PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Quality Assurance	**SOP No.:**
Title: Procedure for the detection of Bacterial Endotoxin in Water for Injection and Pure Steam Condensate by Gel Clot method	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 Objective

To lay down the procedure for the detection of bacterial endotoxin in water for injection and pure steam condensate by Gel clot method.

2.0 Scope

This Standard Operating Procedure is applicable for pharmaceutical formulation plant of ……

3.0 Responsibility

Microbiologist/QC-Officer : Responsible for execution of this SOP.

Head-QC/Designee : Responsible for compliance of the SOP.

4.0 Abbreviations & Definitions

SOP	: Standard Operating Procedure
QC	: Quality Control
LAL	: Limulus Amoebocyte Lysate
LRW	: LAL Reagent Water
EU	: Endotoxin Unit
MVD	: Maximum Valid Dilution
PC	: Product Control
PPC	: Positive Product Control
WPC	: Water Positive Control
WNC	: Water Negative Control
CSE	: Control Standard Endotoxin
QA	: Quality Assurance

5.0 Procedure

5.1 Preparation of Limulus Amoebocyte Lysate:

5.1.1 This reagent must be reconstituted just prior to use or as per manufacturer instructions.

5.1.2 Reconstitute the lyophilized lysate with LRW as per label direction. Swirl gently but thoroughly for at least 30 seconds. Do not shake.

5.1.3 The reconstituted lysate can be stored for 24 hours at 2-8ºC without loss of sensitivity. It can also be stored at -20^{0}C for 28 days and is thawed only once just before use.

5.2 Preparation of Control Standard Endotoxin dilution:

5.2.1 Take a control standard endotoxin vial. Reconstitute it with LRW, as per label directions and vortex the vial for at least 15 minutes. Once reconstituted the CSE can be stored at 2 to 8^{0}C for 28 days.

5.2.2 Prepare the Control standard endotoxin dilutions 2λ, λ, λ/2, λ/4.

5.3 **Preparation of samples:**

5.3.1 Calculate the Maximum Valid Dilution (MVD) of the samples in which endotoxin has to be detected. The formula for calculating the MVD value is given below:

MVD = Endotoxin Limit x potency/sensitivity of lysate.

Then the sample is diluted to its MVD value using LAL Reagent Water.

5.3.2 Prepare the MVD/2 dilution of the sample to be tested in depyrogenated dilution tubes as follows:

For Water for Injection and Pure Steam Condensate:

MVD = 0.25 x 1/0.125 = 2
MVD/2 = 1

The sample is directly used in the test.

5.3.3 Mark the depyrogenated assay tubes for Negative Product Control (NPC), Positive Product Control (PPC), Water Negative Control (WNC) and Water Positive Control (WPC) in duplicates.

5.3.4 Also mark in duplicate the series of control standard endotoxin i.e. 2 λ, λ, λ/2 and λ/4.

5.3.5 To the tubes marked as NPC add 50 µL of the sample and 50 µL of LRW.

5.3.6 Add 50 µL of sample and 50 µL of 4 λ CSE to the tubes marked as PPC.

5.3.7 To the tubes marked as WNC add 100 µL of LRW.

5.3.8 Add 50 µL of LRW and 50 µL of 4λ CSE to the tubes marked as WPC.

5.3.9 To the series of control standard endotoxin add 100 µL of each dilution to the respective assay tube.

5.3.10 Finally add 100 µL of lysate having sensitivity of 0.125 EU / mL to all the tubes.

5.3.11 Following the addition of lysate, Incubate the tubes at 37ºC ±1ºC for 60 ±2 minutes in a tube heating block. Do not disturb the assay tube prior to the specified time of 1 hour.

5.3.12 After 1 hour of incubation examine each reaction tube for gelation.

5.3.13 A positive reaction is indicated by firm gel that remains intact momentarily when the tube is inverted by 180º

5.3.14 **Acceptance criteria & Retest:**

5.3.14.1 The test is not valid unless both the replicates of PPC, NPC are positive and those of WNC are negative.

5.3.14.2 The sample under test complies when both the tubes of NPC & WNC are negative and does not complies when both the tubes are positive.

5.3.14.3 Repeat the test when a positive result is found in one tube of NPC and negative in the other.

5.3.14.4 The sample under test complies only when both the tubes of NPC are found negative in the repeat test.

6.0 Forms and Records

6.1 Report format for bacterial endotoxin test: Annexure-1

7.0 Distribution

6.1 Master Copy: Documentation cell (Quality Assurance)

6.2 Controlled Copies: Quality Control, Quality Assurance

8.0 History

Date	Revision Number	Reason for Revision
	00	New SOP