PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Validation of Oven for Depyrogenation	**Effective Date:**
Supersedes: Nil	**Review Date:**
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1.0 OBJECTIVE

To lay down the procedure for validation of depyrogenation oven using Endotoxin indicator vial.

2.0 SCOPE

This procedure is applicable to validate the oven, for depyrogenation cycle.

3.0 RESPONSIBILITY

3.1 Doing : Technical Assistant (Microbiologist)/Executive.

3.2 Checking : Executive/ Manager

4.0 ACCOUNTABILITY

Head of the Department

5.0 PROCEDURE

FREQUENCY: Every Three Month.

5.1 Reconstitute Endotoxin indicator vial with LRW as per the labelled claim vortex for 1 min at every 10min. interval up to 30minutes.

5.2 Distribute the quantity of Endotoxin indicator in 10 nos. of the Pyrogen free vial under LAF (equivalent to 10,000 EU/vial).

5.2 Label 1-9 vial and keep one vial for positive control wrapping with Para film.

5.3 Keep all above nine vials at different location in oven as per location chart and operate the cycle as per SOP.

5.5 Keep one vial unexposed as a +ve control.

5.6 After completion of cycle take all the nine vials from the oven and bring it to room temperature.

5.7 Reconstitute all vials in 1ml LRW and vortex it for five minutes.

(One vial) For PC➔ Dilute it up to 0.125 and 0.25 EU/ml

(Nine vial) For Sample ➔Assuming 3log reduction (i.e. each vial contain 10 EU/ml). Dilute it up to 0.125 EU/ml with LRW, as per dilution scheme given in Annexure-I.

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5.7 Do the LAL test of all dilution of 0.125 EU/ml and positive control of 0.125 & 0.25 EU/ml and negative control with LRW, in duplicate.

5.8 All 9 vial (0.125 EU/ml) must be negative for gel formation and positive control should be positive.

5.9 Calculate as per the annexure-II and record the results in it.

5.10 Interpretation of results:- The +ve control must be positive & the exposed vial must be negative indicating >3 log reduction of Endotoxin.

5.10.1 If the exposed vial shows +ve results proceed for revalidation.

5.11.2 If revalidation of the exposed vials shows +ve results then rectify the oven problem and repeat the cycle.

5.10.2 **Acceptance Criteria:** There should be >3 log reduction of Endotoxin.

6.0 ABBREVIATIONS

^{0}C = Degree Centigrade
EU =Endotoxin units
ml= Millilitre
LAL = Limulus Amebocyte Lysate
LRW = Lal Reagent Water
PC=Positive Endotoxin Indicator control

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ANNEXURE-I

Dilution scheme

ENDOTOXIN INDICATOR POTENCY

⬇

Reconstitution with LRW

⬇

Distribution of 10,000 EU in all 10 vials. So each vial contain 10,000 EU/vial.

⬇

All vials kept under LAF for drying purpose overnight.

⬇

Nine vials kept in oven as per location chart and Depyrogenation cycle operated as per SOP

⬇

➔ One vial kept as +ve control i.e. (Unexposed)

⬇

Reconstitute all 10 Vials with 1ml LRW

➔ For +ve control:

(10,000 EU/ml) ----➔ 100 EU/ml --➔ 1 EU/ml ----➔ 0.25 EU/ml ----➔ 0.125 EU/ml
1:100 1 :100 1:4 1:2

➔For exposed 9 vials:

(Assuming 3 log reduction) i.e. EU/ml ---➔ 1 EU/ml ----➔ 0.125 EU/ml
1:10 1:8 (Last dilution)

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ANNEXURE-II

Oven Validation for Dehydrogenation

1. **Depyrogenation Cycle Temp.:_____ Time: _____**
2. **Position of Endotoxin Sample ➔ as per location chart.**
3. **Reagent Information.**

LAL: _____ Endotoxin:_____ LRW :_____

Sensitivity:_____ Expiry Date:_____ Expiry Date:_____

Expiry Date: _____ Reconstitution:_____

Reconstitution: _____

Block Temp.: Temp (In) :_____ Temp (Out) :_____Time (In) :_____Time (Out) :_____

S.No.	Location	Results of Last dilution		Remarks
		1	2	
1.				
2.				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
+ve Control 0.25 0.125				
-ve Control				

Analyst :_____ Checked By :_____

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ANNEXURE-III

MICROBIOLOGY LABORATORY

Oven Validation for Depyrogenation

Calculation:

X Log reduction = (Log of recovered EU/ml from PC-Log of recovered EU/ml from sample)

➔ **Log recovered EU/ml from PC =Total EU from PC X Sensitivity of Lysate.**

➔ **Log recovered EU/ml from sample = Remaining EU from sample X Sensitivity of Lysate.**

Antilog (>3 log reduction)

i.e. =

Remarks :-

Analyst :_____ **Checked By :_____**

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LOCATION CHART: VALIDATION OF OVEN FOR DEPYROGENATION

SOP No.:.....