STANDARD OPERATING PROCEDURE

Title: Qualification of Sensitivity of Lysate

SOP No.:		**Department:**	Microbiology
		Effective Date:	
Revision No.:	00	**Revision Date:**	
Supersede Revision No.:	Nil	**Page No.:**	1 of 5

1.0 OBJECTIVE

To lay down procedure for qualification of sensitivity of Lysate.

2.0 SCOPE

This SOP is applicable for Lysate used in microbiology laboratory.

3.0 RESPONSIBILITY

Prepared by - Executive Microbiology

Checked by - Assistant Manager Microbiology / QC

Approved by - Head QA, QC

4.0 PROCEDURE

4.1 Preparation of Endotoxin free equipment

4.1.1 All the materials coming in direct contact with samples or test material shall be free of Endotoxin contamination.

4.1.2 Glassware's shall be first cleaned / flushed with purified water, and rinsed with water for injection and are subjected to depyrogenation at validated temperature and time.

4.2 Endotoxin control standard series

4.2.1 Reconstitute the lyophilized control standard Endotoxin (CSE) with volume of Endotoxin free LRW as indicated on the certificate of analysis (COA) supplied by the vendor.

4.2.2 Vortex intermittently for 30 minutes and use this concentrate to prepare dilutions as described under Endotoxin standard dilutions as per Table - I.

4.2.3 Prepare a series of two fold dilutions of the CSE as mentioned in Table - I, to give concentrations of 2 λ, λ, λ/2 and λ/4, where, λ is the labeled sensitivity of the LAL reagent in EU per ml.

TABLE - I

For example, if the LAL sensitivity is 0.125 EU/ml and the CSE vial concentration is 20 EU/ml prepare the dilutions as follows.

Tube No.	LRW	CSE Added from tube	Final Concentration of CSE	
01	0.9 ml	0.1 ml of 20 EU/ ml	2.0 EU/ml	
02	1.2 ml	0.4 ml of Tube No. 1	0.5 EU/ml	- 4λ
03	1.0 ml	1.0 ml of Tube No. 2	0.25 EU/ml	- 2λ
04	1.0 ml	1.0 ml of Tube No. 3	0.125 EU/ml	- λ
05	1.0 ml	1.0 ml of Tube No. 4	0.06 EU/ml	- λ/2
06	1.0 ml	1.0 ml of Tube No. 5	0.03 EU/ml	- λ/4

Note: Vortex each dilution for a minimum of not less 30 second before addition of the next concentration.

4.3 Test for confirmation of labeled claim Sensitivity of LAL reagent

4.3.1 Confirm the labeled claim sensitivity of each lot of LAL reagent prior to use in the test as described below.

4.3.2 Prepare a series of two fold dilutions of the CSE as mentioned above to give concentrations of 2 λ, λ, λ/2 and λ/4 , where, λ is the labeled sensitivity of the LAL reagent in EU per ml.

4.3.3 Remove the LAL reagent vial from the refrigerator and place it under LAF to acclimatize with the room temperature.

4.3.4 Collect LAL powder into the bottom of the vial by tapping it on the LAF bench.

4.3.5 Aseptically reconstitute the lyophilized LAL with the quantity of LRW as indicated on the vial label.

4.3.6 Swirl gently to dissolve, avoiding liquid contact with stopper.

4.3.7 Perform the test in quadruplicate including negative control as described in the following example.

4.3.8 The geometric mean end point concentration shall be within the ± 2 fold of the labeled LAL sensitivity.

4.3.9 Calculate the Geometric mean as given below.

Example:

Replicate No.	Observed Result				LRW (Negative Control)	End Point
	2λ (0.25)	λ (0.125)	λ/2 (0.06)	λ/4 (0.03)		
01	+ Ve	+ Ve	- Ve	- Ve	- Ve	0.125
02	+ Ve	+ Ve	- Ve	- Ve	- Ve	0.125
03	+ Ve	+ Ve	+ Ve	- Ve	- Ve	0.06

04	+ Ve	+ Ve	- Ve	- Ve	- Ve	0.125

Replicate No	End Point (EU/ml)	Log_{10} (End Point)	Mean	Geometric Mean End Point
01	0.125	-0.903	- 0.983	Anti log_{10} of Mean = Anti log_{10} of -0.983 = 0.104 EU/ml. (Complies with labeled LAL sensitivity λ = 0.125)
02	0.125	-0.903		
03	0.06	-1.222		
04	0.125	- 0.903		

5.0 SAFETY & PRECAUTIONS

Use depyrogenated glassware's for testing.

6.0 REVISION HISTORY

Revision No.	Reason for Revision	Superseded from & Date
00	First Issue	--------

7.0 REFERENCES

Not applicable

8.0 ABBREVIATIONS

LRW : LAL Reagent Water

CSE : Control Standard Endotoxin

λ. : Labelled Sensitivity

LAL : Limulus Amoebocyte Lysate

EU : Endotoxin Unit

9.0 ANNEXURES

Annexure - I : Test for confirmation of lable claim sensitivity of Lysate

ANNEXURE - I

TEST FOR CONFIRMATION OF LABLE CLAIM SENSITIVITY OF LYSATE

Date of testing: _____ Date of Report: _____

Control Standard Endotoxin Ref.No. -_____

1. Details of LAL Reagents, Control Standard Endotoxin and LAL Reagent Water -

LAL Reagent		CSE		LRW	
Lot No.		**Lot No.**		**Lot No.**	
Sensitivity		**Potency**		**Opened on**	
Reconstituted on		**Reconstituted on**		**Expiry Date**	
Expiry date		**Expiry Date**			
Use before date		**Use before date**			

2. Endotoxin Control Standard Series (Dilution Details) –

Tube No.	LRW	CSE added from tube	Final Concentration of CSE (EU / mL)

Dry block Incubator ID: _____

Incubation in Time / Temp: _____ / _____

Incubation out Time / Temp: _____ / _____

3. Control Curve Details -

Sr. No.	Dilution	LRW (µl)	CSE (µl)	Lysate (µl)	OBSERVATION			
					1	2	3	4
1.	Blank (Negative Control)	100	-	100				
2.	2λ (0.25)	-	100 (of 2λ)	100				
3.	λ (0.125)	-	100 (of 1λ)	100				
4.	λ/2 (0.06)	-	100 (of λ/2)	100				
5.	λ/4 (0.03)	-	100 (of λ/4)	100				
End point								
Log_{10} of Endpoint								
Average of Log_{10} of Endpoint								
Geometric Mean End Point = Anti log of Average of Log_{10} of Endpoint								

+ ve : Denotes gel formation; **- ve :** Denotes no gel formation

3. Remark - The sensitivity of the LAL Reagent is with in or not with in ± 2 fold concentration with that of labeled sensitivity.

LAL - Limulus Amoebocyte Lysate, **LRW -** LAL Reagent Water,
CSE - Control Standard Endotoxin.

Tested by:
(Date & Sign)

Reviewed by:
(Date & Sign)