PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Endotoxin Challenge Test	**Effective Date:**
Supersedes: Nil	**Review Date:**
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1.0 OBJECTIVE:

To lay down a procedure for Endotoxin Challenge Test.

2.0 SCOPE:

This SOP is applicable for Endotoxin Challenge Test in Microbiology Section of Quality Control Area.

3.0 RESPONSIBILITY:

Officer / Executive – Microbiology

4.0 ACCOUNTABILITY:

Head – QC

5.0 ABBREVIATIONS:

CSE	Control Standard Endotoxin
No.	Number
Ltd.	Limited
LRW	LAL Reagent Water
LAL	Limulus Amebosyte Lysate
QA	Quality Assurance
QC	Quality Control
SOP	Standard Operating Procedure

6.0 PROCEDURE:

6.1 MATERIAL AND INSTRUMENTS:

6.1.1 Limulus Amebocyte Lysate Reagent.

6.1.2 Endotoxin Indicator Vial (100000 EU/Vial) or as per received from vendor.

6.1.3 LAL Reagent Water.

6.1.4 Ampoules or Vial according to requirement.

6.1.5 Depyrogenated Dilution Tubes (12 x 75 mm, 16 x 100mm)

6.1.6 Depyrogenated LAL Assay Tube (10 x 75 mm)

6.1.7 Micropipette with Pyrogen free tip (20-200 µl)

6.1.8 Micropipette with Pyrogen free tip (100-1000 µl)

6.1.9 Vortex Mixer.

6.1.10 Heating Block.

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6.2 Switch **"ON"** the Heating Block and set the temperature at 37° ± 1°C.

6.3 PREPARATION OF CHALLENGE VIALS:

6.3.1 Reconstitute the challenge vial of Endotoxin with 1ml LRW or as per vendor COA to yield 100000 EU/ml and vortex according to manufacturers instructions.

6.3.2 Transfer 0.1ml aliquot into ampoules or vials used for endotoxin challenge test.

6.3.3 Keep the ampoules/vial in Laminar Air Flow hood for overnight. Each ampoule/vial now contains 10,000 EU. Mark the above prepared ampoules/vial as 1 to 10 numbers.

6.3.4 Keep at least 1 ampoules/vial as positive control (do not expose through oven/tunnel).

6.3.5 Mark the remaining ampoules/vial as NPC.

6.3.6 Expose these ampoules/vials to appropriate location in DHS/Tunnel as per depyrogenation cycle.

6.4 DILUTION OF POSITIVE CONTROL AMPOULE / VIAL:

6.4.1 Reconstitute the ampoules / vial with 1ml LRW and vortex vigorously for 5 minutes and each subsequent dilution for 3-4 minutes.

6.4.2 Now the concentration of Endotoxin in the PPC will be 10000 EU/ml.

6.4.3 Prepare 1:100 dilution of the above to obtain 100 EU/ml.

6.4.4 Prepare 1:100 dilution of the above to obtain 1 EU/ml.

6.4.5 From the above 1 EU/ml preparation, prepare a two fold dilution series upto 2λ, λ, λ/2, λ/4.where **λ=Labelled Lysate sensitivity, if** λ= 0.125 EU/ml

DILUTION TABLE

Sr. No.	Endotoxin	LRW	Endotoxin Con.(EU/ML)
1.	10000 EU/Vial	1 ml	10000 EU/ml
2.	0.1 ml of 10000 EU/ml	9.9 ml	100 EU/ml
3.	0.1 ml of 100 EU/ml	9.9 ml	1 EU/ml 8λ
4.	0.5 ml of 1 EU/ml	0.5 ml	0.5 EU/ml 4λ
5.	0.5 ml of 0.5 EU/ml	0.5 ml	0.25 EU/ml 2λ
6.	0.5 ml of 0.25 EU/ml	0.5 ml	0.125 EU/ml λ

7.	0.5 ml of 0.125 EU/ml	0.5 ml	0.06 EU/ml λ/2
8.	0.5 ml of 0.06 EU/ml	0.5 ml	0.03 EU/ml λ/4

6.5 DILUTION OF NPC AMPOULES / VIAL :

6.5.1 Reconstitute each of the ampoules/vial with 1ml LRW and vortex vigorously for 5 minutes.

6.5.2 It is assumed that three log reduction is achieved after exposure of ampoules/vial in oven/Tunnel, the Endotoxin concentration in the vial is 10 EU/ml.

6.5.3 Prepare 1:10 dilution of each ampoules/vial to obtain 1 EU/ml.

6.5.4 Further prepare 1:8 dilution to obtain 0.125 EU/ml.

DILUTION TABLE

Tube No.	Endotoxin Indicator	LAL reagent Water	Endotoxin Concentration
1.	0.1 ml of 10 EU/ml	0.9 ml	1 EU/ ml
2.	0.1 ml of 1 EU/ml	0.7 ml	0.125 EU/ml

6.6 LAL TEST PROCEDURE:

6.6.1 Test the two fold dilution series prepared from the positive controls ampoules/vial in duplicate.

6.6.2 Test the 0.125 EU/ml dilutions prepared from each of ampoules/vial.

6.6.3 Test should be carried out in clean depyrogenated 10x 75 mm assay tubes only.

6.6.4 Procedure for Positive Control:

S. No.	Dilutions	CSE Dilution Used	LRW	Lysate in µl	No. of Replicates
1.	2 λ	100 µl of 2 λ	–	100 µl	2
2.	λ	100 µl of λ	–	100 µl	2
3.	λ/2	100 µl of λ/2	–	100 µl	2
4.	λ/4	100 µ of λ/4	–	100 µl	2
5.	Negative Water Control (NWC)	–	100 µl	100 µl	2

6.6.5 Procedure for Negative Product Control (Challenged ampoules/vial):

Location No.	Dilutions	Endotoxin Indicator Dilution (0.125 EU/ml)	Lysate in µl	No. of Replicates
1.	NPC	100 µl	100 µl	2
2.	NPC	100 µl	100 µl	2
3.	NPC	100 µl	100 µl	2
4.	NPC	100µl	100µl	2
5.	NPC	100µl	100µl	2
6.	NPC	100µl	100µl	2
7.	NPC	100µl	100µl	2
8.	NPC	100µl	100µl	2
9.	NPC	100µl	100µl	2
10.	NPC	100µl	100µl	2

6.7 CALCULATION:

Log Reduction =Log value of recovered Endotoxin from positive control – Log value of recovered sample from heat treated sample

Recovered EU/ml from heat treated sample (X) = Reciprocal of last dilution x λ

Recovered EU/ml from Positive Control (Y) = Reciprocal of last dilution x λ

6.8 INTERPRETATION OF RESULTS/ACCEPTANCE CRITERIA:

6.8.1 Test results are valid if recovery of Endotoxin in unexposed vials is within a two fold dilution of the labeled claim**.**

6.8.2 The depyrogenation cycle is considered as successfully validated if there is more than 3 log reduction is achieved in challenge Endotoxin vials exposed into Oven/ tunnels at specified place.

6.8.3 For a valid Depyrogenation cycle, the PPC must be positive and NPC's must be negative indicating a greater than 3-log reduction of endotoxin.

6.9 Record the "Endotoxin Challenge Test Record in **Annexure – I**.

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7.0 ANNEXURES:

ANNEXURE No.	TITLE OF ANNEXURE	FORMAT No.
Annexure – I	Endotoxin Challenge Test Report	

ENCLOSURES: SOP Training Record

8.0 DISTRIBUTION:

- Controlled Copy No. 01 — Quality Assurance
- Controlled Copy No. 02 — Microbiology Laboratory
- Master Copy — Quality Assurance

9.0 REFERENCES:

Not Applicable.

10.0 REVISION HISTORY:

CHANGE HISTORY LOG

Revision No.	Change Control No.	Details of Changes	Reason for Change	Effective Date	Updated By

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ANNEXURE-I
ENDOTOXIN CHALLENGE TEST REPORT

Report. No.			
Date of Vial Exposure		**No. of Exposed Vial**	
Date of Testing		**Date of Release**	
Tunnel/oven Location		**Tunnel/oven ID No.**	
Performed By		**Date of Analysis**	
Shift		**BET Kit Manufacturer**	
Heating Block Temperature	37°C ± 1°C	**Incubation Time**	60 ± 2 Minutes
Incubation Started at		**Incubation Completed at**	

REAGENTS DETAILS:

Reagent Details	Lysate	CSE	LRW
Lot No.			
Sensitivity/Potency			
Date of Reconstitution /Opening			
Use Before			
Expiry Date			
Manufacturer			

DILUTION PREPARATION FOR HEAT TREATED VIAL (10 EU/ VIAL ASSUMED)

S.No.	Test Dilution	Test	LRW	Endotoxin Concentration
-	-	10 EU/Vial or Ampoule Assumed	1 ML	10 EU/ml
1.	1:10	0.1ml	0.9 ml	1 EU/ml
2.	1:8	0.1ml	0.7 ml	0.125 EU/ml
Reciprocal of last dilution		10 X 8 =80		

DILUTION PREPARATION FOR POSITIVE CONTROL VIAL (10,000 EU/ VIAL)

S.No.	Test Dilution	Test	LRW	Endotoxin Concentration
-	-	10,000 EU/Vial or Ampoule	1ML	10,000 EU/ml
1.	1:10	0.1ml	0.9ml	10,00 EU/ml
2.	1:10	0.1ml	0.9ml	100 EU/ml
3.	1:10	0.1ml	0.9ml	10 EU/ml

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4.	**1:10**	**0.1ml**	**0.9ml**	**1 EU/ml**
5.	**1:2**	**0.5ml**	**0.5ml**	**0.5Eu (4λ) EU/ml**
6.	**1:2**	**0.5ml**	**0.5ml**	**0.25Eu (2λ) EU/ml**
7.	**1:2**	**0.5ml**	**0.5ml**	**0.125Eu (λ) EU/ml**
Reciprocal of last dilution		**10 x 10 x 10 x 10 x 2 x 2 x 2 = 80,000**		

CALCULATION OF LOG REDUCTION:

Vial No.	Recovered EU/ml = Reciprocal of last dilution x λ		Log reduction=Recovered EU from positive control –Recovered EU from Heat treated vial
	Heat treated vial	Positive control	
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			
11.			
12.			

PREPARATION OF TEST SOLUTION:

Solution	Tube No.	Product Dilution (1:80)	LAL Water	LAL Reagent	Total Volume
Negative Product Control	01	100 µl	----	100 µl	200 µl
	02	100 µl	----	100 µl	200 µl
Negative Water Control	01	----	100 µl	100 µl	200 µl
	02	----	100µl	100µl	200µl

OBSERVATIONS:

Results of Negative Product Control & Negative Water Control:

	Tube No.	Observation Vial No.											
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Negative Product Control	1												
	2												
Negative Water Control	1												
	2												

Results of Positive Control:

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Tube No.	Endotoxin Dilution				Negative Water Control
	2λ	λ	λ/2	λ/4	
01					
02					

+Ve: Gel Formation -Ve: No Gel Formation

Remark: The depyrogenation cycle of Tunnel complies (three log reduction)/does not comply for Endotoxin challenge Test

Microbiologist: **Checked By:**

Date: **Date:**