SOP: LFA 00064
SOP For Bacterial Endotoxin (LAL) Test

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1 Objective

Procedure to follow to perform Bacterial Endotoxin (LAL) test for detection of endotoxin in materials to be tested

2 Scope

Quality Control Department

3 Responsibility

By: Microbiologist

4 Accountability

Head of the Department

5 Procedure

5.1 Precaution to be observe during LAL Testing

5.1. Check the calibration status of the triobloc

5.2. Check the triobloc temperature before putting the sample tubes to the well (Temperature should be 37 ±1 °C).

5.3. Glassware and accessories must have proper certificate for endotoxin free quality.

5.4. Use only depyrogenated glassware for the test.

5.5. Follow the manufacturer?s recommendations for storage and reconstitution of control standard endotoxin and lysate.

5.6. Check that the reagents used are not expired.

5.7. Do not vortex the lysate on reconstitution, gently shake to mix properly.

5.2 Depyrogenation of Glassware

5.1. Depyrogenation of LAL reaction tubes/dilution tubes/accessories

5.2. Use aluminium foil large enough to cover the tubes/accessories that are to be pyrogen free.

5.3. Place the tube/accessories inside the aluminium foil and wrap the tubes so that they are completely covered.

5.4. Place the tubes in dry heat sterilizer and follow the SOP.

5.5. Once depyrogenation is done, unload the tube/accessories and check to make sure that there are no tears in the foil. If there are any tears, replace the foil, rewrap and repeat the depyrogenation process again.

5.6. Ready to use depyrogenated tubes/accessories can be used directly.

5.3 Calculation of Maximum Valid Dilution (MVD) and Minimum Valid Concentration (MVC)

5.1. Raw Material MVC

$$\frac{\lambda(EU/ml)}{EL(EU/MG)}$$

5.2. Finished Products MVC

$$\frac{EL \times Potencyofproduct}{\lambda(EU/ml)}$$

5.3. Finished Products MVD

$$\frac{E.L.C}{\lambda}$$

Whereas:

EL = Endotoxin limit as specified in the individual product monograph, in EU/ml or EU/mg

Potency of product = Concentration of product in units or mg/ml

λ = Labelled sensitivity lysate

E.L.C = Endotoxin Limit Concentration, specified in monograph and specified ml of the product If the endotoxin limit of the product is not indicated or it is a new product, calculate the endotoxin limit by Endotoxin limit = K/M

K = 5.0 EU/kg for parenteral preparation (0.2 EU/kg for drugs administered intrathecally)

M = Maximum human dose/kg/hr

5.4 Reconstitution of LAL Reagant and Storage Condition

5.1. Store the lyophilised lysate as per manufacturer?s instructions.

5.2. Reconstitute the lysate with LAL Reagant water (LRW) as per manufacturer's instructions.

5.3. Reconstituted lysate may be stored as per manufacturer?s instructions.

5.4. Reconstituted lysate may only be frozen and thawed once (follow manufacturer?s instructions).

5.5. Reconstitution of Controlled Standard Endotoxin (CSE) and Storage Condition.

5.6. Reconstituted CSE with LRW as per the manufacturer?s instruction and vortex it as per the given time period.

5.7. Reconstituted CSE may be stored as per manufacturer?s instructions.

5.8. Vortex the CSE at least 5 minutes before use and vortex the Endotoxin at least one minute before use and after every 10 minutes of standing.

5.5 Confirmation of Labelled Sensitivity of LAL Reagent

5.1. Dilute the CSE with the LRW to obtain the $2\lambda, \lambda, \lambda/2, \lambda/4, (EU/ml)$ concentration of the Endotoxin or other available dilution step.

5.2. Add 100ml of each Endotoxin concentration to the respective tube marked as $2\lambda, \lambda, \lambda/2, \lambda/4$ Prepare 4 sets of each of the concentration.

5.3. Add 100ml lysate to each tube.

5.4. Negative control - add 100ml of LRW and 100ml of lysate to the test tube.

5.5. Vortex each tube gently and insert the triobloc, maintained at temperature of 37 ± 1 °C, incubate for 60 minutes $\pm$ 2 minutes.

5.6. Remove the tube from the triobloc after the incubation and check the tubes by inverting it gently at 180° for either positive or negative results.

5.7. A firm gel clot that maintains its integrity upon the inversion at 180° will indicate a positive result.

5.8. Tubes that show visible increase in viscosity or no firm gel will indicate a negative result.

Details	Test Solution	LRW	C.S.E	Lysate
Negative LRW control (NC)		$100\mu L$		$100\mu L$
Negative product control (NPC)	$100\mu L$			$100\mu L$
Positive product control (PPC)	$100\mu L$		$10\mu L(20\lambda)$	$100\mu L$
Positive LRW control (PC)		$100\mu L$	$10\mu L(20\lambda)$	$100\mu L$

5.9. Record the results. Check and find the endpoint, which is the lowest concentration that provides the positive result. Look for the log endpoint.

5.10. Find the geometric means by following the formula below:

$$GeometricMean = Antilog\frac{\Sigma}{f}$$

Σ = sum of log end points f = Number of replicates

5.11. The label claim is verified as okay if the Geometric Mean of the endpoint is between 0.5λ and 2λ

5.6 Test Procedures

Note: If there is any interference, the sample preparation is carried out by pH adjustment using acid, base or other suitable buffers. Use dispersing agents in cases of viscous products or use of reagent of hypersensitivity.

5.1. Samples will be diluted or prepared as instructed and the same sample is used for further testing.

5.2. 50/50 Method

5.3. As mentioned above in 5.6.1and 5.6.2, reagents shall be added or other suitable combination.

5.4. Shake the tube gently before placing it in the triobloc.

5.5. Insert the tubes in the triobloc, maintained at 37 $\pm$1 °C incubate for 60 minutes $\pm$2 minutes.

5.6. A firm gel clot that maintains its integrity upon the 180° inversion indicates as a positive result.

5.7. Tubes with increased viscosity or turbidity and shows no firm gel will indicate a negative result.

5.8. Record the said observations.

5.7 Validation

5.1. As per validation protocol, validation should be done for three batches of each product. The revalidation shall be carried out as per the validation protocol.

5.2. Validation is satisfactory if both series Endotoxin/Product and Endotoxin/LRW conforms not less than 0.5λ and not more than 2λ of the label, claimed sensitivity of lysate as per the validation protocol.

5.8 Interpretation of the Results

5.1. Product complies with LAL test if (-) results are found in both tubes of NPC, (+) results are found in both tubes of PPC. The Positive Control should show positive result in both tubes and Negative control should show negative results in both tubes.

5.2. The instrument usage details shall be recorded in the Standard Operating Procedure (SOP).

6 Abbreviations

SOP Standard Operating Procedure

EU Endotoxin Unit

EL Endotoxin Limit

NC Negative Control

MVD Maximum Valid Dilution

MVC Maximum Valid Concentration

ELC Endotoxin Limit Concentration

LRW LAL Reagent Water

CSE Controlled Standard Endotoxin

GM Geometric Means

NPC Negative Product Control

PPC Positive Product Control

PC Positive Control