VALIDATION PROTOCOL

FOR

BACTERIAL ENDOTOXIN TEST

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1.0 PROTOCOL APPROVAL

This document is prepared by the Microbiology Department of bacterial endotoxin test product validation .Hence this document before being effective shall be approved by the QC Head & QA. If any modification shall be desired it design as addendum and approved before execution.

PREPARED BY		
NAME/ FUNCTIONAL AREA	**DESIGNATION**	**SIGNATURE /DATE**
MICROBIOLOGY		

CHECKED BY		
NAME/ FUNCTIONAL AREA	**DESIGNATION**	**SIGNATURE /DATE**
MICROBIOLOGY		
QUALITY ASSURANCE		

APPROVED BY		
NAME/ FUNCTIONAL AREA	**DESIGNATION**	**SIGNATURE /DATE**
QUALITY CONTROL-HEAD		
QUALITY ASSURANCE-HEAD		

2.0 OBJECTIVE

To validate the product for bacterial endotoxin test.

3.0 SCOPE

The scope of this protocol shall be limited to validate bacterial endotoxin test validation for all finished products and Sterile powder for injection that require to be tested.

4.0 RESPONSIBILITY

Responsibility of different department/personnel involved in different activities related to the valuation study.

Functions	Responsibility
Microbiology	Preparation of protocol
Microbiology	Review of the protocol & Execution
Quality assurance	Review & Approval of protocol
Microbiology	Preparation of report
Quality assurance	Approval of the executed protocol and report

5.0 REFERENCE DOCUMENT

Following documents are referred during preparation of the protocol:

Document Name	Document Number
USP 37 (Bacterial Endotoxin Test)	Chapter No. 85
General Test Procedure (Bacterial Endotoxin Test)	

6.0 METHODOLOGY

6.1 Pre-Requisites

6.1.1 Accessories:

- Endotoxin free 10 X 75 mm reaction (Assay) tubes
- Endotoxin free 13 X 100 mm dilution tubes
- Micropipettes and Depyrogenated Tips
- Heating block
- Calibrated Stop Watch

- Weighing Balance
- Test tube stand racks
- Endotoxin Free acid, base or suitable buffer for adjusting the pH if required

6.1.2 Reagents

- LAL Reagent
- Control Standard Endotoxin
- LAL Reagent Water (LRW)

6.1.3 Sample

- Perform the Bacterial Endotoxin test, Product validation for three batches of the each product.

6.1.4 System/ Process description:

The validation will be performed under the following subheadings:

- pH Measurement
- Determining the Maximum Valid dilution
- Determination of Non-Interfering Dilution (NID)
- Determination of Inhibition or Enhancement

6.2 pH Measurement:

6.2.1 Check the pH of three batches before Starting the activity. pH should be within the range of 6.0 to 8.0. If the pH is not within range then adjust by use of an acid, base or suitable buffer.

6.3 Determining the Maximum Valid Dilution:

6.3.1 Determine the Maximum valid dilution of the product e.g.; “Omeprazole sodium” by using following method.

Sterile Omeprazole Sodium Endotoxin limit: 1.25 EU/mg

Labelled claim Lysate sensitivity: 0.125 EU/ml

$$MVD = \frac{\text{Endotoxin Limit}}{\text{Sensitivity of lysate } (\lambda)} \text{ X Concentration of the Product (mg/ml)}$$

$$MVD = \frac{1.25}{0.125} \text{ X } 4 \quad = \text{MVD is } 40$$

Note: The sample if diluted 40 times is the maximum valid dilution.

6.3.2 Record the MVD of product as per data Sheet.

6.4 Determination Of Non-Interfering Dilution (NID)

6.4.1 Objective:

6.4.1.1 To determine a suitable dilution at which the sample or product does not interfere with the activity of endotoxin during routine LAL testing. e.g.; Omeprazole sodium sterile by using following method.

6.4.2 Procedure:

6.4.2.1 Reconstitute the sample vial with LRW as per labeled claim.

6.4.2.2 Vortex for 2 minutes to reconstitute the entire contents.

6.4.2.3 Perform the non interfering dilution at MVD/32, MVD/16, MVD/8, MVD/4, MVD/2 and MVD

6.4.2.4 Add the samples and the lysate as given below in Table-1 for testing at MVD/32, MVD/16, MVD/8, MVD/4, MVD/2 and at MVD.

Table –1

S.No.	Volume of Product (Sample) Added	Volume of LRW Added	Amount of Endotoxin Added	Final Conc. of Product	Volume of Lysate Added	Total Volume
01 (PWC)	---	50µl	50µl of 4λ	---	100µl	200µl
NWC	---	100µl	---	---	100µl	200µl
02 (PPC)	50µl of MVD/32	---	50µl of 4λ	MVD/16 + 2 λ	100µl	200µl
NPC	50µl of MVD/32	50µl	---	MVD/16	100µl	200µl
03 (PPC)	50µl of MVD/16	---	50µl of 4λ	MVD/8 + 2 λ	100µl	200µl
NPC	50µl of MVD/16	50µl	---	MVD/8	100µl	200µl
04 (PPC)	50µl of MVD/8	---	50µl of 4λ	MVD/4 + 2 λ	100µl	200µl
NPC	50µl of MVD/8	50µl	---	MVD/4	100µl	200µl
05 (PPC)	50µl of MVD/4	---	50µl of 4λ	MVD/2 + 2 λ	100µl	200µl
NPC	50µl of MVD/4	50µl	---	MVD/2	100µl	200µl
06 (PPC)	50µl of MVD/2	---	50µl of 4λ	MVD + 2 λ	100µl	200µl
NPC	50µl of MVD/2	50µl	---	MVD	100µl	200µl

6.4.2.5 Incubate the tubes for 60±2 minutes at 37±1°C.

6.4.2.6 Observe and Record the results in respective data sheet.

6.4.2.7 The Non – Interfering Dilution (NID) is the first set of dilution (PPC) containing 2 λ Endotoxin that shows a positive gel.

6.4.2.8 Routine testing is to be performed at the concentration validated as per method and at a concentration less than NID.

6.5 Determination Of Inhibition or Enhancement

6.5.1 Objective: To demonstrate that the product when tested by Gel Clot Method should not have any properties to inhibit or enhance the reaction of the LAL.

6.5.2 Procedure:

6.5.2.1 Prepare 4 λ, 2λ, λ, λ /2 Endotoxin dilutions. (Where λ is labeled lysate sensitivity)

6.5.2.2 Prepare a product dilution of NID.

6.5.2.3 Test all the dilutions in quadruplicate.

6.5.2.4 Perform negative test for CSE Dilutions with LRW and CSE Dilutions with Product Dilution.

6.5.2.5 Perform the test dilutions as given below in Table-2 & 3.

TABLE-2

CSE Dilutions with LRW

S.No.	Volume of CSE	Volume of LRW	Final CSE Concentration	Volume of Lysate	Total Volume
1.	50µl of 4 λ	50µl of LRW	2 λ	100 µl	200 µl
2.	50µl of 2 λ	50µl of LRW	λ	100 µl	200 µl
3.	50µl of λ	50µl of LRW	λ/2	100 µl	200 µl
4.	50µl of λ/2	50µl of LRW	λ/4	100 µl	200 µl

Table-3

CSE Dilutions with Product

S.No.	Volume of CSE	Volume of Product Sample NID	Final CSE Concentration	Volume of Lysate	Total Volume
1.	50µl of 4 λ	50µl of NID	2 λ	100 µl	200 µl
2.	50µl of 2 λ	50µl of NID	λ	100 µl	200 µl
3.	50µl of λ	50µl of NID	λ/2	100 µl	200 µl
4.	50µl of λ/2	50µl of NID	λ/4	100 µl	200 µl

6.5.2.6 Record the inhibition or enhancement test results of product as per respective data sheet.

7.0 VALIDATION PARAMETERS AND ACCEPTANCE CRITERIA

7.1 This test must be repeated when any condition that is likely to influence the test results changes.

7.2 The test is not valid unless Negative result of **CSE Dilutions with LRW** and Negative result of **CSE Dilutions with Product** show no reaction. In addition the positive control i.e. 2 λ of both CSE with LRW and CSE with NID should show gel formation and λ/4 concentrations should show no gel formation and the result of Solution **CSE Dilutions with LRW** confirms the labeled sensitivity. The CSE dilutions with NID should also show the sensitivity of the lysate within 2 λ to λ/2.

7.3 If the sample under test does not comply with the test at a dilution less than the MVD, repeat the test using a greater dilution, not exceeding the MVD. The use of a more sensitive lysate permits a greater dilution of the sample to be examined and this may contribute to the elimination of interference.

7.4 Revalidation

7.4.1 A new source of vendor is identified for LAL Reagents.

7.4.2 If the method for testing of product is revised.

7.4.3 If the product manufacturing process is modified.

7.4.4 If the Endotoxin limit of the product revised.

8.0 Summary of Validation Report:

The validation report shall consist of a summary document, in the narrative form, which briefly describes the work as well as conditions regarding acceptability. This validation report shall also include the raw data, which shall be completed at the time of validation activity as per annexure.

9.0 Approval of Validation Report:

All validation parameters should comply with acceptance criteria as per protocol, Reviewed and signed by Quality assurance.

10.0 Annexure:

Following documents (Annexures) are enclosed as a part of protocol and shall be pre-approved as a part of main protocol.

S. No.	Document	Title	Data sheet Number
1	Data Sheet -1	Observation Sheet for Bacterial Endotoxin Test	

11.0 Abbreviations:

Abbreviation	Terms
NID	Non Interfering Dilution
MVD	Maximum Valid Dilution
LAL	Limulus Amoebocyte Lysate
LRW	LAL Reagent Water
CSE	Control Standard Endotoxin

12.0 Change history details:

Version no.	Reason for revision	CRF no.	Effective date
00	First Issue	Not Applicable	