PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Bacterial Endotoxin Test (LAL)	**Effective Date:**
Supersedes: Nil	**Review Date:**
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1.0 PURPOSE

To lay down the procedure to perform Bacterial Endotoxin (LAL) test for detection of endotoxin in the material to be tested.

2.0 SCOPE

It is applicable to Quality control department.

3.0 RESPONSIBILITY

Microbiologist

4.0 PROCEDURE

4.1 Precaution to be taken during LAL testing

4.1.1 Check the calibration status of the triobloc.

4.1.2 Check the temperature of triobloc before keeping the sample tubes into the wells, it should be 37±1°C

4.1.3 All ready to use glassware/accessories must have proper certification for endotoxin free quality.

4.1.4 Depyrogenated glassware only shall be used for test.

4.1.5 Storage and reconstitution of control standard endotoxin and lysate shall be according to manufacturer recommendations.

4.1.6 All the reagents to be used for test must be within its expiry date.

4.1.7 Do not vortex lysate on reconstitution; shake gently to mix it properly.

4.2 Depyrogenation of glassware's

4.2.1 Depyrogenation of LAL reaction tubes / dilution tubes / accessories

4.2.1.1 Tear off piece of aluminium foil large enough to be fold and cover the required number of tubes /

accessories to be depyrogenated.

4.2.1.2 Place prepared tubes/accessories on aluminium foil, Wrap foil around tubes/accessories so that they are completely covered.

4.2.1.3 Put the tubes/accessories in Dry heat steriliser and run as per the SOP.

4.2.1.4 After depyrogenation is completed unload the tubes/accessories and observe the pack, no tears in foil should be observed. If tears are noted rewrap the tubes/accessories again and put for depyrogenation.

4.2.1.5 Alternatively depyrogenated tubes/accessories (ready to use) can be directly used.

4.3 Calculation of Maximum valid dilution (MVD) and Minimum valid concentration (MVC)

For Raw material $MVC = \frac{\lambda\ (EU / ml)}{EL\ (EU / mg)}$

For Finished products $MVD = \frac{EL \times Potency\ of\ product}{\lambda\ (EU / ml)}$

For Finished products $MVD = \frac{E.L.C.}{\lambda}$

Where as,

EL = Endotoxin limit specified in individual monograph of product, in EU/ml or EU/mg.

Potency of Product = Concentration of product in Units or mg/ml

λ = Labelled sensitivity of lysate.

E.L.C. = Endotoxin limit concentration Specified in monograph, in specified ml of product.

If Endotoxin limit of product is not specified or for a new product, calculate Endotoxin limit as follows:

$$\text{Endotoxin limit} = \frac{K}{M}$$

K = 5.0 EU/kg for parenteral preparation (0.2 EU/kg. for those drugs administered intrathecally)

M = Maximum human dose /kg/hour

4.4 Reconstitution of LAL Reagent and Storage condition.

4.4.1 Store the lyophilised lysate as mentioned by the manufacturer.

4.4.2 Reconstitute the lysate with LAL Reagent water (LRW) as per the instruction given by the manufacturer.

4.4.3 Reconstituted lysate may be stored as per manufacturer.

4.4.4 Reconstituted Lysate may be frozen and thawed once only for use (Follow as per the manufacturer instruction)

4.5 Reconstitution of Controlled Standard Endotoxin (CSE) and storage condition.

4.5.1 Reconstitute the CSE with LRW as per the manufacturer instruction and vortex it for the given time period.

4.5.2 Reconstituted CSE may be stored per manufacturer temperature / time period mentioned.

4.5.3 Vortex the CSE at least for 5 minutes before using and vortex the Endotoxin at least for one minute before use and after every ten minutes of standing.

4.6 Confirmation of Labelled Sensitivity of LAL Reagent.

4.6.1 Dilute the CSE with LRW to get 2λ, λ, λ/2, λ/4 (EU/ mL) concentration of Endotoxin or any suitable dilution step.

4.6.2 Add 100 µl of each Endotoxin concentration to respective tube marked as 2λ, λ, λ/2, λ/4. Prepare four sets for each concentration.

4.6.3 Add 100 µl of Lysate to each tube.

4.6.4 For negative control add 100 µl of LRW to test tube and add 100 µl of Lysate.

4.6.5 Vortex gently each tube and insert in triobloc maintained at a temperature 37°C ± 1°C and incubate for 60 min ± 2 min.

4.6.6 After completion of incubation remove the tubes from triobloc and observe the tubes by inverting gently at 180° for positive or negative results.

4.6.7 A firm gel clot that maintains its integrity upon inversion at 180° indicates positive results.

4.6.8 Tubes, which indicate no firm gel or show visible increase in turbidity or viscosity, are reported as negative.

4.6.9 Note down the results in annexure-I. Find out the end point, the lowest concentration that gives positive results is the end point. Find out the log end point.

4.6.10 Find out the Geometric mean as follows,

$$\text{Geometric Mean} = \text{Antilog} \frac{\sum e}{F}$$

e = sum of log end points f = Number of replicates

4.6.11 Label claim is verified ok when, Geometric Mean of the endpoint confirms between 0.5λ and 2λ of the label claimed sensitivity of lysate. Record the results in Annexure-I

4.7 Test Procedure

Note: In case of any interference, the sample preparation shall be carried out by pH adjustment using acid, base or suitable buffers, using dispersing agents in case of viscous products or using the reagents of higher sensitivity.

4.7.1 Sample shall be diluted / prepared as per the requirement and the same sample shall be taken for further testing.

4.7.2 50 : 50 method

Details	Test Solution	LRW	C.S.E	Lysate
Negative LRW control (NC)		100µL		100µL
Negative product control (NPC)	50µL	50µL		100µL

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Positive Product control (PPC)	50µL		50µL (4λ)	100µL
Positive LRW control (PC)		50µL	50µL (4λ)	100µL

NA: Not applicable µL: Micro litre LRW: LAL reagent water

4.7.3 Hot spike method

Details	Test Solution	LRW	C.S.E	Lysate
Negative LRW control (NC)		100µL		100µL
Negative product control (NPC)	100µL			100µL
Positive Product control (PPC)	100µL		10µL (20λ)	100µL
Positive LRW control (PC)		100µL	10µL (20λ)	100µL

4.7.4 Like the above mentioned in point number 4.7.1 & 4.7.2 reagents shall be added or otherwise any suitable combinations can be added.

4.7.5 Gently shake each tube before putting it into triobloc.

4.7.6 Insert tubes in triobloc maintained at 37° C ± 1° C incubate for a period of 60 ± 2 minutes.

4.7.7 After completion of incubation remove the tubes from triobloc and observe the tubes by inverting gently at 180° for results.

4.7.8 A firm gel clot that maintains its integrity upon inversion at 180° indicates positive results.

4.7.9 Tubes, which indicate no firm gel or show visible increase in turbidity or viscosity, are reported as negative.

4.7.10 Record the observation in Annexure - II

4.8 Validation

4.8.1 Validation should be done for 3 batches of each product as per validation protocol. Revalidation shall be carried out as per the validation protocol.

4.8.2 Validation is satisfactory if both series endotoxin / product and endotoxin/LRW conforms not less than 0.5λ and not more than 2λ of the label claimed sensitivity of lysate. (As per the validation protocol)

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4.9 Interpretation of Results.

4.9.1 The product complies with LAL test if negative results are found in both tubes of NPC and positive results are found in both tubes of PPC. Positive control (PC) should show positive results in both tubes and negative control (NC) should show negative results in both tubes.

4.10 Instrument usage details shall be recorded in SOP

5.0 ABBREVIATIONS AND DEFINITIONS

SOP	Standard Operating Procedure
QCM	Quality Control Microbiology
QAD	Quality Assurance Department
QCC	Quality Control Chemical
EU	Endotoxin Unit
EL	Endotoxin Limit
NC	Negative Control
MVD	Maximum Valid Dilution
MVC	Maximum Valid Concentration
ELC	Endotoxin Limit Concentration
LRW	LAL Reagent Water
CSE	Controlled Standard Endotoxin
GM	Geometric Mean
NPC	Negative Product Control
PPC	Positive Product Control
PC	Positive Control

6.0 REFERENCE DOCUMENTS

SOP

7.0 ANNEXURE / ATTACHMENTS

Annexure I: Form 1- Confirmation of labelled Sensitivity of LAL Reagent.

Annexure II: Form 2- Bacterial Endotoxin (LAL) test Report.

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8.0 REVISION LOG

Revision Number	Effective Date	Reason for Revision