substrate reagent, placed in an incubating microplate reader, and automatically monitored over time for the appearance of a yellow color. The time required before the appearance of a yellow color (Reaction Time) is inversely proportional to the amount of endotoxin present. That is, in the presence of a large amount of endotoxin the reaction occurs rapidly; in the presence of a smaller amount of endotoxin the Reaction Time is increased. The concentration of endotoxin in unknown samples can be calculated from a standard curve.

The use of LAL for the detection of endotoxin evolved from the observation by Bang[1] that a Gram-negative infection of *Limulus polyphemus*, the horseshoe crab, resulted in fatal intravascular coagulation. Levin and Bang[2,3] later demonstrated that this clotting was the result of a reaction between endotoxin and a clottable protein in the circulating amebocytes of Limulus. Following the development of a suitable anticoagulant for Limulus blood, Levin and Bang[4] prepared a lysate from washed amebocytes, which was an extremely sensitive indicator of the presence of endotoxin. Solum[5,6] and Young, Levin, and Prendergast[7] have purified and characterized the clottable protein from LAL and have shown the reaction with endotoxin to be enzymatic.

The present LAL method utilizes the initial part of the LAL endotoxin reaction to activate an enzyme, which in turn releases p-nitroaniline (pNA) from a synthetic substrate, producing a yellow color.

Principle



Gram-negative bacterial endotoxin catalyzes the activation of a proenzyme in the LAL[7]. The initial rate of activation is determined by the concentration of endotoxin present. The activated enzyme catalyzes the release of pNA from the colorless substrate Ac-Ile-Glu-Ala-Arg-pNA. The free pNA is measured photometrically, at 405 nm continuously throughout the incubation period. The concentration of endotoxin in a sample is calculated from its Reaction Time by comparison to a standard curve.

Reagents Supplied and Storage Conditions

Kinetic-QCL™ Reagent (K50-643) Yellow-Labeled Vial

Each vial contains a lyophilized mixture of lysate prepared from the circulating amebocytes of the horseshoe crab, *Limulus polyphemus*, and chromogenic substrate.

Reconstitute immediately before use with 2.6 ml of LAL Reagent Water per vial. If the contents of more than one vial are required, pool two or more vials before use. Swirl gently to avoid foaming. Lyophilized Kinetic-QCL™ Reagent is to be stored at 2–8°C. Protect from long term exposure to light.

Reconstituted Kinetic-QCL™ Reagent should be used promptly. Reconstituted Kinetic-QCL™ Reagent is stable for 8 hours at 2–8°C or can be stored at -10°C or colder for up to two weeks. Freeze and thaw only once.

***E. coli* 055:B5 Endotoxin (E50-643)** Red-Labeled Vial

The reconstitution volume of the vial is stated on the Certificate of Analysis (CoA) and is calculated to yield a solution containing 50 EU (or IU)/ml. Reconstitute with the specified volume of LAL Reagent Water. Shake vigorously for at least 15 minutes at high speed on a vortex mixer. Prior to subsequent use, a stored stock solution must be warmed to room temperature and vigorously vortexed for 15 minutes. This is important because the endotoxin tends to attach to glass. The COA is available at **www.lonza.com/coa**.

Lyophilized *E. coli* 055:B5 Endotoxin is to be stored at 2–8°C. Reconstituted stock endotoxin is stable for four weeks at 2–8°C.
Note: Endotoxin is not included but required for lysate-only kits.

This endotoxin is provided for the user's convenience. Other endotoxin preparations may be used to prepare the standards; however, their performance in the chromogenic assay relative to the Reference Standard Endotoxin (RSE) must be determined.

LAL Reagent Water (W50-640) Yellow-Labeled Vial
Each vial contains 30 ml of LAL Reagent Water. This water should be used to rehydrate the Kinetic-QCL™ Reagent and *E. coli* Endotoxin and to prepare endotoxin and sample dilutions. LAL Reagent Water is equivalent to Water for Bacterial Endotoxins Test (BET).

LAL Reagent Water should be stored at 2–8°C.
Note: LAL Reagent Water is not included but required for lysate-only kits.

Materials and Equipment NOT Provided

1. Disposable endotoxin-free glass dilution tubes (13 × 100 mm, #N207 or equivalent).
2. Individually wrapped serological pipettes.
3. Automatic hand-held pipettes with sterile, individually wrapped or racked tips.

4. Disposable sterile microplates.
   Note: Prior to routine use, microplates should be pre-qualified[8] (#25-340 or equivalent).
5. Reagent reservoirs (#00190035 or equivalent).
6. Eight channel pipettor.
7. Sodium hydroxide, 0.1N, or Hydrochloric acid, 0.1N, dissolved in LAL Reagent Water, for pH adjustment of sample if necessary.
8. Microplate reader (ELx808™ IU Reader, #25-315 or equivalent).
9. WinKQCL™ Software.
10. Timer.
11. Vortex mixer.
12. For kits without water: LAL Reagent Water (#W50-640, #W50-100, #W50-500, or equivalent).
13. Endotoxin Standard (Control Standard Endotoxin that has been matched with the LAL).

Sample Collection and Preparation

Careful technique must be used to avoid microbial or endotoxin contamination. All materials coming in contact with the sample or test reagents must be endotoxin-free. Clean glassware and materials may be rendered endotoxin-free by heating at 250°C for 30 minutes. Appropriate precautions should be taken to protect depyrogenated materials from subsequent environmental contamination.

From experience, most sterile, individually wrapped, plastic pipettes and pipette tips are endotoxin-free. However, these materials should be tested before regular use.

It may be necessary to adjust the pH of the sample to within the range 6.0–8.0 using endotoxin-free sodium hydroxide or hydrochloric acid. Always measure the pH of an aliquot of the bulk sample to avoid contamination by the pH electrode. Do not adjust unbuffered solutions.

Samples to be tested must be stored in such a way that all bacteriological activity is stopped or the endotoxin level may increase with time. For example, store samples at 2–8°C for less than 24 hours and frozen for periods greater than 24 hours. It is the responsibility of the end-user to validate the proper container and storage conditions for their samples.

If the container of diluent used to rehydrate the Kinetic-QCL™ Reagent has been opened previously or was not supplied by Lonza , the diluent alone must be tested for endotoxin contamination.

Types of Kinetic-QCL™ Assays

The incubating microplate reader and WinKQCL™ Software are an integral part of the Kinetic-QCL™ Assay. It is important to become familiar with the operation of the incubating microplate reader and the features of the WinKQCL™ Software. Please refer to the incubating microplate reader and WinKQCL™ Software Manuals or Help for more detailed information.

There are four (4) basic types of Kinetic-QCL™ Assays, each of which is designed to perform a different aspect of LAL testing.

1. **Routine**
   A Routine assay calculates the concentration of endotoxin in unknowns by comparison to the performance of a series of endotoxin standards.

   As part of a Routine assay, the user has the option to include a Positive Product Control (PPC) as a monitor for product inhibition or enhancement (section 2 below). A PPC is a sample of product to which a known amount of endotoxin spike has been added. The WinKQCL™ Software automatically calculates the amount of endotoxin recovered in the PPC, allowing for a comparison to the known amount of endotoxin spike.

2. **Inhibition/Enhancement**
   The LAL reaction is enzyme mediated and, as such, has an optimal pH range and specific salt and divalent cation requirements. Occasionally test samples may alter these optimal conditions to an

extent that the lysate is rendered insensitive to endotoxin. Negative results with samples which inhibit the LAL test do not necessarily indicate the absence of endotoxin.

An Inhibition/Enhancement assay is designed to determine what level of product dilution overcomes inhibition or enhancement. Each product dilution must be accompanied by a Positive Product Control (PPC). The WinKQCL™ Software automatically calculates the amount of endotoxin recovered in the PPC for comparison to the known amount of endotoxin spike. In this manner it can be determined which product dilutions are non-interfering.

3. **RSE/CSE**
   An RSE/CSE assay is designed to determine the potency of a Control Standard Endotoxin (CSE) in terms of the concentration units of the Reference Standard Endotoxin (RSE).

   The assay requires a single series of RSE dilutions and one or more sets of dilutions of the CSE. Depending on the concentration units of the CSE, the WinKQCL™ Software automatically computes mean potency values in terms of EU/ng or EU/ml. The user also has the option to enter units other than EU or ng.

4. **Initial Qualification**
   An Initial Qualification assay is designed according to the requirements described in the Pharmacopeia[8]. **This assay is required as part of the validation of the LAL assay and is also to be performed with each new lot of Kinetic-QCL™.**

   The Initial Qualification assay performs a log/log linear correlation of the individual Reaction Time values for each replicate of each endotoxin standard. The other assays use the average Reaction Time of all the replicates of each standard.

   The Initial Qualification assay does not provide for the inclusion of any samples.

Reagent Preparation

Allow reagents to equilibrate to room temperature prior to use.

In order to calculate endotoxin concentrations in unknown samples each Kinetic-QCL™ Test must be referenced to a valid standard curve.

Because of the large concentration range over which endotoxin values can be determined, it is possible to adjust the quantitative range of any given test by adjusting the concentration of endotoxin standards used to generate the standard curve. A minimum of three standards is required.

The Kinetic-QCL™ Assay has been optimized to be linear from 0.005 EU/ml to 50.0 EU/ml. However, the individual user may choose to truncate the standard curve depending on specific product requirements. Data indicates that truncating a kinetic chromogenic LAL standard curve may improve the accuracy of predicted endotoxin values for test samples. It is recommended that the user be familiar with the Pharmacopeial requirements for kinetic LAL techniques prior to establishing a kinetic chromogenic LAL standard curve range to be used for routine testing of product samples[8].

The following table suggests a dilution scheme for constructing a series of endotoxin dilutions from the endotoxin supplied in the kit. Not all dilutions must be used to generate a standard curve. Alternative dilution schemes can be used as well as other endotoxins not supplied in this kit. If the endotoxin used is not supplied in the kit, an RSE/CSE test to determine the CSE potency may be required.

Note: Plastic tubes are not recommended for making endotoxin dilutions.

Endotoxin Concentration (EU/ml)	Volume of LAL Reagent Water	Volume of Endotoxin Solution Added to LAL Reagent Water
5.0	0.9 ml	0.1 ml of 50.0 EU/ml solution
0.5	0.9 ml	0.1 ml of 5.0 EU/ml solution
0.05	0.9 ml	0.1 ml of 0.5 EU/ml solution
0.005	0.9 ml	0.1 ml of 0.05 EU/ml solution

1. Prepare a solution containing 5.0 EU/ml endotoxin by adding 0.1 ml of the 50.0 EU/ml endotoxin stock into 0.9 ml of LAL Reagent Water in a suitable container and label 5.0 EU/ml. This solution should be vigorously vortexed for at least 1 minute before proceeding.

2. Transfer 0.1 ml of the 5.0 EU/ml endotoxin solution into 0.9 ml of LAL Reagent Water in a suitable container and label 0.5 EU/ml. The solution should be vigorously vortexed for at least 1 minute before proceeding.

3. Transfer 0.1 ml of the 0.5 EU/ml endotoxin solution into 0.9 ml of LAL Reagent Water in a suitable container and label 0.05 EU/ml. This solution should be vigorously vortexed for at least 1 minute before proceeding.

4. Transfer 0.1 ml of the 0.05 EU/ml endotoxin solution into 0.9 ml of LAL Reagent Water in a suitable container and label 0.005 EU/ml. This solution should be vigorously vortexed for at least 1 minute before proceeding.

Test Procedure

Refer to the microplate reader and WinKQCL™ Software Manuals for more detailed information on performing a Kinetic-QCL™ Test.

1. Create a specific **Template** for the test to be run. A Template contains the name of the analyst, type of assay, lot numbers of reagents, the number and concentration of endotoxin standards, number of replicates, and how standards and samples will be organized on the microplate.

2. The **Assay Type** must be selected as **Kinetic-QCL**. The default Template Parameters that follow should not be changed without prior qualification:

Delta t (seconds)	150
Measurement filter (nm)	405
Delta mOD	200
Number of Reads	40

3. Print the Template for use as a guide in placing standards and samples into the microplate.

4. "Run" the Template, following the WinKQCL™ Software prompts.

5. Carefully dispense 100 µl of the LAL Reagent Water blank, endotoxin standards, product samples, positive product controls (see pages 22–24 for positive product control instructions), etc. into the appropriate wells of the microplate.

6. Place the filled plate in the microplate reader and close the lid.

7. Pre-incubate the plate for ≥10 minutes at 37°C ± 1°C.

8. Near the end of the pre-incubation period reconstitute each of the appropriate number of Kinetic-QCL™ Reagent vials with 2.6 ml LAL Reagent Water per vial. Mix gently but thoroughly.
   Note: Do not vortex the lysate.

9. Pool the reagents into a reagent reservoir and mix by gently rocking the reservoir from side to side.

10. Using an eight channel pipettor dispense 100 µl of the Kinetic-QCL™ Reagent into all wells of the microplate beginning with the first column (A1-H1) and proceeding in sequence to the last column used. Add reagent as quickly as possible.
    Note: Avoid causing bubbles!

11. Immediately click on the OK button in the WinKQCL™ Software to initiate the test.
    Note: The Kinetic-QCL™ Assay is performed with the microplate cover removed.

Performance Characteristics

Linearity

The linearity of the standard curve within the concentration range used to determine endotoxin values should be verified. No less than three endotoxin standards, spanning the desired concentration range, and an LAL Reagent Water blank should be assayed at least in triplicate according to the test parameters of an **Initial Qualification** assay. Additional standards should be included to bracket each log interval over the range of the standard curve.

The absolute value of the correlation coefficient (r) of the calculated standard curve should be ≥0.980.

Reproducibility

Replicate samples should be run in order to establish good technique and low coefficient of variation. The coefficient of variation (C.V.) equals the "sample" standard deviation of the reaction times divided by the mean and is usually expressed as a percent. The %C.V. of the reaction times for the replicates should be less than 10%. With experience, values of 3–4% should be attainable.

Calculation of Endotoxin Concentration

Continuously throughout the assay, the microplate reader/WinKQCL™ Software monitors the absorbance at 405 nm of each well of the microplate. Using the initial absorbance reading of each well as its own blank, the reader determines the time required for the absorbance to increase 0.200 absorbance units. This time is termed **Reaction Time**. The WinKQCL™ Software automatically performs a log/log linear correlation of the Reaction Time of each standard with its corresponding endotoxin concentration. The standard curve parameters are printed on the report printout. If the absolute value of the correlation coefficient (r) is ≥0.980, a polynomial model can be used to construct a standard curve and in turn predict endotoxin concentrations of test samples. This polynomial curve-fitting model (POWERCURVE™) is an important feature of the WinKQCL™ Software (see POWERCURVE™, page 20).

Linear Regression

The information provided below is an example of how the WinKQCL™ Software performs the log/log linear correlation and computes endotoxin concentrations in unknowns. It is not necessary to perform these calculations independently. For each sample of each product, the WinKQCL™ Software calculates the corresponding endotoxin concentration from the Reaction Time for that sample. The software automatically adjusts the final **Test Result** value to account for any product dilution.

Linear Correlation

Example Calculations

Standards	Concentration	Mean Reaction Time (Sec)	Log Concentration	Log Mean Reaction Time
Neg. Control	—	Unreactive	—	—
S1	0.005 EU/ml	4351	-2.301	3.639
S2	0.05 EU/ml	2496	-1.301	3.397
S3	0.5 EU/ml	1406	-0.301	3.148
S4	5.0 EU/ml	895	0.699	2.952
S5	50.0 EU/ml	561	1.699	2.749
Samples				
1	—	1576	—	3.198
2	—	943	—	2.975

$$\text{Slope} = \left(\frac{Sy}{Sx}\right)r$$

$$\text{Y-intercept} = \Sigma y / N - (\Sigma x / N \times \text{slope})$$

$$r = \frac{N\Sigma xy - (\Sigma x)(\Sigma y)}{N(N-1)SxSy}$$

$$\text{Endotoxin concentration} = \text{antilog}\left[\frac{\log \text{Mean Reaction Time} - \text{Y int.}}{\text{slope}}\right]$$

$x = \log_{10}$ Endotoxin concentration in EU/ml.

$y = \log_{10}$ Mean Reaction Time.

N = Number of standards used.

Σx = Summation of $\log_{10}$ concentration of standards used in EU/ml.

Σy = Summation of $\log_{10}$ Reaction Time.

Σxy = Summation of the $\log_{10}$ standard concentrations times $\log_{10}$ Mean Reaction Time.

$$Sx = \text{Standard deviation of } x = \sqrt{\frac{N\Sigma x^2 - (\Sigma x)^2}{N(N-1)}}$$

$$Sy = \text{Standard deviation of } y = \sqrt{\frac{N\Sigma y^2 - (\Sigma y)^2}{N(N-1)}}$$

Calculations using Example Data:

$N = 5$

$\Sigma x = -1.505 = (-2.301 - 1.301 - 0.301 + 0.699 + 1.699)$

$\Sigma y = 15.885 = (3.639 + 3.397 + 3.148 + 2.952 + 2.749)$

$\Sigma xy = -7.006 = (-2.301 \times 3.639) + (-1.301 \times 3.397) + (-0.301 \times 3.148) + (0.699 \times 2.952) + (1.699 \times 2.749)$

$Sx = 1.581$

$Sy = 0.352$

$$r = \frac{5(-7.006) - (-1.505)(15.885)}{5(5-1)(1.581)(0.352)} = -0.999$$

$$\text{Slope} = \frac{0.352}{1.581} \times -0.999 = -0.222$$

$$\text{Y-intercept} = \frac{15.885}{5} - \left[\frac{-1.505}{5} \times (-0.222)\right]$$

$$= 3.177 - [(-0.301) \times (-0.222)] = 3.110$$

Sample 1

$$\text{Endotoxin Conc. EU/ml} = \text{antilog}\left[\frac{3.198 - 3.110}{-0.222}\right]$$

$$= \text{antilog}(-0.396)$$

$$= 0.402 \text{ EU/ml}$$

Sample 2

$$\text{Endotoxin Conc. EU/ml} = \text{antilog}\left[\frac{2.975 - 3.110}{-0.222}\right]$$

$$= \text{antilog}(0.608)$$

$$= 4.056 \text{ EU/ml}$$

POWERCURVE™

If the absolute value of the correlation coefficient (r) is ≥0.980, a polynomial model can be used to construct a standard curve and predict endotoxin concentrations of test samples. It has been determined that this polynomial model (POWERCURVE™) improves the accuracy of predicting endotoxin concentrations over the entire (5-log) endotoxin range. The use of the POWERCURVE™ Model requires the use of WinKQCL™ Software.

When using POWERCURVE™, a standard curve is generated using the $\log_{10}$ Reaction Time values and their corresponding $\log_{10}$ endotoxin concentration to define a polynomial equation. The order of the polynomial equation used to generate the regression curve is determined by the number of endotoxin standards in the assay. The order of the polynomial will always be one less than the number of endotoxin standards, with a maximum of a fourth order polynomial for assays with five or more endotoxin standards and a minimum of a second order polynomial for assays with three standards.

Finding solutions to these polynomial equations is readily accomplished using the WinKQCL™ POWERCURVE™ Software. The information provided below is an example of a solution to a polynomial equation using the same set of data from the linear correlation example on page 18.

Polynomial (POWERCURVE™) Model

Y	=	$A + BX + CX^2 + DX^3 + EX^4$
A	=	3.08374
B	=	-0.20432
C	=	0.02894
D	=	-0.00596
E	=	-0.00503

The standard curve parameters are printed on the report printout. The WinKQCL™ POWERCURVE™ Software uses these parameters to calculate the corresponding endotoxin concentration from the Reaction Time of each sample. The software automatically adjusts the final **Test Result** value to account for any product dilution.

It is important to note that the POWERCURVE™ polynomial model **CANNOT** be used for **Initial Qualification** assays. Linear regression must still be used in those cases. Additionally, the POWERCURVE™ Polynomial Model has only been evaluated for the Kinetic-QCL™ and PYROGENT™-5000 Reagents supplied by Lonza.

Product Inhibition

Product inhibition occurs when substances in the test sample interfere with the LAL reaction. In the Kinetic-QCL™ Assay, this inhibition results in a longer Reaction Time, indicating lower levels of endotoxin than may actually be present in the test sample. The lack of product inhibition should be determined for each specific sample, either undiluted or at an appropriate dilution.

To verify the lack of product inhibition, an aliquot of test sample (or a dilution of test sample) is spiked with a known amount of endotoxin.

It is recommended that the endotoxin spike result in a final endotoxin concentration in the sample is equal to 0.5 EU/ml. For samples which may contain a background endotoxin level >1 EU/ml, the endotoxin spike should result in a final endotoxin concentration of 5.0 EU/ml.

In an Inhibition/Enhancement assay, the spiked solution (PPC) is assayed along with the unspiked sample, and their respective endotoxin concentrations, as well as the endotoxin recovered in the spiked sample are automatically calculated. The endotoxin recovered should equal the known concentration of the spike within 50 – 200%[8].

A spiked aliquot of the test sample (or dilution) may be prepared as in one of the following examples:

Tube Method

Transfer 50 µl of the 50.0 EU/ml solution into 4.95 ml of test sample (or dilution). This solution contains an endotoxin concentration of 0.5 EU/ml in test sample (or dilution). This sample should be vigorously vortexed for one minute prior to use.

Transfer 100 µl of this solution into the 96-well plate as directed by the assay template.

Plate Method #1

Transfer 10 µl of the 5.0 EU/ml solution into each of the PPC wells in the 96-well plate, as directed by the assay template. To these wells add 0.1 ml of test sample (or dilution). Each well will now contain a 0.5 EU/ml solution. Mix gently by tapping the side of the plate.

Plate Method #2

Place 0.1 ml of test sample (or dilution) into the PPC wells in the 96-well plate, as directed by the assay template. To these wells, add 10 µl of the 5.0 EU/ml solution. Each well will now contain a 0.5 EU/ml solution. Mix gently by tapping the side of the plate.

If the test sample (or dilution) is found to be inhibitory to the Kinetic-QCL™ Reaction, the sample may require further dilution until the inhibition is overcome.

Example: Determination of a Non-Inhibitory Dilution

Sample Dilution	Endotoxin Recovered
1/10	0.125 Inhibitory
1/20	0.212 Inhibitory
1/40	0.550 Non-Inhibitory

Initially, one may want to screen for product inhibition by testing 10-fold dilutions of test sample. Once the approximate non-inhibitory dilution is determined, the exact dilution can be found by testing two-fold dilutions around this dilution.

Limitations and Indications

The degree of inhibition or enhancement will be dependent upon the concentration of product. If several concentrations of the same product are to be assayed, it is necessary to establish performance characteristics for each independently.

Patterns of inhibition or enhancement different from those seen with the traditional LAL gelation test may be found.

It may be necessary to adjust the pH of the sample to within the range 6.0 to 8.0 using endotoxin-free sodium hydroxide or hydrochloric acid to overcome inhibition.

Colored Samples

Since the initial absorbance reading of each well is used as its own blank, samples which possess significant color on their own do not present a special problem. If the background color is ≥1.5 absorbance units, the sample should be diluted and reassayed.

Archived Standard Curve

The WinKQCL™ Software may be run using an archived standard curve. Provided that the current reagent lot numbers for the Kinetic-QCL™ Reagent, LAL Reagent Water, and endotoxin, as well as microplate reader parameters match those used to generate the valid archived standard curve, the archived standard curve may be used instead of placing new endotoxin standards on the 96-well plate.

If an archived standard curve is used, a single standard-control containing an endotoxin concentration equal to the mid-point, on a log basis, between the endotoxin concentration of the highest and lowest endotoxin standards in the archived standard curve should be assayed. The predicted endotoxin concentration should be within ± 25% of its known value.

For example, in an assay with a standard curve spanning from 50.0 to 0.005 EU/ml, a standard-control equal to 0.5 EU/ml should be assayed.

log 50.0	=	1.6990
log 0.005	=	-2.3010
log average	=	-0.3010
antilog -0.3010	=	0.5

In an assay with a standard curve spanning from 1.0 to 0.01 EU/ml, a standard-control equal to 0.1 EU/ml should be assayed.

log 1.0	=	0.0000
log 0.01	=	-2.0000
log average	=	-1.0000
antilog -1.0000	=	0.1

Correlation with Other Methods

The FDA regulates the official use of LAL testing in the United States. The potency of different endotoxin preparations varies in both the traditional gel test and the chromogenic method. The endotoxin standard supplied in this kit has been compared to the USP Reference Standard Endotoxin (RSE) using the Kinetic-QCL™ Assay, and the potency is 50.0 EU/ml when reconstituted using the volume specified on the lot-specific Certificate of Analysis. The calibration curve diluted from this standard will yield a range of 0.005 to 50.0 Endotoxin Units/ml relative to the RSE. It should be remembered, however, that the traditional gel test is standardized by two-fold dilutions, so that variations will appear quite large in comparison to those in the Kinetic-QCL™ Test where standardization is continuous and variations are minimal.

A Note for Our International Customers

Other regulatory agencies may adopt other performance standards which will need to be satisfied in order to be in compliance in their jurisdictions.

References

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11. U.S Department of Health and Human Services, Food and Drug Administration, *Guidance for Industry Pyrogen and Endotoxins Testing: Questions and Answers* (June 2012).

Notes

www.lonza.com/pharmabiotech

Certificate of Analysis: www.lonza.com/coa

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08293 P50-650U-14 10/13 RT-MN008