PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE

Title: Preparation and Testing of Endotoxin Indicators

SOP No.:		**Department:**	Microbiology	
		Effective Date:		
Revision No.:	00	**Revision Date:**		
Supersede Revision No.:	Nil	**Page No.:**	1 of 4	

1.0 OBJECTIVE

To lay down procedure for preparation and testing of Endotoxin indicators.

2.0 SCOPE

This SOP is applicable for Endotoxin indicators 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 Apparatus Used

4.1.1 Test tubes.

4.1.2 Endotoxin dilution tubes.

4.1.3 Reaction test tubes (size: 10 X 75 mm).

4.1.4 Heating block for incubation.

4.1.5 Glass pipettes.

4.1.6 Calibrated Micropipettes (range: 10 - 100µl, 20 - 200µl and 200 - 1000µl).

4.1.7 Depyrogenated micropipette tips (capacity: 200µl and 1000µl).

4.1.8 Stop watch.

4.2 Reagents Used

4.2.1 Lyophilized Endotoxin Indicators.

4.2.2 LAL Reagent Water (LRW).

4.2.3 LAL Reagent.

4.3 Testing Procedure

4.3.1 Preparation of Endotoxin Free Equipment -

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

4.3.1.2 Glassware's shall be cleaned and depyrogenated as per SOP.

4.3.2 Preparation of Endotoxin Indicators -

43.2.1 Reconstitute the Endotoxin indicator vial containing 11250000 EU with 5.0 ml of LRW.

43.2.2 Vortex the vial for 2 minutes initially, and then for 1.0 minute and every 10 minutes for 30 minutes.

43.2.3 From the above-reconstituted Endotoxin vial take 0.1 ml reconstituted Endotoxin and dilute it with 2.15 ml LRW in a test tube.

43.2.4 Vortex the tests tube for 2 minutes initially.

43.2.5 Dispense 0.1 ml diluted Endotoxin in small vials, which is subjected to depyrogenation cycle.

43.2.6 Air-dry the vials containing 0.1 ml Endotoxin by keeping it under LAF over night.

43.2.7 Now the Endotoxin content per vial will become 10,000EU.

43.2.8 Use these indicators for Endotoxin challenge study.

43.2.9 If not used immediately after preparation, wrap them and stored in the refrigerator at 2 - 8^0C for not more than 24 hrs.

43.2.10 Perform the recovery validation using one number of unexposed vial along with the testing of exposed vial.

43.2.11 The recovery should be within ± two fold of the labeled claim potency.

4.3.3 Recovery Validation of Endotoxin Indicators -

4.3.3.1 Dilute the Endotoxin indicator containing 10,000 EU/vial with 4 ml of LRW ---(2500 EU/ml - 1:4) and vortex for a minimum of 5min. and make further dilutions as shown below [vortex each dilution for at least 1 min. before making further dilution.

Sr.No.	Endotoxin Concentration	Total	Dilution
1.	0.1 ml of 2500 EU/ml + 0.9 ml LRW	250 EU/ml	1: 40
2.	0.1 ml of 250 EU/ml + 0.9 ml LRW	25 EU/ml	1: 400
3.	0.1 ml of 25 EU/ml + 0.9 ml LRW	2.5 EU/ml	1: 4000

4.	0.2 ml of 2.5 EU/ml + 1.8 ml LRW	0.25 EU/ml	1: 40000
5.	1.0 ml of 0.25 EU/ml + 1.0 ml LRW	0.125 EU/ml	1: 80000
6.	1.0 ml of 0.25 EU/ml + 1.0 ml LRW	0.06 EU/ml	1: 160000

4.3.3.2 Test the dilutions at Sr.No. 4.3.2.3 to 4.3.2.6 in duplicate using the LAL having confirmed labeled sensitivity equal to 0.125 EU/ml.

4.3.3.3 Analyze the sample as per the SOP for Endotoxin testing.

4.3.4 Acceptance Criteria -

4.3.4.1 The dilution no.4 containing 0.25 EU/ml must show positive test, with a LAL of sensitivity 0.125 EU/ml.

4.3.5 Acceptance Criteria For Recovery Tests -

4.3.5.1 If the unexposed indicator [Recovery validation] is showing positive at 0.25 EU/ml [1:40,000 dilution] and negative at 0.125 EU/ml [1:80,000 dilution], it means the recovery is 50%.

4.3.5.2 In this case, the exposed indicator must show negative at 0.25 EU/ml [1:40 dilution], thus meeting the 3-log reduction during depyrogenation cycle.

4.3.5.3 If the unexposed vial is positive at both 0.25 EU/ml [1:40000] and 0.125 [1:80000] dilution, it means the recovery is 100%.

4.3.5.4 In this case, the exposed indicator must show -ve at 0.125 EU/ml [1:80 or 1:40 dilution] thus meeting 3-log reductions during depyrogenation cycle.

5.0 SAFETY & PRECAUTIONS

Not applicable.

6.0 REVISION HISTORY

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

7.0 REFERENCES

United State Pharmacopoeia.

8.0 ABBREVIATIONS

SOP	:	Standard Operating Procedure
No.	:	Number
%	:	Percentage
mm	:	Millimeter
µl	:	Micro liter
LRW	:	LAL Reagent Water
LAL	:	Limulus Amoebocyte Lysate
EU	:	Endotoxin unit
ml	:	Milliliter
LAF	:	Laminar Airflow
^{0}C	:	Degree Centigrade

9.0 ANNEXURES

Not applicable.