PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: SOP for Recovery of Endotoxins from Rubber Stoppers, Vials, Aluminium Containers and Rubber Bungs	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1. **Purpose:**

   The purpose of this SOP is to lay down the procedure for recovery of Endotoxins from Spiked and unspiked, Rubber Stoppers, Vials and Rubber Bungs.

2. **Scope:**

   This SOP is applicable to all the spiked and unspiked rubber stoppers, vials Aluminium Containers and rubber bungs.

3. **Responsibility:** Microbiologist.

4. **Accountability:** Head of Quality Control

5. **Material and Equipments:** Biological Indicators.

6. **Procedure:**

   6.1. Estimation of Endotoxin content in incoming Rubber Stopper, Vials, Aluminium containers and Rubber Bungs from the supplier.

   6.1.1. Rubber Stopper:

   6.1.1.1. Sample randomly 10 rubber stoppers from the bag received from the supplier for processing.

   6.1.1.2. Take the sample (taking care not to contaminate) in a beaker previously depyrogenated in DHS.

   6.1.1.3. Test the rubber stoppers at 1.0 EU, 2 EU, 4 EU and 8 EU respectively.

   6.1.1.4. Carry out the test in the following way.

   6.1.1.5. Take 1 rubber stopper in a test tube and add 1.0 ml of LRW.

   6.1.1.6. Vortex it for 10 minutes.

   6.1.1.7. Proceed the test as per the following dilutions.

S.No.	Vol. of LRW	Sample vol.	Final Conc.	Vol. from the final dilution	Vol. of Lysate added
1.	0.7 ml	0.1 ml	1 : 8 (1 EU)	0.1 ml	0.1 ml
2.	0.1 ml	0.1 ml of 1 : 8	1:16 (2 EU)	0.1 ml	0.1 ml
3.	0.1 ml	0.1ml of 1 : 16	1: 32 (4 EU)	0.1 ml	0.1 ml
4.	0.1 ml	0.1ml of1: 32	1: 64 (8 EU)	0.1 ml	0.1 ml

6.1.1.8. Incubate the samples for 60 minutes ± 2 minutes for $37^{O}C$.

6.1.1.9. Note down the observations in the format given as Annexure I.

6.1.2. Vials:

6.1.2.1. Sample randomly 10 vials from the bag received from the supplier for processing.

6.1.2.2. Take the sample (taking care not to contaminate) in a beaker previously depyrogenated in a validated DHS.

6.1.2.3. Test the vials at 1.0 EU, 2 EU, 4 EU and 8 EU.

6.1.2.4. Take 2 vials and add 2.0 ml of LRW in each vial.

6.1.2.5. Apply Para film on the mouth of each vial.

6.1.2.6. Vortex it for 10 minutes.

6.1.2.7. Pipette out 1.0ml from each vial in depyrogenated test tube.

6.1.2.8. Vortex the sample for 5.0 minutes.

6.1.2.9. Proceed the test as per the following dilutions.

S.No.	Vol. of LRW	Sample vol.	Final Conc.	Vol. from the final dilution	Vol. of Lysate added
1.	0.7 ml	0.1 ml	1:8 (1 EU)	0.1 ml	0.1 ml
2.	0.1 ml	0.1 ml of 1 : 8	1:16 (2 EU)	0.1 ml	0.1 ml
3.	0.1 ml	0.1ml of1 : 16	1: 32 (4 EU)	0.1 ml	0.1 ml
4.	0.1 ml	0.1ml of1 : 32	1: 64 (8 EU)	0.1 ml	0.1 ml

6.1.2.10. Incubate the samples for 60 minutes ± 2 minutes for $37^{O}C$.

6.1.2.11. Note down the observations in the format given as Annexure II.

6.1.3. **Rubber Bungs:**

6.1.3.1. Sample three rubber bungs.

6.1.3.2. Place one rubber bung in a S.S. vessel previously depyrogenated in a Dry Heat Sterilizer.

6.1.3.3. Add 400 ml into the beaker.

6.1.3.4. Swirl it for 30 minutes.

6.1.3.5. Check the rubber bung at 50 EU.

6.1.3.6. Carry out three such trials.

6.1.3.7. Follow the procedure for dilutions.

S.No.	Vol. of LRW	Sample vol.	Final Conc.	Vol. from the final dilution	Vol. of Lysate added
1.	400 ml	----	50 EU /ml	0.1 ml	0.1 ml

2.1.1.1 Incubate the samples for 60 minutes ± 2 minutes for 37 ^{O}C .
2.1.1.2 Note down the observations in the format given as Annexure IV.

2.2 Spiking of Rubber Stoppers, Vials and Bungs.

2.2.1 Rubber Stoppers / Vials.

2.2.1.1 Take out the Endotoxin Indicator from the refrigerator and place it over the LAF bench.

2.2.1.2 Open the seal of the vial very carefully.

2.2.1.3 With the help of a calibrated micropipette, pipette out 2.0 ml of the LAL Reagent Water.

2.2.1.4 Vortex the indicators vial for 30 minutes.

2.2.1.5 Cut one portion of the rubber stopper so as to differentiate from the unspiked stoppers or spike in rubber stopper of different colors.

2.2.1.6 Label the vial by numbering to differentiate between the spiked and unspiked vials.

2.2.1.7 Pipette out 0.2 ml of the indicator into the surface, which will come in contact with the powder in rubber stopper / vial.

2.2.1.8 Place all the rubber stoppers / vial under the LAF overnight to dry.

2.2.1.9 Retain three no. of rubber stoppers / vial for performing the recovery of the spiked endotoxin indicator.

2.2.1.10 Send all the other rubber stoppers / vials to the plant for performing the validation.

2.2.2 Aluminium Containers and Rubber Bungs:

2.2.2.1 Take out the Endotoxin Indicator from the refrigerator and place it over the LAF bench.

2.2.2.2 Open the seal of the vial very carefully.

2.2.2.3 With the help of a calibrated micropipette, pipette out 1.0 ml of the LAL Reagent Water.

2.2.2.4 Vortex the indicators vial for 30 minutes.

2.2.2.5 Cut one end of the rubber bung to differentiate from other unspiked rubber bungs.

2.2.2.6 Pipette out 1.0 ml of the indicator into the aluminium container and rubber bung, which will come in contact with the powder.

2.2.2.7 Place the aluminium containers and rubber bungs under the LAF for overnight to dry.

2.2.2.8 Retain three no. of aluminium containers for performing the recovery of the spiked endotoxin indicator.

2.2.2.9 Send all the other containers to the plant for performing the validation.

2.3 Recovery of Endotoxin from the spiked Rubber Stopper, Vials, Aluminium Containers and Bungs.

2.3.1 Rubber Stopper.

2.3.1.1 Take 1 rubber stopper which was previously spiked (endotoxin content at the inner portion of the stopper which comes in contact with the rubber stopper) and place it in a depyrogenated beaker.

2.3.1.2 Add 20 ml of the LAL reagent water and keep it in the sonicator for 30 mins.

2.3.1.3 Assuming that after washing a three log reduction must have taken place, the total concentration on each rubber stopper will be =20 EU/r. stopper.

2.3.1.4 Volume of LRW used for Dilution = 20 ml

2.3.1.5 Therefore the total concentration will be = 20 EU / 20
= 1 EU/ ml

2.3.1.6 Perform the following procedure to determine the endotoxin content.

S.No.	Endotoxin Conc.	Volume of LRW added	Total Conc.	Dilution
1	2.0 ml of 1.0 EU	2.0 ml of LRW	0.5 EU /ml	1: 20
2	1.0 ml of 0.5 EU	1.0 ml of LRW	0.25EU /ml	1: 40
3	0.5 ml of 0.25 EU	0.5 ml of LRW	0.125 EU /ml	1 : 80

2.3.1.7 Perform the analysis in duplicate for 1 : 40 and 1 : 80 dilutions in duplicate.

2.3.1.8 Incubate the samples for 60 minutes ± 2 minutes for 37 ^{O}C.

2.3.1.9 Note down the observations in the format given as Annexure V.

2.3.2 Vials:

2.3.2.1 Take 1 vial that was spiked with endotoxin.

2.3.2.2 Add 4.0 ml of LRW into the vial with the help of a calibrated pipette.

2.3.2.3 Apply parafilm over the mouth of the vial.

2.3.2.4 Vortex it for 30 minutes.

2.3.2.5 Assuming that after depyrogenation a three log reduction must have taken place, the total concentration on each vial will be 10 EU / vial.

2.3.2.6 Add 4.0 ml of LRW to the vial.

2.3.2.7 The total concentration in vial will be 2.5 EU / ml.

2.3.2.8 Follow the dilutions as per the table for determining the content of endotoxin in the vial.

S.No.	Endotoxin Conc.	Volume of LRW added	Total Conc.	Dilution
1.	0.2 ml of 2.5 EU / ml	1.8 ml of LRW	0.25 EU /ml	1: 40
2.	1.0 ml of 0.25 EU / ml	1.0 ml of LRW	0.125 EU /ml	1 : 80

2.3.2.9 Perform the analysis from 1: 40 and 1 : 80 dilutions in duplicate.

2.3.2.10 Incubate the samples for 60 minutes ± 2 minutes for 37 ^{O}C.

2.3.2.11 Note down the observations in the format given as Annexure-VI.

2.3.3 Aluminium Containers:

2.3.3.1 Take 1 aluminium container.

2.3.3.2 Assuming that there has been a three-log reduction during depyrogenation the endotoxin content in the container will be 10 EU.

2.3.3.3 Add 40 ml of LRW with the help of calibrated pipette.

2.3.3.4 Place it in the sonicator for 30 minutes.

2.3.3.5 After the addition of 40 ml LRW the concentration in the container will be 0.25 EU/ml (1:40).

2.3.3.6 Perform the dilutions as per the following.

2.3.3.7 Take 0.1 ml of 0.25 EU / ml (1:40) and add 0.1 ml of lysate.

2.3.3.8 Take 0.05 ml of 0.25 EU / ml (1:40) and add 0.05 ml of LRW.

2.3.3.9 The final concentration will 0.125 EU / ml (1: 80).

2.3.3.10 Perform the analysis in duplicate.

2.3.3.11 Incubate the samples for 60 minutes ± 2 minutes for 37 ^{O} C.

2.3.3.12 Note down the observations in the format given as Annexure VII.

2.3.4 Rubber Bungs:

2.3.4.1 Take the rubber bung and place it under the LAF.

2.3.4.2 Assuming that there has been a three-log reduction during washing and sterilization the endotoxin content in the rubber stopper will be 10 EU.

2.3.4.3 Add 4.0 ml of the LRW with the calibrated pipette.

2.3.4.4 Gently stir the LRW in the groove of the bung with the help of a depyrogenated glass rod.

2.3.4.5 After the addition of 4.0 ml the concentration in the rubber bung will be 2.5 EU / ml.

2.3.4.6 Perform serial dilutions as per the following table.

S.No.	Vol. of LRW	Sample vol.	Final Conc.	Vol. from the final dilution	Vol. of Lysate added
1.	4.0 ml	----	2.5 EU /ml	----	----
2.	0.1 ml	0.9 ml of 2.5 EU	0.25 EU /ml	0.1 ml	0.1 ml
3.	1.0 ml	1.0 ml of 0.25 EU	0.125EU /ml	0.1 ml	0.1 ml

2.3.4.7 Perform the analysis from 1: 40 and 1 : 80 dilutions in duplicate.

2.3.4.8 Incubate the samples for 60 minutes ± 2 minutes for 37 ^{O}C.

2.3.4.9 Note down the observations in the format given as Annexure VIII

2.3.5 FREQUENCY:

2.3.5.1 As per the validation plan in the production and Microbiology lab.

2.3.5.2 New vendors are approved.

2.3.5.3 Any major breakdown in the equipment.

6.1.4. Take three biological indicator paper strips.

7. Distribution and control:

7.1. *Master copy*:

Master copy should keep in lock and key in QA department

7.2. Controlled copy:

Controlled copy should keep with HOD of Quality control department.

7.3. Reference copy :

Reference copy should be present in departmental file.