STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Procedure for Performing LAL test	**Effective Date:**
Supersedes: Nil	**Review Date:**
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1.0 OBJECTIVE

To lay down the procedure for performing LAL test.

2.0 RESPONSIBILITY

QA Personnel/Microbiologist/Executive.

3.0 ACCOUNTABILITY

Head-Quality Control.

4.0 PROCEDURE

4.1 RECONSTITUTION OF CONTROL STANDARD ENDOTOXIN (CSE)

4.1.1 Reconstitute the CSE by adding LRW to the CSE vial as per the Manufacturer Instruction provided on the vial or along the with party COA.

4.1.2 Vortex the vial for 30 minutes.

4.2 Label six 12 x 75 mm glass tubes as follows and keep them inn the test tube rack.

01) 1.0 EU - 8λ

02) 0.50 EU - 4λ

03) 0.250 EU - 2λ

04) 0.125 EU - λ

05) 0.060 EU - λ/2

06) 0.030 EU - λ/4

4.3 Make Suitable dilutions for the given 6 labelled test tubes as per manufacture potency given. (e.g. If potency of CSE is 20 EU/ML*)

***** Potency of CSE will vary as per party COA

Test Tubes No.	Concentration	CSE in ml	LRW in ml
1	8λ	0.1 (STD)	1.9
2	4λ	1.0 of 01 Tube	1.0
3	2λ	1.0 of 02 Tube	1.0
4	λ	1.0 of 03 Tube	1.0
5	λ/2	1.0 of 04 Tube	1.0
6	λ/4	1.0 of 05 Tube	1.0

4.3.1 Vortex all the six test tubes for 1 minutes

4.4 PREPARATION OF NEGATIVE AND POSITIVE PRODUCT CONTROL (NPC & PPC)

4.4.1 For making test dilution refer to individual STP of product. Make assay tubes for any product as mentioned below.

PRODUCT	SAMPLE	LRW	CSE	LAL
NPC	50µl	50µl	-	100µl
PPC	50µl	-	50µl	100µl

4.4.2 Ensure that the final dilution of the product must be within the maximum valid dilution (MVD) for that in PPC.

4.4.2 Prepared the sample as per STP of individual raw material or finish product.

4.4.3 Label two 10x75 mm glass test tubes for each standard, PPC and sample to be tested and include two test tube of LRW for blank.

4.4.4 Place the tubes in the test tube rack.

4.4.5 Place the clean pipette tips on micropipette.

4.4.6 Check that the volume of the micropipette is set at 100µl.

4.4.7 Prepare the control curve as given below.

S.no.	Concentration	CSE	LRW	LAL
1	Blank	-	100µl	100µl
2	2λ	100µl of 2λ	-	100µl
3	λ	100µl of λ	-	100µl
4	λ/2	100µl of λ/2	-	100µl
5	λ/4	100µl of λ/4	-	100µl

4.4.8 Reconstitute the Lysate with LRW as per the manufacturer instructions and swirl gently for 10 seconds.

4.4.9 Place the clean tip on micropipette and pipette out 100 µl of the lysate into each tube using the same tip.

4.4.10 Mix gently for 3 second by shaking the tube in the tube rack itself.

4.4.11 Place the tube in the Heating Block temperature maintained previously 37ºC± 1ºC for 60 minutes.

4.4.12 After the completion of incubation, take the tubes out of the incubator and gentle invert them to 180º and record the gelled and ungel tubes.

4.4.13 A positive is indicated by gel formation which remains momentarily at the base of the tube when inverted to 180º.

4.4.14 Ensure that MVD is calculated for each product to be tested and the sample processed accordingly shall be taken as test sample.

4.4.15 Formula for the calculation of MVD is given below.

$$MVD = \frac{\text{Potency of Product MG/ML} \times \text{Endotoxin Limit of Product EU/MG}}{\text{Lysate sensitivity in EU/ML}}$$

$$MVD = \frac{\text{Endotoxin Limit of Product EU/ML}}{\text{Lysate sensitivity in EU/ML}} \text{ (for Sample given in EU/ML)}$$

4.5 ABBREVIATIONS

CSE	-	Control Standard Endotoxin
LRW	-	Lal Reagent Water
LAL	-	Limulus Amoebocyte Lysate
BET	-	Bacterial Endotoxin Test
NPC	-	Negative Product Control
PPC	-	Positive Product Control
MVD	-	Maximum Valid Dilution
µl	-	Microlitre

5.0 REASON FOR REVISION

Testing procedure for making serial dilution of individual product is now added in STP instead of LAL SOP.

6.0 TRAINING

Trainer	-	Manager –Quality Control
Trainees	-	Microbiologist and QA inspector
Period	-	One Day

7.0 DISTRIBUTION

Certified copy no 1	:	Head of Department –Quality Control
Certified copy no 2	:	Microbiology Department

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Certified copy no 3	:	Record File
Reference Copy no 4	:	Display near LAL Test area
Original Copy	:	Head –Quality Assurance

8.0 ANNEXURE

Annexure –I	:	Bacterial Endotoxin Test Format

9.0 REFERENCES

USP,BP,IP AND INHOUSE

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

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ANNEXURE-01

BACTERIAL ENDOTOXIN TEST
TEST REPORT

1. Details of Product being Tested Reference No: E- ----------

Name:---------- Batch No:----------

Potency:----------

Endotoxin Limit:----------

2. Incubation Temperature and Time of Product: At 37ºC for 60 minutes

3. Details of Reagent, Endotoxin and Pyrogen free water (LRW):

LAL Lot No:---------- Sensitivity:----------EU/ML Exp. Date:----------

Endotoxin Lot no:---------- Potency:----------EU/ML Exp. Date:----------

Endotoxin Reconstituted On:---------- there after stored at 4ºC

LRW Lot No:---------- Date of Testing:----------

PRODUCT	I RESULT II
Negative Product Control (Test Sample) Positive Product Control	
Control curve Concentration	
1. Blank 2. 0.03 EU/ML 3. 0.06 EU/ML 4. 0.125 EU/ML 5. 0.25 EU/ML	

Remarks: Sample Passes/Does not Passes the test for Endotoxin.

Microbiologist
Sign & Date

Chief Microbiologist
Sign & Date