# PHARMA DEVILS

**MICROBIOLOGY DEPARTMENT**

| STANDARD OPERATING PROCEDURE | |
|---|---|
| **Department:** Microbiology | **SOP No.:** |
| **Title:** Preparation of Endotoxin challenge vials and to evaluate log reduction value in Endotoxin challenged test vials | **Effective Date:** |
| **Supersedes:** Nil | **Review Date:** |
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## 1.0 Objective

To lay down a procedure for preparation of endotoxin challenge vials and to evaluate log reduction value in Endotoxin challenged test vials.

## 2.0 Scope

This Standard Operating Procedure is applicable for preparation of endotoxin challenge vials and to evaluate log reduction value in endotoxin challenged vials in Microbiology lab.

## 3.0 Responsibility

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| Executive/Officer – QC | : | Shall be responsible to follow the procedure of preparation of endotoxin challenge vials and to evaluate log reduction value in Endotoxin challenged test vials |
| Head - QC/Designee | : | Shall be responsible for the compliance of this SOP. |

## 4.0 Abbreviations and Definitions

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| QC | : | Quality Control |
| SOP | : | Standard Operating Procedure |

## 5.0 PROCEDURE:

### 5.1 Preparation of Endotoxin Challenged vials.

5.1.1 Wash the tubes/vials/bottles/Ampoules (used for challenged study) with Water For Injection and allow them to dry. Depyrogenate the tubes/vials/bottles/ Ampoules in the dry heat sterilizer as per the validated cycle.

5.1.2 Reconstitute the Endotoxin Challenge vial (containing 10000000 EU) with 5.0 ml LAL Reagent Water. The endotoxin content in the reconstituted vial is 2000000 EU/ml. Mark the vial as “A”. Vortex the vial for about 30 minutes. If standard endotoxin of higher concentration or lower concentration is procured, prepare

challenged vials/tubes/bottles/Ampoules containing 10,000 EU of endotoxin by making suitable dilutions of the Endotoxin Challenge Vial.

5.1.3 Pipette out 1.9 ml of LAL Reagent Water in a 13 X 100 mm test tube and add 0.1 ml of solution "A", mark the test tube as "B" so that each ml contains 100000EU. Vortex the test tube for not less than 1 min. (Refer Annexure –I for preparation of dilutions of standard Endotoxin).

5.1.4 Pipette out 0.1 ml quantities of the diluted endotoxin solution (Tube B) i.e., 10000EU into each of the depyrogenated vial/tube/bottle/Ampoule to be challenged.

5.1.5 Rotate the vials/tubes/bottles/Ampoules to spread the endotoxin solution on the walls of the vials/bottles/tubes/Ampoules and allow it to dry under LAF for overnight. These vials/tubes/bottles/ Ampoules contain 10,000 EU of endotoxin. Use these vials/tubes/bottles/Ampoules for endotoxin challenge test. Keep one unbaked vial as positive control.

5.1.6 Put the challenged vials/tubes/bottles/Ampoules in the airtight SS container and transfer it to the respective area.

5.1.7 Place the challenged vials/bottles/tubes/Ampoules along with the temperature scanning probes in the equipment as per the locations described in the respective protocol. Run the cycle at time and temperature mentioned in the respective protocol.

5.1.8 After completion of cycle, remove the vials/tubes/bottles/Ampoules from the equipment and place in the airtight SS container and bring it to the microbiology lab for bacterial endotoxin test.

**5.2 Analysis of endotoxin challenged vials.**

5.2.1 Preparation of dilutions of unbaked vial.

5.2.1.1 Reconstitute the unbaked vial/tube/bottle/Ampoule with 1 ml LAL Reagent Water. In case of 50 ml & 100 ml vials, reconstitute the unbaked vials with 5 ml of LRW. Vortex for 5 min.

5.2.1.2 For 7.5 ml – 30 ml vials, pipette out 0.1 ml solution of the reconstituted vial and dilute it with 1.9ml of LAL reagent Water(1:20 Dilution). Incase of 50 ml & 100 ml vials, pipette out 0.1 ml solution from the reconstituted vial and dilute it with 0.3 ml of LAL reagent water(1:4 Dilution) ---[I] - 500Eu/ml).

5.2.1.3 Pipette out 0.1 ml from dilution [I] and dilute it with 1.9 ml LAL Reagent Water --- [II] (1:400 Dilution - 25Eu/ml).

5.2.1.4 Pipette out 0.1 ml from dilution [II] and dilute it with 2.4 ml LAL Reagent Water ---[III] (1:10000 Dilution - 1Eu/ml).

5.2.1.5 Prepare the following dilutions from [III] as follows,

0.5ml [III](1Eu/ml) + 0.5ml LRW = 0.5 Eu/ml (a)

0.5m l(a) + 0.5ml LRW = 0.25 Eu/ml (b)

0.5ml (b) + 0.5ml LRW = 0.125 Eu/ml (c)

0.5ml (c) + 0.5ml LRW = 0.06 Eu/ml (d)

0.5ml (d) + 0.5ml LRW = 0.03 Eu/ml (e)

5.2.1.6 Perform the test in duplicate in a 10 X 75 mm assay tube as shown in Annexure – II or as follows,

0.1 ml (b) + 0.1 ml of lysate (2λ)

0.1 ml (c) + 0.1 ml of lysate (λ)

0.1 ml (d) + 0.1 ml of lysate (λ/2)

0.1 ml (e) + 0.1 ml of lysate (λ/4)

5.2.1.7 Run a Negative Water Control with the above test, by adding 0.1ml of LRW to 0.1ml of Lysate in duplicate in 10 X 75 mm assay tubes.

5.2.2 Preparation of dilutions for challenged vials.

5.2.2.1 Add 1 ml of LRW to each of the baked vials and vortex for about 5 minutes. In case of 50 ml & 100 ml vials, add 5 ml of LAL reagent water and vortex for about 5 minutes.

5.2.2.2 Take 0.1 ml from each of the baked vial and add 0.1 ml of lysate in duplicate in 10 X 75 mm assay tubes.

5.2.2.3 Run a Negative Water Control with the above test, by adding 0.1ml of LRW to 0.1ml of Lysate in duplicate in 10 X 75 mm assay tubes.

5.2.2.4 Incubate the reaction tubes at 37ºC ± 1ºC for 60 Min ± 2 min, avoiding any vibrations.

5.2.2.5 After completion of incubation period, gently invert the tubes at an angle of $180^0$ and observe for gel formation.

5.2.2.6 If a Positive result is observed, dilute the solution further and perform the test. Record the results in Annexure – III.

**5.3 Interpretation of results:**

5.3.1 To test the integrity of the gel, take each tube in turn directly from the Heating Block and invert it through about 180º in one smooth motion.

5.3.2 If a firm gel is formed that remain in place upon inversion, record the result as positive. A result is negative if an intact gel is not formed.

**5.4 Calculation of Log Reduction:**

5.4.1 Calculate the log reduction by the following formula.

Minimum Log Reduction = Log Endotoxin Concentration of the Unbaked Control vial - Log Endotoxin Concentration of the Baked Vials.

Where,

Endotoxin Concentration = Lysate Sensitivity X reconstituted volume X dilution

**5.5 Acceptance Criteria:**

5.5.1 A minimum of 3 Log reductions is required for the test to comply.

**6.0 Forms and Records**

6.1 Flow Diagram for Preparation of Endotoxin Challenge Vials : Annexure-1

6.2 Bacterial Endotoxin Test for Unbaked and Challenged Vials : Annexure-2

6.3 Endotoxin Challenge Test Report : Annexure-3

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## 7.0 Distribution

7.1 Master Copy : Documentation Cell (Quality Assurance)

7.2 Controlled Copies : Quality Control, Quality Assurance

## 8.0 History

| Date | Revision Number | Reason for Revision |
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