STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Efficacy Test for Confirmation of Labeled LAL reagent	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 OBJECTIVE:

1.1 To lay down a procedure for testing of each lot of Lysate received from the manufacturer for its labeled sensitivity.

2.0 SCOPE:

2.1 This SOP is applicable for testing of each lot of Lysate received from the manufacturer for its labeled sensitivity.

3.0 RESPONSIBILITY

3.1 Microbiologist

4.0 ACCOUNTABILITY

4.1 QC-Manager

5.0 PROCEDURE

5.1 Depyrogenate the materials required for the test as per the respective SOP.

5.2 Enter the details of the Lysate received from the manufacturer in the BET Stock Register.

5.3 Perform the test for efficiency for every new lot of lysate's received and enter the test details in Annexure-I.

5.4 Reconstitute Lysate vial by gentle taping to collect the Lysate powder into the bottom of the vial, carefully remove the stopper, rehydrate with LAL Reagent water as per the direction given on the vial & close the vial.

5.5 Mix the LAL powder gently until it dissolves.

5.6 Storage: Rehydrated LAL reagent solution can be stored at 2ºC to 8ºC for up to 24 hours otherwise store at -20°C for up to28 days. LAL can be frozen and thawed only once.

5.7 Using the Control Standard Endotoxin (CSE) specific for the lot number of the Lysate received, prepare a series of two fold dilutions of the CSE to give concentrations of 2λ, λ, λ/2 and λ/4, where, λ is the labeled sensitivity of the LAL reagent in EU per ml.

5.8 For Example: - Preparation of two folds dilution series for the confirmation of Lysate of 0.125 Endotoxin Units/ml end point.

If λ = 0.125Endotoxin Units/ml.

20 Eu/ml (after reconstituted with required amount of LRW vortex for 5 min)

↓

0.1ml + 1.9ml LRW (vortex for 1 min) = 8λ (1EU/ml)

↓

0.5ml+0.5ml LRW (Vortex for 1 min) = 4λ (0.5EU/ml)

↓

0.5ml+0.5ml LRW (Vortex for 1 min) = 2λ (0.25EU/ml)

↓

0.5ml+0.5ml LRW (Vortex for 1 min) = λ (0.125EU/ml)

↓

0.5ml+0.5ml LRW (Vortex for 1 min) = λ/2 (0.06EU/ml)

↓

0.5ml+0.5ml LRW (Vortex for 1 min) = λ/4 (0.03EU/ml)

5.9 After the dilution series of CSE is prepared, perform the test on the four standard concentrations (2 λ, λ, λ/2 and λ/4) in quadruplicate and include negative control as described in the following example:

Example:

Replicate No	Endotoxin dilutions (EU/ml)				LRW [Negative Control]	Log End point (e)
	2λ (0.25)	λ (0.125)	λ/2 (0.06)	λ/4 (0.03)		
1	+	+	-	-	-	-0.903
2	+	+	-	-	-	-0.903
3	+	+	+	-	-	-1.2218
4	+	+	-	-	-	-0.903
Mean of log end point						**∑e**

Geometric Mean = Antilog ∑e/f

Where,

∑e = Mean value of log end point.
f = Number of replicates

5.10 TEST PROCEDURE:

5.10.1 Pipette 100µl of the CSE series of each standard (2λ,λ, λ/2 and λ/4) dilutions into four endotoxin tubes respectively.

5.10.2 Pipette 100µl of Lysate for which the confirmation is being done.

5.10.3 Incubate the tubes in a calibrated heating block at 37^0C ± 1^0C for 60 minutes ± 2 minutes.

5.11 RESULT INTERPRETATION:

5.11.1 Positive reaction is indicated by a “Firm Gel” that remains intact even when the tube is inverted to 180°C.

5.11.2 Negative reaction is observed by “No Gel Formation”, if any Gel clot occurs in negative control the test is considered invalid.

5.12 Record the endpoint concentration, for each replicate series dilutions in the Annexure-I.

5.13 Determine the log of end point concentration, e and calculate the geometric mean end point concentration using the following formula:

Geometric mean end point concentration = antilog (Σe/f)

Where "Σe" is the sum of the logs of end point concentrations of the dilution series used and "f" is the number of replicates.

5.11 The geometric mean end point concentration should be within ± two fold of the labelled claim sensitivity of the Lysate.

6.0 ABBREVIATIONS

6.1	CSE	Control Standard Endotoxin
6.2	BET	Bacterial Endotoxin Test
6.3	LAL	Limulus Amoebocyte Lysate
6.4	LRW	LAL Reagent Water
6.5	λ	Lambda

7.0 ANNEXURES

7.1	Annexure-I	Confirmation of Lysate Label Claim Sensitivity