Chapter 5

“SORTING OUT OF INTERFERENCE IN DETECTION OF ENDOTOXINS IN BIOThERAPEUTIC DRUGS, LACTOBIONIC ACID & MEDICAL DEVICES”
5.1 Biotherapeutic drugs

Abstract:

The effect of varying interference factors responsible for false positive results was investigated. The interfering factors inhibiting the Endotoxins in activated lymphocytes sample was sorted by heat denaturation. These activated lymphocytes are Biotherapeutic drugs which are used for autologous immune enhancement therapy. The technique used for denaturation can be applied to protein samples as well, to get rid of inhibition while quantifying Endotoxins.

Introduction:

Water is the principal source of Endotoxin in parenteral products. In general, as per the United State Pharmacopeias (USP) the threshold pyrogenic dose is 5 EU/kg/hr for parenteral drugs and 0.2 EU/kg/hr for intrathecal drugs. When Endotoxin enters into human blood these toxins induces white blood cells (WBC) to release cytokines, such as Tissue Necrosis Factor (TNF), Interleukin-1 and Interleukin-8, which mediate a complex biological response including pyrogensity, shock, coagulation and inflammation (Bang FB, 1956). So it is mandatory to check the presence of Endotoxin level in Biotherapeutic drugs before passing the product into market.

Bacterial Endotoxin is one of the most potent activator of mammalian immune system. Gram negative bacterial outer membrane Lipopolysaccharide (LPS) induces a cascade of defense mechanism that is known as fever and inflammation (C.M.Good and H.E. Lane, 1977). In incomparable fashion, for parenteral drugs administered into cerebrospinal fluid (CSF) known as intrathecal administration, K is reduced to 0.2 EU/kg. The most common toxic route of entry of Endotoxin into human system via intrathecal administration. Endotoxins are negatively charged macromolecules as small as 20-30 kDa, varying in size due to bacterial origin, the presence of divalent cations or biological detergents. Bacterial Endotoxin is the significant pyrogen that has been identified as a contaminant in
5.1 Biotherapeutic drugs

parenteral products. The LAL reaction with Endotoxin requires pH neutrally and optimum levels of divalent cations\(^2\). A uniform temperature of 37\(^\circ\)C optimizes the rate of reaction. Most Biotherapeutic drug products require dilution with LAL Reagent Water (LRW) (J.F. Cooper, 1990) before testing to avoid interference. Testing of serum, plasma, protein sample is subjected to inhibition from serine protease inhibitors and this interference creates problems in both Biotech and research fields\(^5\).

Materials and Methods:

Materials:

Limulus Amoebocyte Lysate (LAL), Control Standard Endotoxin (CSE), LAL Reagent Water (LRW) were purchased from Endosafe U.S. Depyrogenated 10 X 75 mm assay tubes, 16X100mm dilution tubes and pyrogen free Micropipette tips.

Principle of BET (Bacterial Endotoxins test):

The principle of Bacterial Endotoxin test is to detect presence of Endotoxin in the given sample to which the test is applied using Lysate derived from the animal *Limulus Polyphemus*. *Limulus polyphemus* is also called as Horseshoe crab. It is found in the eastern coast of America. This animal is living fossil as since last 150 million years there has been no physical or physiological change taken place in the animal. The blood of the animal is blue in colour due to the presence of Hemocyanin instead of Hemoglobin. The unique property of the blood is to react with Lipopolysaccharide (LPS) present in the membrane of Gram-negative bacteria.

Methods:

Procedure of LAL test:

Equal volume of test sample and LAL reagent is added in a depyrogenated test tube of 10 X 75 mm and incubate this mixture at 37\(\pm\)1\(^\circ\)C for 60\(\pm\)2 min. Then invert the tube by 180\(^\circ\) and look for gel formation. If a gel inside the test tube is able to maintain its integrity after inverting the tube to 180\(^\circ\) then it is a positive reaction which indicates presence of Endotoxin in the sample. Other than this any condition is considered as negative which
5.1 Biotherapeutic drugs

Indicates absence of Endotoxin in the sample (lesser than the lysate sensitivity).

**Endotoxin Limit:**

Since Endotoxin is ubiquitous in nature there has to be some safety limit to pass the product. The Endotoxin limit should be such that it will not cause any harmful effects to patient. Appendix E of USFDA (United States Food and Drug Administration) gives Endotoxin limits for various products. It also gives a formula through which Endotoxin limit for new product can be calculated.

The formula is as follows.

\[ \text{Endotoxin Limit} = \frac{K}{M} \] (USP 32, 2009)

Where, ‘K’ is threshold pyrogenic dose in humans and animals. 5 EU/kg body weight for parenteral drug and 0.2EU/kg body weight for intrathecal drug.

‘M’ Maximum dose administered to a patient per Kg body weight per hour (Not heroic dose).

The limit formula for radiopharmaceuticals is:

175/V except for intrathecally-administered products.

14/V for intrathecal drugs.

‘V’ equals the maximum recommended dose, in ml, at the expiration date or time. For drugs administered on a per Square Meter of Body Surface:

\[ 5 \text{ EU/ } \left( \frac{\text{dose} \times 1.8 \text{ sq. m.}}{70 \text{ Kg}} \right) \]

**Product Testing:**

For testing products equal volume of drug (sample) and LAL reagent is taken and following tubes are prepared

Negative Product Control (NPC) - Sample + LAL
Positive Product Control (PPC) - Sample + CSE (2λ) + LAL
Negative Water Control (NWC) - LRW + LAL
Positive Water Control (PWC) - LRW + CSE (2λ) + LAL

Majority of times it has been a common observation that if a product is tested directly it inhibits the LAL test and thus shows interference (J. van Noordwijk et al., 1997).
5.1 Biotherapeutic drugs

Interference:

Interference is defined as a significant difference between the end points of positive water control and positive product control using standard Endotoxin.

This interference could be either inhibition wherein the recovery of Endotoxin is below than the expected or enhancement wherein the recovery of Endotoxin is higher than expected.

Interference may occur due to following reasons:

Suboptimal pH:

The optimal pH for LAL reaction is in between about 6.8 to 7.4. If the sample is too acidic or too basic it will inhibit enzymes involved in LAL reaction. Hence it is very necessary to bring pH of reaction to neutral range. This can be done using 1N HCl or 1N NaOH for basic and acidic product respectively.

Endotoxin modification:

Purified Endotoxin has tendency to form micelle formation which is due to hydrophilic and hydrophobic interactions between LPS and water. This aggregated Endotoxin escapes LAL test. Hence to avoid such problem vortex mixing for samples is performed.

Container effects:

Adsortion of Endotoxin on tube wall causes poor recovery of Endotoxin in LAL test. So it is suggested to use high quality borosilicate glass tubes because of its inert nature hence adsorption of Endotoxin is least.

Unbalanced cation levels:

Divalent cations play important role in LAL reactivity and dispersion of Endotoxins. LAL test requires optimum concentration of Ca** and Mg** ions. If divalent cations are insufficient then Ca** and Mg** ions are added externally.

Protein or enzyme modification:

Enzyme inactivation due to oxidants, proteolytic agents or specific inactivators will cause inhibition.

Non-specific LAL activation:

Some molecules other than Endotoxin are known to react with LAL reagent and give gel formation. This is enhancement reaction and is very rare.
5.1 Biotherapeutic drugs

Subjects:

Activated lymphocyte sample which is used for autologous immune enhancement therapy for myeloid leukemia has been tested for sorting out of interference problem.

90% of interference problems are solved by just diluting the sample. But how much sample can be diluted so that it can still detect Endotoxin limit is given by formula for Maximum Valid Dilution (MVD).

\[ \text{MVD} = \text{concentration of sample} \times \text{Endotoxin Limit} / \text{Lysate sensitivity} \]

Example:

Drug : Activated Lymphocytes
Endotoxin Limit : NMT 0.25 EU/mL
Lysate sensitivity : 0.03125 EU/mL

\[ \text{MVD} = \text{potency} \times \text{E.L} \]

\[ \lambda \]

\[ = \frac{1 \text{mL/mL} \times 0.25 \text{EU/mL}}{0.03125 \text{EU/mL}} \]

MVD = 8

Product Validation:

Product needs to be validated before start for routine testing. Validation is a test condition where an Endotoxin standard is detected with the same efficiency in a test sample as it is in LRW. This validation study consists of two different phases wherein in Phase I (Preliminary screening) involve interference testing and Phase II consists of validation of product. Significance of product validation is that it gives information on whether there are any interfering factors in the drug product to the LAL test and also it gives an idea of the approximate levels of Endotoxin content in the drug product. It also covers manufacturing of product and formulation of the product.

It is always advisable to carry out revalidation if product formulation is changed and which is likely to affect the interference pattern of the product for LAL test. Also revalidation is to be conducted for any product if there is any change in manufacturing procedures or in vendor.
5.1 Biotherapeutic drugs

**Phase I: Preliminary Screening / interference Study**

In this two identical series of product dilutions (two-fold dilutions), one spiked with $2\lambda$, and one left unspiked. The result of Phase I will tell you the non-interfering dilution (NID) of the product, which is used for the actual validation (Phase II). The non-interfering dilution (NID) is the first set of PPC that shows a gel.

Example:

Drug: Activated Lymphocytes  
Endotoxin Limit: NMT 0.25 EU/mg  
Lysate sensitivity: 0.03125 EU/mL

\[ \text{MVD} = \text{potency} \times \text{E.L} \]
\[ \lambda \]
\[ = 1\text{mL/mL} \times 0.25 \text{EU/ml} \]
\[ = 0.03125 \text{EU/mL} \]

\[ \text{MVD} = 8 \]

**Results and discussions:**

<table>
<thead>
<tr>
<th>Sample Dilution</th>
<th>1:1</th>
<th>1:2</th>
<th>1:4</th>
<th>1:8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unspiked</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Spiked</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Table: 1

This assay shows that there is inhibition up to 1:8 (MVD). Due to inhibition LAL is unable to detect the Endotoxins even in spiked sample. After analyzing the sample using different procedures, finally in order to sort out this inhibition problem then the activated sample is heated at 55°C for 15min to coagulate the proteins in the sample and this heat denaturation technique is applicable to all protein samples. The denaturation won’t affect the Endotoxin because Endotoxins can be denatured at 250°C for 30 minutes as per USP.

Assay results after Heat denaturation of the sample.

<table>
<thead>
<tr>
<th>Sample Dilution</th>
<th>1:1</th>
<th>1:2</th>
<th>1:4</th>
<th>1:8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unspiked</td>
<td>--</td>
<td>++</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Spiked</td>
<td>--</td>
<td>+ +</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Table: 2
5.1 Biotherapeutic drugs

This assay shows that there is inhibition up to 1:1 dilution and the spike recovery at 1:2 dilutions onwards. Therefore the NID is 1:2. It is advisable to validate the product at not less than 1:4 dilution to take care of any batch to batch variation during regular production. So 1:4 dilution is chosen for product validation.

Phase II: Validation of Product

For validation, test and compare two identical series of Endotoxin dilutions bracketing $\lambda$; One prepared in LRW and another prepared in product diluted to the proposed test dilution. Here dilution selected for validation is 1:4. (Hot spike method).

Example of results:

<table>
<thead>
<tr>
<th>Replicates</th>
<th>0.0625 EU/mL</th>
<th>0.03125 EU/mL</th>
<th>0.015 EU/mL</th>
<th>0.007 EU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table: 3

Negative product control: --Geometric Mean = 0.03 EU/ml

<table>
<thead>
<tr>
<th>Replicates</th>
<th>0.0625 EU/mL</th>
<th>0.03125 EU/mL</th>
<th>0.015 EU/mL</th>
<th>0.007 EU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table: 4

Blank: -- Geometric Mean = 0.03 EU/ml

Successful validation requires that both series confirm label claim (Geometric mean) within +/- one two-fold dilution. Validation is conducted at this dilution on three batches of product.
5.2 Lactobionic Acid

Abstract:
Lactobionic acid used in organ preservative during organ transplant. The effect of varying the pH and ionic strength in Lactobionic acid was investigated. The interfering factors inhibiting the Endotoxins in Lactobionic acid while quantifying with Limulus Amoebocyte Lysate was sorted out by neutralizing the ionic concentration in sample using alkali and validated the test sample. Lactobionic acid is weak acid and having pH in the range 1 to 2. Since it is a weak acid its ionic dissociation is very less. As we add strong alkali like NaOH in to it, the pH rises immediately and after some time it comes down to acidic range. After adjusting the pH the sample to be analyzed within an hour to avoid interference.

Introduction:
Bacterial Endotoxin is one of the most potent activator of mammalian immune system. In general, as per the United State Pharmacopeias (USP) the threshold pyrogenic dose is 5 EU/kg/hr for parenteral drugs and 0.2 EU/kg/hr for intrathecal drugs. When Endotoxin enters into human blood these toxins induces white blood cells (WBC) to release cytokines, such as tissue necrosis factor (TNF), interleukin-1 and interleukin-8, which mediate a complex biological response including pyrogensis, shock, coagulation and inflammation. Gram negative bacterial outer membrane Lipopolysacchride (LPS) induces a cascade of defense mechanism that is known as fever and inflammation. So it is mandatory to check the presence of Endotoxin level in Organ preservative before using it for preservation of organs during transplant.

The LAL reaction with Endotoxin requires pH neutrality and optimum levels of Na+ and divalent cations. A uniform temperature of 37°C optimizes the rate of reaction. Most therapeutic drug products require dilution with LAL Reagent Water (LRW) before testing to avoid interference, where inhibition is failure to recover the positive control, and enhancement is excess recovery. There are 3 principle causes of invalid or inhibitory results in gel clot testing are 1. Loss of purified Endotoxin used for product positive controls (PPC). 2. Adverse
5.2 Lactobionic Acid

chemical conditions such as non-neutral pH or sub optimal levels of sodium ions and divalent cations (Mg++ and Ca++). 3. Inadequate controlled test parameters including testing accessories, reagents and analyst proficiency.

The aim of the study is to sort out the interfering factors which lead to the diverse results in Lactobionic acid. The false positive results may cause severe complication in the patients as discussed in literature.

Materials and Methods:

Materials:
Lyophilized Limulus Amoebocyte Lysate of 0.0312 sensitivity (LAL), Control Standard Endotoxin 5 Eu/ng (CSE), LAL Reagent Water (LRW) of Endosafe US, Depyrogenated (250°C for 30 min) 10 X 75 mm assay tubes, 16X100 mm dilution tubes, pyrogen free Micropipette tips, vortex mixture, 1N NaOH and Lactobionic acid were used for determination of Endotoxin content by the gel clot technique.

The sensitivity of the Lysate (labeled 0.0312 Eu/mL) was determined by using known amount of E.coli Control Standard Endotoxin.

In the gel-clot techniques, the reaction end point is determined from dilutions of the material under test in direct comparison with parallel dilutions or a reference Endotoxin, and quantities of Endotoxins are expressed in Endotoxin units.

1. Preparation of Standard stock solution and standard solutions: The CSE having a defined potency of 50 EU/Vial was reconstituted with 5ml of LRW and mixed intermittently for 30 minutes using a vortex mixture and this concentrate was used to prepare $2\lambda$, $\lambda$, $\lambda/2$ & $\lambda/4$, where $\lambda$ is the labeled claim sensitivity of Lysate.

2. Preparation of sample solution: Test samples were diluted to the required concentrations based on the formulae MVD. MVD is the maximum valid dilution, which is allowable dilution of the specimen at which the Endotoxin limit can be determined. The general equation to determine MVD is

$$MVD = \frac{\text{Endotoxin limit} \times \text{Concentration of sample solution}}{\lambda}.$$ 

Where E.L is the Endotoxin limit of the test sample, which is specified in the individual monograph/ based on the E.L formula if not mentioned in the monograph, in terms of volume or units of active drug (in EU/mg).
5.2 Lactobionic Acid

3. Lactobionic acid sample preparation: Batch No: LBA-0109, Potency=100mg/mL, E.L=0.005 Eu/mg, Lysate sensitivity is 0.0312 Eu/mL and MVD = 16. The following test dilutions are prepared by 1:16 (6.25 mg/mL), 1:8 (12.5 mg/mL), 1:4 (25 mg/mL) & 1:2 (50 mg/mL). Lactobionic acid is weak acid and having pH in the range 1 to 2.

Method:
Equal volume of test sample and LAL reagent is added in a depyrogenated test tube of 10 X 75 mm and incubate this mixture at 37 ± 1°C for 60 ± 2 min. Then invert the tube by 180° and look for gel formation. If a gel inside the test tube is able to maintain its integrity after inverting the tube to 180° then it is a positive reaction which indicates presence of Endotoxin in the sample greater than the limit. Other than this any condition is considered as negative which indicates absence of Endotoxin in the sample (lesser than the lysate sensitivity).

Product Testing:
For testing products equal volume of drug (sample) and LAL reagent is taken and following tubes are prepared:

Negative Product Control (NPC) - Sample + LAL
Positive Product Control (PPC) - Sample + CSE (2λ.) + LAL
Negative Water Control (NWC) - LRW + LAL
Positive Water Control (PWC) - LRW + CSE (2λ.) + LAL

Majority of times it has been a common observation that if a product is tested directly it inhibits the LAL test and thus shows interference. Interference: Interference is defined as a significant difference between the end points of positive water control and positive product control using standard Endotoxin.

This interference could be either inhibition wherein the recovery of Endotoxin is below than the expected or enhancement wherein the recovery of Endotoxin is higher than expected.
5.2 Lactobionic Acid

**Product Validation:**

Product needs to be validated before start for routine testing. Validation is a test condition where an Endotoxin standard is detected with the same efficiency in a test sample as it is in LRW. This validation study consists of two different phases wherein in Phase I (Preliminary screening) involve interference testing and Phase II consists of validation of product.

Significance of product validation is that it gives information on whether there are any interfering factors in the drug product to the LAL test and also it gives an idea of the approximate levels of Endotoxin content in the drug product. It also covers manufacturing of product and formulation of the product.

It is always advisable to carry out revalidation if product formulation is changed and which is likely to affect the interference pattern of the product for LAL test. Also revalidation is to be conducted for any product if there is any change in manufacturing procedures or in vendor.

**Phase I: Preliminary Screening / interference Study**

In this two identical series of product dilutions (two-fold dilutions), one spiked with $2\lambda$, and one left unspiked. The result of Phase I will tell you the non-interfering dilution (NID) of the product, which is used for the actual validation (Phase II). The non-interfering dilution (NID) is the first set of PPC that shows a gel.

**Results and discussions:**

**Lactobionic acid:**

<table>
<thead>
<tr>
<th>Sample Dilution</th>
<th>1:2</th>
<th>1:4</th>
<th>1:8</th>
<th>1:16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unspiked</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Spiked</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Table: 1
5.2 Lactobionic Acid

This assay shows that there is inhibition up to 1:16 (MVD) in Lactobionic acid. Due to inhibition LAL is unable to detect the Endotoxins even in spiked sample. After analyzing the sample using different procedures, finally in order to sort out this inhibition problem the acidic pH of the Lactobionic acid (1-2) is adjusted to 7-8 with 1N NaOH.

**Lactobionic acid:** (Results after adjusting the Acidic pH to the range of 7-8 with 1N NaOH).

<table>
<thead>
<tr>
<th>Sample Dilution</th>
<th>1:2</th>
<th>1:4</th>
<th>1:8</th>
<th>1:16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unspiked</td>
<td>++</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Spiked</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Table: 2

This assay shows no inhibition up to 1:2 dilution in Lactobionic acid and the spike recovery at 1:2 dilutions onwards. Therefore the NID is 1:2 (Lactobionic acid). It is advisable to validate the product at not less than MVD/4 to take care of any batch to batch variation. So MVD/4 dilution is chosen for product validation.

**Phase II:**

Validation of Product

For validation, test and compare two identical series of Endotoxin dilutions bracketing $\lambda$; One prepared in LRW and another prepared in product diluted to the proposed test dilution. Here dilution selected for validation is 1:4. (Hot spike method).

Example of results:

<table>
<thead>
<tr>
<th>Endotoxin/product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicates</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
</tbody>
</table>

Table: 3

Negative product control: --Geometric Mean = 0.0312 EU/ml
5.2 Lactobionic Acid

<table>
<thead>
<tr>
<th>Replicates</th>
<th>0.0625 Eu/mL</th>
<th>0.0312 Eu/mL</th>
<th>0.0156 Eu/mL</th>
<th>0.0078 Eu/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table: 4
Blank: – Geometric Mean = 0.0312 EU/ml
Successful validation requires that both series confirm label claim (Geometric mean) within +/- one two-fold dilution.
5.3 Quantification of Bacterial Endotoxins in Medical devices:

Abstract: Like drug products, each lot of medical devices need to be checked for bacterial Endotoxins for compliance before releasing into the market. Endotoxins are found to be variable absorptive to the device surfaces and leads to false negative results, to overcome this issue the desired test device need to be filled with WFI/LRW and incubate it at 37°C for 30 min, so that the Endotoxins stick to the walls of the medical device will be extracted into the WFI/LRW. Then the WFI/LRW rinse will be tested and report the value per ml of rinse. Then the reported value will be multiplies by total volume and divide the same with the number of devices taken will gives us the Endotoxin content in each medical device.

Materials and Methods:

Materials:
Lyophilized Limulus Amoebocyte Lysate of 0.125 sensitivity (LAL), Control Standard Endotoxin 5 Eu/ng (CSE), LAL Reagent Water (LRW) of Endosafe US, Depyrogenated (250°C for 30 min) 10 X 75 mm assay tubes, 16X100 mm dilution tubes, pyrogen free Micropipette tips, vortex mixture, Blood sets, I.V Set, 1mL Syringe, 5mL Syringe and Sutures were used for determination of Endotoxin content by the gel clot technique.

The sensitivity of the Lysate (labeled 0.125 Eu/mL) was determined by using known amount of E.coli Control Standard Endotoxin.

In the gel-clot techniques, the reaction end point is determined from dilutions of the material under test in direct comparison with parallel dilutions or a reference Endotoxin, and quantities of Endotoxins are expressed in Endotoxin units.

1. Preparation of Standard stock solution and standard solutions: The CSE having a defined potency of 50 EU/Vial was reconstituted with 5ml of LRW and mixed intermittently for 30 minutes using a vortex mixture and this
5.3 Quantification of Bacterial endotoxins in Medical devices

concentrate was used to prepare $2\lambda$, $\lambda$, $\lambda/2$ & $\lambda/4$, where $\lambda$ is the labeled claim sensitivity of Lysate.

2. Preparation of sample solution: Test samples were diluted to the required concentrations based on the formulae MVD. MVD is the maximum valid dilution, which is allowable dilution of the specimen at which the Endotoxin limit can be determined. The general equation to determine MVD is $MVD = (\text{Endotoxin limit} \times \text{Concentration of sample solution})/ (\lambda)$. Where E.L is the Endotoxin limit of the test sample, specified for Medical devices.

3. Blood set rinse sample preparation: Potency 1 mL/mL, E.L= 0.73 Eu/mL, Lysate sensitivity is 0.125 Eu/mL and MVD = 96. The following test dilutions are prepared by 1:1, 1:2, 1:3, 1:4 & 1:5.

4. I.V set rinse sample preparation: Potency 1 mL/mL, E.L= 1.25 Eu/mL, Lysate sensitivity is 0.125 Eu/mL and MVD = 10. The following test dilutions are prepared by 1:1, 1:2, 1:4, 1:8 & 1:10.

5. 1mL Syringe rinse sample preparation: Potency 1 mL/mL, E.L= 18.75 Eu/mL, Lysate sensitivity is 0.125 Eu/mL and MVD = 150. The following test dilutions are prepared by 1:9, 1:18, 1:37, 1:75 & 1:150.

6. 5mL Syringe rinse sample preparation: Potency 1 mL/mL, E.L= 3.75 Eu/mL, Lysate sensitivity is 0.125 Eu/mL and MVD = 30. The following test dilutions are prepared by 1:2, 1:4, 1:8, 1:16 & 1:30.

7. Sutures rinse sample preparation: Potency 1 mL/mL, E.L= 12 Eu/mL, Lysate sensitivity is 0.125 Eu/mL and MVD = 30. The following test dilutions are prepared by 1:6, 1:12, 1:24, 1:48 & 1:96.

Methods

Equal volume of test sample and LAL reagent is added in a depyrogenated test tube of 10 X 75 mm and incubate this mixture at 37 ± 1°C for 60±2 min. Then invert the tube by 180° and look for gel formation. If a gel
5.3 Quantification of Bacterial endotoxins in Medical devices

inside the test tube is able to maintain its integrity after inverting the tube to 180° then it is a positive reaction which indicates presence of Endotoxin in the sample greater than the limit. Other than this any condition is considered as negative which indicates absence of Endotoxin in the sample (lesser than the lysate sensitivity).

Product Testing: For testing products equal volume of drug (sample) and LAL reagent is taken and following tubes are prepared:

- Negative Product Control (NPC) - Sample + LAL
- Positive Product Control (PPC) - Sample + CSE (2λ) + LAL
- Negative Water Control (NWC) - LRW + LAL
- Positive Water Control (PWC) - LRW + CSE (2λ) + LAL

Product Validation: Product needs to be validated before start for routine testing. Validation is a test condition where an Endotoxin standard is detected with the same efficiency in a test sample as it is in LRW. This validation study consists of two different phases wherein in Phase I (Preliminary screening) involve interference testing and Phase II consists of validation of product.

Significance of product validation is that it gives information on whether there are any interfering factors in the drug product to the LAL test and also it gives an idea of the approximate levels of Endotoxin content in the drug product. It also covers manufacturing of product and formulation of the product.

It is always advisable to carry out revalidation if product formulation is changed and which is likely to affect the interference pattern of the product for LAL test. Also revalidation is to be conducted for any product if there is any change in manufacturing procedures or in vendor.
5.3 Quantification of Bacterial endotoxins in Medical devices

(i) Blood Set:

Preliminary Screening Test

Product : Blood set
Concentration : 1 ml / mL

Endotoxin Limit : \( \frac{K \times N}{V} \)

- \( K = 20 \text{ EU/Device} \)
- \( N = \text{No of Devices} \)
- \( V = \text{Volume of solution rinsed/ taken} \)

E.L = 20 EU/Device \( \times \) 3 Devices

82 mL

\[ = \frac{82}{1000} = 0.73 \text{ EU/ml} \]

MVD = Potency \( \times \) E.L

Sensitivity of Lysate

\[ = \frac{1\text{ml/ml} \times 0.73 \text{EU/ml}}{0.125 \text{EU/ml}} = 5 \]

Test Dilution : 1:1, 1:2, 1:3, 1:4 and 1:5

Test sample Rinse: Three blood bags from each lot are taken and completely filled with WFI and incubated it at 37°C for 30 min. Then the Endotoxins stick to the walls of the bag are extracted into the WFI. Then the WFI rinse was tested and reported the value per ml of rinse. Then the reported value was multiplied by total volume and divided the same with the number of devices taken, it gives us the Endotoxin content in each Blood bag.

<table>
<thead>
<tr>
<th>Test Dilutions</th>
<th>NPC</th>
<th>PPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>1:2</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>1:3</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>1:4</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>1:5</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

++: Gel Formation   − − : No Gel Formation
5.3 Quantification of Bacterial endotoxins in Medical devices

**Conclusion:** Carried out screening and observed Non Interfering Dilution (NID) at MVD/8 (1:1). Non Interfering dilution is 1ml/ mL. Endotoxins content is < 4 EU/ device.

**End-Product Endotoxins Test:**

**Product** : Blood set

**Preparation** : Product Concentration : 1mL / mL

Endotoxin Limit : 20 EU/Device

MVD : 5

Test Dilution : 1:2

**Results** :

<table>
<thead>
<tr>
<th>Material</th>
<th>Test Endotoxin Concentration EU/mL</th>
<th>NEG Control</th>
<th>NEG Product Control</th>
<th>Test Endpoint EU/mL</th>
<th>Log of End point</th>
<th>Geometric Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSE</td>
<td>+       +       -       -       -</td>
<td>0.125</td>
<td>-0.903</td>
<td>Antilog (-3.612/4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>+       +       -       -       -</td>
<td>0.125</td>
<td>-0.903</td>
<td>Antilog –0.903</td>
<td>0.125EU/mL</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>+       +       -       -       -</td>
<td>0.125</td>
<td>-0.903</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>+       +       -       -       -</td>
<td>0.0625</td>
<td>-1.2041</td>
<td>Antilog (-4.2142/4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Product</td>
<td>+       +       -       -       -</td>
<td>0.0625</td>
<td>-1.2041</td>
<td>Antilog –1.05355</td>
<td>0.088EU/mL</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>+       +       -       -       -</td>
<td>0.125</td>
<td>-0.9030</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Interpretation:**

Test results are : Valid

**Comments** : Product validation for Blood set was carried out at MVD/8 (1:2). The GM of the end points in Endotoxin / LRW was 0.125EU/mL and Endotoxin/product was 0.088 EU/mL which is in between 2λ and 1/2 λ, which indicates no inhibition and enhancement at test dilution 1:2.
5.3 Quantification of Bacterial endotoxins in Medical devices

(II) I.V SET

Preliminary Screening Test

Product : I.V Set
Concentration : 1 ml / mL
Endotoxin Limit : \( K \times N \)
\[ K = 20 \text{ EU/Device} \]
\[ N = \text{No of Devices} \]
\[ V = \text{Volume of solution rinsed/ taken} \]

E.L. = \( 20 \text{ EU/Device} \times 3 \text{ Devices} \)
48mL
= 1.25 EU/ml

MVD = Potency \times E.L
Sensitivity of Lysate

\[ = 1\text{ml/ml} \times 1.25 \text{ EU/ml} \]
\[ = 0.125 \text{ EU/ml} \]
\[ = 10 \]

Test Dilution : 1:1, 1:2, 1:4, 1:8 and 1:10

Test sample Rinse: Three I.V Sets from each lot are taken and completely filled with WFI and incubated it at 37°C for 30 min, Then the Endotoxins stick to the walls of the bag was extracted into the WFI. Then the WFI rinse was tested and reported the value per ml of rinse. Then the reported value was multiplied by total volume and divide the same with the number of devices taken, it gives us the Endotoxin content in each I.V bag.

<table>
<thead>
<tr>
<th>Test Dilutions</th>
<th>NPC</th>
<th>PPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1:2</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>1:4</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>1:8</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>1:10</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

+++: Gel Formation    --- : No Gel Formation
5.3 Quantification of Bacterial endotoxins in Medical devices

Conclusion: Carried out screening and observed Non Interfering Dilution (NID) at 1:2 dilution, at 1:1 the rinse of the I.V set is showing inhibition. Non Interfering dilution is 1:2 dilution. Endotoxins content is < 2.5 EU/ device.

End-Product Endotoxins Test:

Product: I.V set
Preparation: Product Concentration: 1mL / mL

<table>
<thead>
<tr>
<th>Endotoxin Limit</th>
<th>20 EU/Device</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVD</td>
<td>10</td>
</tr>
<tr>
<td>Test Dilution</td>
<td>1:2</td>
</tr>
</tbody>
</table>

Results:

<table>
<thead>
<tr>
<th>Test</th>
<th>Material</th>
<th>Endotoxin Concentration EU/mL</th>
<th>NEG Control</th>
<th>NEG Product Control</th>
<th>Test Endpoint EU/mL</th>
<th>Log of Endpoint</th>
<th>Geometric Mean = A log (log of Endpoint.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CSE</td>
<td>+ + - - - -</td>
<td>-</td>
<td>-</td>
<td>0.125</td>
<td>-0.9030</td>
<td>Antilog (-3.612/4) = Antilog -0.903 = 0.125EU/mL</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>+ + - - -</td>
<td>-</td>
<td>-</td>
<td>0.125</td>
<td>-0.9030</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>+ + - - -</td>
<td>-</td>
<td>-</td>
<td>0.125</td>
<td>-0.9030</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>+ + - - -</td>
<td>-</td>
<td>-</td>
<td>0.0625</td>
<td>-1.2041</td>
<td>Antilog (-4.2142/4) = Antilog -1.05355 = 0.088EU/mL</td>
</tr>
<tr>
<td></td>
<td>Product</td>
<td>+ + - - -</td>
<td>-</td>
<td>-</td>
<td>0.0625</td>
<td>-1.2041</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>+ + - - -</td>
<td>-</td>
<td>-</td>
<td>0.125</td>
<td>-0.9030</td>
<td></td>
</tr>
</tbody>
</table>

Interpretation:
Test results are: Valid

Comments: Product validation for I.V set was carried out at 1:4 dilution. The GM of the end points in Endotoxin / LRW was 0.125EU/mL and Endotoxin/product was 0.088 EU/mL which is in between $2\lambda$ and $\frac{1}{2}\lambda$, which indicates no inhibition and enhancement at test dilution 1:4 dilution.
5.3 Quantification of Bacterial endotoxins in Medical devices

(iii) 1 mL SYRINGE

Preliminary Screening Test

Product : 1 ml Syringe
Concentration : 1 ml / mL

Endotoxin Limit : \( K \times N \)

\[
K = 20 \text{ EU/Device} \\
N = \text{No of Devices} \\
V = \text{Volume of solution rinsed/taken}
\]

\[
E.L = 20 \text{ EU/Device} \times 3 \text{ Devices} \\
3.2 \text{ mL} \\
= 18.75 \text{ EU/ml}
\]

\[
MVD = \text{Potency} \times E.L \\
\text{Sensitivity of Lysate} \\
= 1\text{ml/ml} \times 18.75 \text{ EU/ml} \\
0.125 \text{ EU/ml} \\
= 150
\]

Test Dilution : 1:9, 1:18, 1:37, 1:75 and 1:150

Test sample Rinse: Three 1 ml Syringes from each lot are taken and completely filled with WFI and incubated it at 37°C for 30 min. Then the Endotoxins stick to the walls of the Syringe was extracted into the WFI. Then the WFI rinse was tested and report the value per ml of rinse. Then the reported value was multiplied by total volume and divide the same with the number of devices taken, it gives us the Endotoxin content in each 1 ml Syringe.

<table>
<thead>
<tr>
<th>Test Dilutions</th>
<th>NPC</th>
<th>PPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:9</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>1:18</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>1:37</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>1:75</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>1:150</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

++: Gel Formation  -- : No Gel Formation
5.3 Quantification of Bacterial endotoxins in Medical devices

**Conclusion:** Carried out screening and observed Non Interfering Dilution (NID) at 1:9 dilutions. Non Interfering dilution is 1:9 dilution. Endotoxins content is < 1.5 EU/ device.

**End-Product Endotoxins Test:**

**Product**: 1 ml Syringe  
**Preparation**: Product Concentration : 1mL / mL  
- Endotoxin Limit : 20 EU/Device  
- MVD : 150  
- Test Dilution : 1:18

**Results**:

<table>
<thead>
<tr>
<th>Material</th>
<th>Test Endotoxin Concentration EU/mL</th>
<th>NEG Control</th>
<th>NEG Product Control</th>
<th>Test End point. EU/mL</th>
<th>Log of End point.</th>
<th>Geometric Mean =A log (log of End point.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSE</td>
<td>+ + - - -</td>
<td></td>
<td></td>
<td>0.125</td>
<td>-0.903</td>
<td>Antilog (-3.612/4) =Antilog –0.903 =0.125EU/mL</td>
</tr>
<tr>
<td>Water Control</td>
<td>+ + - - -</td>
<td></td>
<td></td>
<td>0.125</td>
<td>-0.903</td>
<td></td>
</tr>
<tr>
<td>Positive Product Control</td>
<td>+ + - - -</td>
<td></td>
<td></td>
<td>0.125</td>
<td>-0.903</td>
<td></td>
</tr>
</tbody>
</table>

**Interpretation:**
Test results are : Valid  
**Comments** : Product validation for 1 ml Syringe was carried out at 1:8 dilution. The GM of the end points in Endotoxin / LRW was 0.125EU/mL and Endotoxin/product was 0.088 EU/mL which is in between $2\lambda$ and $\frac{1}{2}\lambda$, which indicates no inhibition and enhancement at test dilution 1:8 dilution.
(iv) 5 mL SYRINGE

Preliminary Screening Test

<table>
<thead>
<tr>
<th>Product</th>
<th>5 ml Syringe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>1 ml / mL</td>
</tr>
<tr>
<td>Endotoxin Limit</td>
<td>$K \times N$</td>
</tr>
</tbody>
</table>

\[
K = 20 \text{ EU/Device} \\
N = \text{No of Devices} \\
V = \text{Volume of solution rinsed/taken}
\]

\[
\text{E.L} = 20 \text{ EU/Device} \times 3 \text{ Devices} \\
\quad = 3.75 \text{ EU/ml}
\]

\[
\text{MVD} = \text{Potency} \times \text{E.L} \\
\quad = 1 \text{ ml/ml} \times 3.75 \text{ EU/ml} \\
\quad = 3.75 \times 1 \text{ ml/ml} \\
\quad = 0.125 \text{ EU/ml} \\
\quad = 30
\]

**Test Dilution**: 1:1, 1:2, 1:4, 1:8 and 1:16

**Test sample Rinse**: Three 5 ml Syringes from each lot are taken and completely filled with WFI and incubated it at 37°C for 30 min. Then the Endotoxins stick to the walls of the Syringe was extracted into the WFI. Then the WFI rinse was tested and report the value per ml of rinse. Then the reported value was multiplied by total volume and divide the same with the number of devices taken, it gives us the Endotoxin content in each 5 ml Syringe.

<table>
<thead>
<tr>
<th>Test Dilutions</th>
<th>NPC</th>
<th>PPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1:4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1:8</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>1:16</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>1:30</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

++: Gel Formation  --: No Gel Formation
Conclusion: Carried out screening and observed Non Interfering Dilution (NID) at MVD/8 (1:2) dilution. Non Interfering dilution is 1:2 dilution. Endotoxins content is < 1.6 EU/ device.

End-Product Endotoxins Test:

Product: 5 ml Syringe

Preparation: Product Concentration: 1mL/mL
Endotoxin Limit: 20 EU/Device
MVD: 30
Test Dilution: 1:4

Results:

<table>
<thead>
<tr>
<th>Material</th>
<th>Test Concentration EU/mL.</th>
<th>2λ</th>
<th>λ</th>
<th>½λ</th>
<th>¼λ</th>
<th>NEG Control</th>
<th>NEG Product Control</th>
<th>Test Endpoint. EU/mL</th>
<th>Log of Endpoint.</th>
<th>Geometric Mean =A log (log of Endpoint.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSE Water Control</td>
<td>+ + - - -</td>
<td>0.125</td>
<td>-0.903</td>
<td>Antilog (-3.612/4) = Antilog -0.903 = 0.125EU/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive Product Control</td>
<td>+ + - - -</td>
<td>0.0625</td>
<td>-1.2041</td>
<td>Antilog (-4.2142/4) = Antilog -1.05355 = 0.088EU/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Interpretation:
Test results are: Valid
Comments: Product validation for 5ml Syringe was carried out at MVD/4 (1:4). The GM of the end points in Endotoxin / LRW was 0.125EU/mL and Endotoxin/product was 0.088 EU/mL which is in between 2λ and ½ λ, which indicates no inhibition and enhancement at test dilution 1:4 dilution.
5.3 Quantification of Bacterial endotoxins in Medical devices

(V) SUTURES

Preliminary Screening Test

<table>
<thead>
<tr>
<th>Product</th>
<th>Suture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>1 ml / mL</td>
</tr>
<tr>
<td>Endotoxin Limit</td>
<td>K X N</td>
</tr>
<tr>
<td>V</td>
<td>K = 20 EU/Device</td>
</tr>
<tr>
<td></td>
<td>N = No of Devices</td>
</tr>
<tr>
<td></td>
<td>V = Volume of solution rinsed/ taken</td>
</tr>
<tr>
<td>E.L</td>
<td>20 EU/Device x 3 Devices</td>
</tr>
<tr>
<td></td>
<td>5mL</td>
</tr>
<tr>
<td></td>
<td>= 12 EU/ml</td>
</tr>
<tr>
<td>MVD</td>
<td>Potency x E.L</td>
</tr>
<tr>
<td></td>
<td>Sensitivity of Lysate</td>
</tr>
<tr>
<td></td>
<td>= 1ml/ml x 12 EU/ml</td>
</tr>
<tr>
<td></td>
<td>0.125 EU/ml</td>
</tr>
<tr>
<td></td>
<td>= 96</td>
</tr>
</tbody>
</table>

Test Dilution : 1:6, 1:12, 1:24, 1:48 and 1:96

Test sample Rinse: Three Sutures sets from each lot are taken and soaked them in 5 ml WFI and incubated it at 37°C for 30 min, and then the Endotoxins stick to the suture roll was extracted into the WFI. Then the WFI rinse was tested and report the value per ml of rinse. Then the reported value was multiplied by total volume and divide the same with the number of devices (Sutures), it gives us the Endotoxin content in each Suture roll.

<table>
<thead>
<tr>
<th>Test Dilutions</th>
<th>NPC</th>
<th>PPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:6</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>1:12</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>1:24</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>1:48</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>1:96</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

+++ : Gel Formation  -- : No Gel Formation
5.3 Quantification of Bacterial endotoxins in Medical devices

**Conclusion:** Carried out screening and observed Non Interfering Dilution (NID) at MVD/16 (1:1). Non Interfering dilution is 1:6 (1 Part of Rinse and 5 parts of LRW) Endotoxins content is < 1.25 EU/device (Per suture roll).

**End-Product Endotoxins Test:**

- **Product:** Suture
- **Preparation:** Product Concentration : 1mL / mL
  - Endotoxin Limit : 20 EU/Device
  - MVD : 96
  - Test Dilution : 1:12

<table>
<thead>
<tr>
<th>Test Material</th>
<th>2λ</th>
<th>λ</th>
<th>½λ</th>
<th>¼λ</th>
<th>NEG Control</th>
<th>NEG Product Control</th>
<th>Test End point. EU/mL</th>
<th>Log of End point.</th>
<th>Geometric Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSE Water</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.125</td>
<td>-0.9030</td>
<td>Antilog (-3.612/4) = Antilog –0.903 = 0.125EU/mL</td>
</tr>
<tr>
<td>Control</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.125</td>
<td>-0.9030</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.0625</td>
<td>-1.2041</td>
<td>Antilog (-4.2142/4) = Antilog –1.05355 = 0.088EU/mL</td>
</tr>
<tr>
<td>Product Control</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.0625</td>
<td>-1.2041</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.125</td>
<td>-0.9030</td>
<td></td>
</tr>
</tbody>
</table>

**Interpretation:**

Test results are : Valid

**Comments** : Product validation for Suture (roll) was carried out at MVD/8 (1:12). The GM of the end points in Endotoxin / LRW was 0.125EU/mL and Endotoxin/product was 0.088 EU/mL which is in between 2λ and ½ λ, which indicates no inhibition and enhancement at test dilution MVD / 8 (1:12).