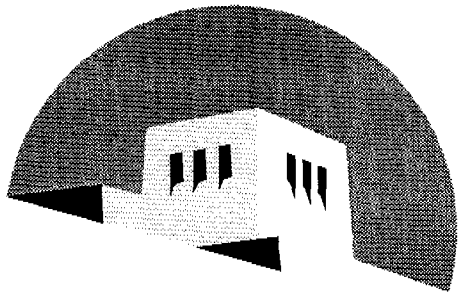

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Pyrogen and Sterility Testing of Radiopharmaceuticals

by:

James F. Cooper, M.S., Pharm.D., BCNP

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James F. Cooper, M.S., Pharm.D., BCNP

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PYROGEN AND STERILITY TESTING OF RADIOPHARMACEUTICALS

STATEMENT OF OBJECTIVES

The purpose of this correspondence lesson is to increase the reader's knowledge of the microbiological tests that demonstrate the freedom of parenteral medications from viable microbes and harmful levels of pyrogen (bacterial endotoxin). The reader should be able to conduct, document, and interpret the results of microbial assessment tests.

Upon successful completion of this material, the reader should be able to:

1. distinguish the requirements for sterility and pyrogen testing.
2. prepare compounding materials that are free of microbiological contamination.
3. discuss the sources of microbial and pyrogenic contamination in parenteral products.
4. select the materials and prepare a setting for endotoxin testing.
5. calculate the endotoxin limit, permissible dilution and other variables in microbial assessment testing.
6. discuss the basic procedures for endotoxin and sterility testing.
7. describe the approaches to rapid endotoxin testing.
8. report and interpret the results of microbial assessment testing.

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PYROGEN AND STERILITY TESTING OF RADIOPHARMACEUTICALS

by:

James F. Cooper, MS, PharmD, BCNP
ENDOSAFE, INC.
Charleston, SC 29407.

INTRODUCTION

Parenteral medications such as radiopharmaceuticals, which are made extemporaneously in the pharmacy setting, must be prepared aseptically and demonstrated to be free of microbes and associated toxic residues such as pyrogen (bacterial endotoxin). The current revision of the ***U. S. Pharmacopeia*** (***USP***) describes the methods required for microbiological testing of parenteral products.[1,2] The best way to make high quality injectables is to develop and utilize formulation procedures that will assure the purity of the ultimate product. After a product is compounded, microbiological purity tests verify that the product was prepared and packaged as planned. This discussion will focus on short-lived radiopharmaceuticals and the unique problems associated with their preparation and purity testing. Whereas the Bacterial Endotoxins Test (BET) may be done at the time of radiotracer synthesis, the incubation period of the sterility test relegates it to a retrospective test.[1,2] Therefore, prospective process validation is required to assure microbiological purity in the final product.

The unique test requirements for short-lived radiopharmaceuticals played a pivotal role in the advent of the BET when radiotracers became the first set of medications to be pyrogen tested by *in vitro Limulus* Amebocyte Lysate (LAL) methods. The obvious unsuitability of the rabbit pyrogen test for radiotracers and intrathecally-administered drugs prompted early governmental approval of the BET as a replacement for rabbit testing. The endotoxins test may be quickened further to meet the needs of cyclotron-produced products by modifying gel-clot methods, or by using spectrophotometer-assisted assays and endotoxin-specific software.

Sterility test media provides an optimal environment for growth of contaminating live microbes and requires 7 or 14 days of incubation, depending on the technique, as described in the *USP*.[1] Sterility tests do not measure pyrogen which is a part of microbial cell-wall debris. However, the BET measures endotoxin from both live and dead microbes. Both tests are necessary because a solution may be sterile but quite pyrogenic, or may be

endotoxin free but contaminated with gram-positive bacteria or fungi. The sterility test is well established in the *USP*, but the *in vitro* endotoxins test is relatively new and, thus, only the BET is discussed in detail.

This discussion will focus on the nature and scope of the BET. Test requirements, detailed procedures, and relevant calculations will be presented so that the reader could set up and conduct BET testing with confidence and dependability. Although the focus is on radiopharmaceuticals, the methods described herein are applicable to the compounding of any parenteral in the pharmacy setting.

LIMULUS AMEBOCYTE LYSATE (LAL) REAGENT

Applications

Bacterial endotoxin is a large molecule that does not normally cross intact skin or epithelial membranes found in the gastrointestinal tract and other orifices. However, medications and medical devices that are injected, infused or implanted into body tissues must be free of endotoxin to prevent the numerous host-response mechanisms that this toxin induces. The LAL test is specific for endotoxin. It became the official test for this toxin in the *USP 23*.

Biological Principles

Bacterial endotoxins are integral constituents of the outer membrane of gram-negative bacteria (GNB) and are among the most toxic of biomolecules. They were initially known as pyrogen because of their capacity for inducing fever. Endotoxin is composed of a lipopolysaccharide (LPS), is filtrable through sterilizing membranes and is difficult to destroy. Endotoxin is present on all surfaces and unprocessed water. The coliform organisms are the most common type of endotoxin-producing bacteria. Nanogram doses of endotoxins can produce fever and hypotension, whereas higher doses can lead to irreversible shock and death.[3]

Limulus is a marine invertebrate and has a blood coagulation system which is sensitive to endotoxin. Development of the *in vitro* endotoxins test began when Levin and Bang[4] showed that blood cells (amebocytes) from the American horseshoe crab (*Limulus* polyphemus) responded to GNB. The need for a suitable pyrogen test for radiopharmaceuticals led Cooper, Levin and Wagner[5] to extend this new approach to testing sterile products.

The LAL test is recognized as the most sensitive and specific means for measuring bacterial endotoxin and is now the test of choice, worldwide. Endotoxin activates a serine-protease enzyme system that modifies a clotting protein (coagulogen) in LAL to form an opaque gel; this is the basis of the gel-clot method.[2] Color reactions are possible by adding a suitable chromogenic substrate that is cleaved by endotoxin-activated LAL enzymes.[6] With the aid of a computer-assisted kinetic microplate or tube reader, increases in turbidity or color may be monitored with time to produce kinetic turbidimetric or chromogenic assays, respectively. LAL reactivity correlates well with pyrogenicity, exceeds rabbit-test sensitivity by several orders of magnitude and is the most convenient test for endotoxin in pharmaceuticals.[5] The LAL reaction requires a neutral pH and is time, temperature, and endotoxin-concentration dependent. Test conditions in radiotracers that slow or inhibit the LAL test generally are resolved by dilution within the permissible range.[7]

Test Summary

For 20 years this *in vitro* test was known as the *Limulus* Amebocyte Lysate or LAL test. When it was adopted into the *USP*, the name was changed to the Bacterial Endotoxins Test (BET) to reflect the objective of the test. The terms LAL test and BET are used interchangeably. In its simplest form, the BET requires a mixture of 0.1 mL of LAL reagent and 0.1 mL of the test sample in a depyrogenated 10x75 mm glass test tube. The assay mixture is promptly mixed and incubated for one hour in a heating block or water bath at 37°C to provide the optimum temperature for the reaction. After incubation, the tubes are gently removed and inverted to determine if there was sufficient endotoxin present to produce a gel. Finally, the results of the test and performance of controls are evaluated by acceptance criteria.

Types of Reagents

Gel-clot is the most common type of LAL reagent. Gel-clot reagents are further differentiated by their labeled sensitivity, represented by the Greek symbol lambda (λ), which is defined as the lowest concentration of endotoxin that will produce a gel under standardized test conditions. There are four sensitivities of gel reagent ranging in two-fold dilutions from the least sensitive, 0.25 Endotoxin Units (EU)/mL, to 0.03 EU/mL, the most sensitive reagent. LAL suppliers produce multi-test vials with 10-test and 50-test being the most common configurations. A convenient test-type for the hospital or pharmacy setting is the single-test vial (STV) where LAL sufficient for one test is lyophilized and sealed in a tube. To conduct a test, the STVs simply need to be rehydrated with 0.2 mL of the test sample or standard, and then incubated and observed as described above. To order LAL reagent, one would need to specify the sensitivity (λ) of the reagent and identify the number of tests per vial.

Other reagent types include kinetic turbidimetric LAL, kinetic chromogenic LAL and endpoint chromogenic LAL. The kinetic LAL reagents and methods

require an incubating 96-well microplate reader or multi-tube reader with endotoxin-specific software to manage data collection and analysis. These methods yield quantitative results within 30 minutes with excellent data management.

The production and marketing of LAL reagent is carefully controlled by the United States Food & Drug Administration (FDA). LAL is extracted from the blood cells (amebocytes) of *Limulus* polyphemus, the American horseshoe crab, which is found abundantly in shallow coastal waters of the Eastern United States.[8] Because of its critical use, LAL reagent is regulated as a licensed biologic drug by the FDA's Center for Biologics Evaluation & Research (CBER) and is batch-released after evaluating sensitivity and purity. It is crucial to use only FDA-licensed LAL reagents for pharmaceutical testing. LAL test products are differentiated by additives that suppliers use to optimize the performance and sensitivity of their reagents. Therefore, some reagents will perform better under certain test conditions than do others. Examples of formulation attributes include extent of buffer capacity and balance of monovalent and divalent cations.[8]

BACTERIAL ENDOTOXINS

Properties

Endotoxins are large complexes from the outer membrane of GNB that are constantly shed into the environment and released when the bacteria disintegrate or multiply. Environmental (unpurified) endotoxins contain lipid, carbohydrate and protein and have a common structure of a hydrophilic polysaccharide covalently bound to a hydrophobic region know as Lipid A. Whereas the LPS region is highly variable among various GNB families, the Lipid A region of the molecule is quite uniform in structure and is responsible for endotoxin's toxic pharmacological properties and LAL activity.[3,9] Endotoxins are negatively charged macromolecules of at least 10-to-30 thousand daltons, but vary in size due to bacterial origin and the presence of divalent cations or biological detergents.

Endotoxin does not penetrate intact skin or escape from the gastrointestinal tract into tissue. Should endotoxin gain access to vascular spaces, however, Lipid A causes dangerous toxic reactions (endotoxicity) including fever, hypotension and a variety of host responses. Lipid A also activates the coagulation system of *Limulus* species. Low doses of endotoxin (approximately one-to-ten thousand EUs) may produce fever; much larger doses may cause an increase in vascular permeability which leads to irreversible shock and death.

Endotoxins vary greatly in potency and LAL activity when compared on a weight basis. Therefore, quantities of endotoxin are expressed by biological activity as a more realistic way to represent endotoxin potency. The EU is an expression of biological activity relating to an intravenous dose of endotoxin that is sufficient to cause rabbit fever. Studies have shown that at least 1 nanogram or 5 EU per kilogram of purified endotoxin is required to produce pyrogenicity in man and rabbit.

Endotoxin Reference Standards

Endotoxin is isolated and purified from GNB by procedures such as phenol-water extraction to yield a LPS that is devoid of protein. All endotoxin reference standards are highly purified and defined in EUs by the U.S. Pharmacopeia or IUs by the European Pharmacopeia (EUs and IUs are equivalent). The U.S. Standard Endotoxin, more commonly known as Reference Standard Endotoxin (RSE), contains 10,000 EU per vial. The LAL suppliers provide secondary standards, known as Control Standard Endotoxins (CSE), that are carefully matched to specific LAL reagents. Reagent producers certify the LAL activity of these standards by an official method, known as RSE/CSE Standardization, and document this task with a Certificate of Analysis (CoA) for CSE. Delegation of RSE/CSE standardization to LAL suppliers reduces the cost and complexity of routine tests. The CSE is supplied with LAL reagent for preparing the Control Dilution Series (CDS) needed to make positive controls for routine tests, product validation, and confirmation of the labeled sensitivity (λ) of LAL.

Preparation of Positive Controls

The most difficult part of LAL testing is successful preparation and maintenance of the endotoxin assay series, also called the control dilution series, which is required for daily testing.[8] LPS is less stable than environmental endotoxin because the purification process eliminates dispersing factors. Further, the hydrophobic Lipid A in purified LPS tends to form micelles or aggregates which are less endotoxic and less LAL-reactive. Therefore, stability of dilute endotoxin solutions ($<$ 1 EU/mL) is limited because of the tendency to both aggregate into less potent forms and bind to containers. Lengthy vortex mixing is required to keep LPS dispersed and to maintain its potency. It is vital to follow the CSE supplier's rehydration, mixing, dilution and storage instructions. In gel-clot testing, the ultimate dilution series is a 4-tube control dilution series (CDS), prepared in 2-fold dilution steps, that brackets the label claim (λ) of the LAL reagent selected by the analyst. The task of daily label-claim verification suggests that the purpose of the assay is to verify the reactivity of the LAL reagent. Experience tells us that LAL reagent is quite stable when rehydrated and stored correctly, and yields highly reproducible results.

Therefore, the principal reason for the daily CDS assay is to confirm that the endotoxin (positive) controls are prepared and performing properly, thus reducing the possibility of invalid controls.

CSE vials may be obtained in amounts from 50 to 5000 EU per vial. Several 10-fold dilutions are required before the endotoxin is diluted to a workable range near the endpoint. The last step in the preparation is to make the four 2-fold dilutions from 2λ through ¼λ. It is common practice to store the endotoxin concentrations that are above 10 EU/mL for as much as one week, whereas CDS should be prepared more often, ideally on a daily basis in volumes from 5 to 10 mL. The greater precision demanded by the quantitative assays require daily preparation of the standard series.

The Positive Product Control (PPC), also termed interference control, assures the absence of inhibition when LAL testing a product. This control consists of a 2λ CSE concentration in the product being tested. The most reliable way to prepare the PPC is a "direct spike" method in which 2λ positive controls are made by dispensing 10 μL of a 20λ CSE solution directly into the 10 x 75 mm assay tube containing 100 μL of the test product. The same method is used to make the Positive Water Control (PWC). For example, a 2λ positive control for a LAL reagent labeled as 0.03 EU/mL is prepared by pipetting 10 μL of 0.6 EU/mL CSE (20λ) into the positive-control assay tubes. Figure 1 provides a representative scheme for preparing a CDS and the 20λ CSE tube needed to make 2λ positive controls for the example above. The CoA must be followed explicitly so that the CSE dilution series is made equivalent to Figure 1. For example, if the stock endotoxin vial contains only 500 EU, then the dilution scheme must be modified to make the spiking tube (0.5 EU/mL, approximately 20 λ) and 4-tube CDS, i.e., 2λ through ¼λ.

Figure 1. Dilution Scheme for Control Standard Endotoxin

5000 EU/vial = 1000 EU/mL (concentr. in stock vial)
5 mL rehydration

1:10 → 100 EU/mL 1:10→ 10 EU/mL 1:20→
0.5 EU/mL (20 λ) 1:8→ 0.06 EU/mL (2 λ)
1:2→ 0.03 EU/mL (λ) 1:2→ 0.015 EU/mL (½ λ)→
1:2→ 0.007 EU/mL (¼ λ)

STANDARD BACTERIAL ENDOTOXINS TEST (BET) METHODS

Sources of Standardized Procedures

The LAL test revolutionized endotoxin testing worldwide because it was simple, economical, reproducible and specific. The gel-clot BET of the *USP* has excessive controls and is considered only as a referee test for arbitration.[2] The most useful reference is the FDA Guideline for End-product LAL Testing (1987) because it describes validation and routine test procedures in greater detail and provides methods for all three types of LAL methods: gel-clot, end-point, and kinetic turbidimetric or kinetic chromogenic tests.[10] An LAL test standard operating procedure (SOP) should reference the LAL Test Guideline and Use Instructions from the reagent supplier as the basis for all LAL test procedures and interpretations. The *USP* BET should be referenced for "information only" so as to avoid the needlessly excessive requirements of this chapter.

Preparations for LAL Testing

The test is easy to conduct and requires minimal equipment.[11] The gel-clot method entails mixing equal parts (100 μL) of test samples or controls and LAL followed by undisturbed incubation for one hour in a water or dry bath set for 37°C.[10] The single-test vials are rehydrated directly with sample or control. Endotoxin-free glassware must be acquired from an LAL vendor or depyrogenated by a validated dry-heat cycle. A valid cycle usually requires exposure to > 200°C for at least three hours. Sterile, disposable polystyrene tubes are usually adequate for sample preparation, but depyrogenated borosilicate glass tubes are ideally suited for maintaining endotoxin standards.[7] One must be cautious of container-related interference. Appendix A contains an SOP for testing radiopharmaceuticals which is in compliance with the FDA LAL Test Guideline.[8,10]

Sterility testing requires a laminar-flow biological cabinet to assure absence of false-positive sterility tests. However, any clean bench space is adequate for LAL testing. Equipment for LAL testing includes an incubator, vortex mixer and appropriate test-tube racks for sample handling. The test requires accurate, aseptic pipetting devices to transfer endotoxin standards and LAL samples and to prepare interference controls. Appendix A contains an idealized method and a list of essential materials and equipment.

Preparing Test Samples

It is the practice in the industry to dilute test materials prior to LAL testing in order to minimize test-related interference, reduce the sensitivity of the test to a realistic level and diminish levels of hazardous materials such as radioactive products. The amount of permissible

dilution is substantial for small-volume parenterals, and is dependent on the product-specific endotoxin limit and route of administration; refer to the section on Endotoxin Limit.

Rehydration and Storage of LAL Reagent

The reagent must be rehydrated with high-purity water known as LAL reagent water (LRW) which is free of trace endotoxin. The reagent must be kept near 0°C during use, and promptly frozen after use. The LAL may be frozen and thawed only once, so it is prudent to purchase the number of tests per vial that can be consumed in two test periods.[2] The vial must have a vacuum when rehydrated and the ensuing solution should be clear and colorless. The vendor's instructions for use in handling LAL reagent must be followed, explicitly.[11]

Routine LAL Test

A routine LAL test is defined in the BET chapter of the *USP* and FDA Guideline as one conducted on an injectable product under validated conditions, that is, in the absence of any interfering conditions.[2,10] The four components of a routine BET include the following:

- Test material, usually diluted with LRW to achieve compatibility;
- Positive Product Control (PPC), a 2λ solution prepared in the test material;
- Positive Water Control (PWC), a 2λ solution in LRW or a CDS;
- Negative Control (NC), LRW used for LAL and dilution of standards and samples.

A routine test is set up by preparing the above items in duplicate, as detailed in Appendix A. The PPC is the same solution as the test material except that it contains an endotoxin standard concentration which is double the label claim (2λ) of the LAL reagent. The PPC is prepared by inoculating 100-μL samples of the test material with 10 μL of a 20λ CSE solution. The PWC is made by diluting the control standard so that it brackets the endpoint of the LAL sensitivity. Finally, the NC is the LRW that was used to rehydrate the LAL reagent and to dilute the test materials and endotoxin standards. Refer to the section on acceptance criteria in Appendix A.

After a one-hour, undisturbed incubation period at 37°C, the assay tubes are inverted to determine whether or not there was sufficient endotoxin present to produce gelation of the assay mixture. A routine LAL test is valid when the PPC and PWC tubes are positive and the test material and negative controls are negative (without gel), as shown in Table 1. If these test conditions are not achieved, the test must be invalidated and a new test begun. If the test material presents a gel, the material may be diluted to the Maximum Valid Dilution (MVD) and retested. The material **passes** if there is no gel at the MVD, but if there is a positive result, the test **fails** and the radiotracer must be remade.

The FDA LAL Test Guideline specifies the conditions for validating or proving that a specific product can be tested by the LAL test without interference.[10] The conditions for interference screening are discussed under the section on validation.

Table 1. Configurations for Routine LAL-Testing of Radiotracers

Component	Routine		20-min test			In-process	
2λ PWC	⊕	⊕	⊕	⊕		⊕	
2λ PPC	⊕	⊕	⊕	⊕		⊕	
Sample	⊖	⊖	⊖	⊖	⊖	⊖	⊖
NC	⊖	⊖	⊖	⊖		⊖	
10 EU/mL PPC			⊕				

The Twenty-minute Test

The test period may be shortened for positron emission tomography (PET) radiopharmaceuticals by modifying the routine gel-clot test and using an LAL reagent of high sensitivity, 0.015 or 0.03 EU/mL. In this case, a routine LAL test is set up in the usual way except that an extra sample tube and a 10 EU/mL PPC is added.[8] A unique PPC is made by in-tube spiking where 10 μL of a 100 EU/mL CSE solution is added to a product sample, as in Figure 1. At 20 minutes, the 10 EU/mL PPC and one of the sample tubes are carefully removed from incubation and observed for gelation. If the PPC is positive and the sample is negative, the product is safely within the 25 EU/mL limit because it is < 10 EU/mL. To verify the short test, the other tubes are incubated for the full hour before observing and recording the final results. Table 1 illustrates how tubes are set up for a routine LAL test that is compatible with the FDA Guideline and also provides for a 20-minute endotoxins test. The pluses and minuses in the circles, representing assay tubes, indicate the expected results when a test meets validity criteria and the test material is free of detectable endotoxin. The PPCs may be reduced to one set per day provided the conditions of the test and product formula have not changed. For in-process testing, the control tubes may be reduced to one each, except for the test material (see Table 1). It is a good practice to cap tubes containing radioactivity to avoid spillage.

How do we interpret and report the findings? If a routine test is valid and the test material is negative, the test should be reported as less than the product of lambda

times the dilution factor. For example, if the labeled sensitivity (λ) is 0.03 EU/mL and the radiotracer dilution factor is 4, the results of a negative LAL test should be reported as (<0.03 EU/mL x 4) or <0.12 EU/mL. If the tracer was tested undiluted, the sensitivity of the test equals lambda. In a 20-minute test, the results can only be reported as less than 10 EU/mL PPC times the dilution factor. For example, if the radiotracer was diluted 1:2 to avoid interference, the test should be reported as (<10 EU/mL x 2) or <20 EU/mL until the 60-minute test is completed. Appendix B constitutes a form for recording results and interpreting the findings.

VALIDATION OF AN LAL TEST

Purpose

The objective of LAL test validation is to conduct and document experiments which prove that a specific product or in-process material can be tested properly with a specific vendor's LAL reagent. The first step is calculation of the endotoxin limit of each product. With this information the permissible dilution is calculated so that dilution may be applied to resolve inhibition problems. The second step is to determine the compatible concentration of each product through a process of interference screening, where a product is diluted with 2λ CSE solution to find a positive endpoint. Finally, the task of validation is completed by selecting a product dilution and proving it to be non-interfering by conducting a validation assay (see Table 2). Re-validation is needed only if the product formulation or LAL vendor is changed.

Table 2. Configuration for Product Validation for LAL Test

	Endotoxin in Water			
rep.	0.25 (2λ)	0.125 (λ)	0.06 (½λ)	0.03 (¼λ)
1	⊕	⊕	⊖	⊖
2	⊕	⊕	⊖	⊖
3	⊕	⊕	⊖	⊖
4	⊕	⊕	⊖	⊖

Geometric Mean = 0.125 EU/mL

	Endotoxin in Product			
rep.	0.25 (2λ)	0.125 (λ)	0.06 (½λ)	0.03 (¼λ)
1	⊕	⊕	⊕	⊖
2	⊕	⊕	⊖	⊖
3	⊕	⊕	⊖	⊖
4	⊕	⊕	⊕	⊖

Geometric Mean = 0.085 EU/mL

Endotoxin Limit

The endotoxin limit (EL) is the maximum amount of bacterial endotoxin allowed in a single dose of a parenteral drug. The general limit for endotoxin is universally accepted as 5 EU/kg/hr or 350 EU/70-kg adult for parenteral products.[2,10] Since endotoxin is more potent when injected directly into cerebrospinal fluid, this administration route is limited to 0.2 EU/kg/hr.[12] Table 3 gives endotoxin limits for various dosage forms.

Table 3. Official Endotoxin Limits

Item	Endotoxin Limit
Drugs, Biologics	5 EU/kg/hr
Radiopharmaceuticals	2.5 EU/kg/hr
Large Volume Parenterals	0.5 EU/mL
Water for Injection	0.25 EU/mL
Intrathecal drugs	0.2 EU/kg/hr
Medical Advice	up to 200 EU per device

The EL is used to determine the safety of an LAL test result and to calculate the permissible dilution that may be required to resolve interference conditions. According to the FDA LAL Test Guideline[10], the Maximum Valid Dilution (MVD) is calculated by dividing the product of the endotoxin limit times the product potency by the sensitivity of the LAL reagent:

$$\text{MVD} = \frac{\text{Endotoxin Limit (EL) x Potency}}{\text{lambda } (\lambda)}$$

Radiopharmaceuticals were the first group of parenterals assigned an endotoxin limit in the *USP*.[2] They were arbitrarily given a doubly stringent limit of 2.5 EU/kg/hr. The Guideline recognized that the volume of a radiotracer dose increases with time for a given preparation because of radioactive decay. For the purpose of selecting a uniform calculation for radiotracers, the FDA set the maximum dose volume at 7 mL/70 kg adult dose giving an endotoxin limit concentration of 25 EU/mL for a total adult dose of 175 EU. This limit is found in Appendix E of the FDA LAL Test Guideline[10]; it applies to traditional radioactive injectables containing Tc-99m as well as new PET-imaging injectables such as Ammonia N-13, F-18 Fluorodopa and F-18 Fluorodeoxyglucose (FDG). Therefore, the radiopharmaceutical MVD equals the EL of 25 EU/mL divided by lambda, or:

$$\text{Radiopharmaceutical MVD} = \frac{25 \text{ EU/mL}}{\text{lambda } (\lambda)}$$

The EL for radiotracers is described somewhat differently in the *USP* in contrast to the FDA Guideline, but the calculation is quite similar. The endotoxin limit for radiopharmaceuticals which have a monograph in the *USP* 23 is defined as 175EU/V, where V is the maximum dose volume at the time of expiration. The difference between the BET chapter of the *USP* and the FDA Guideline is that the V component is variable in the former and fixed at 7 mL in the FDA Guideline. The exceptions are In-111 and Yb-169 Pentetate which have a limit of 14 EU per dose because of their intrathecal route of administration.

When the LAL sensitivity (λ) and EL are known, we have the values necessary to calculate the MVD for a radiopharmaceutical. For example, when testing F-18 FDG with a 0.125 EU/mL reagent, the MVD equals 200 (25 EU/mL ÷ 0.125 EU/mL). This means that this radiotracer may be tested at any test-compatible dilution up to the MVD, a 1:200 dilution of the radiotracer with LRW. As the sensitivity of the LAL reagent increases, the MVD logically becomes greater. For example, if F-18 FDG was tested with the most sensitive LAL reagent, the MVD would be 800 (25 EU/mL ÷ 0.0325 EU/mL). In actual practice, most PET tracers are water-like and produce no interference. Dilution may be used simply to increase the volume of sample needed to conduct the BET.

Validation of LAL Test Methods

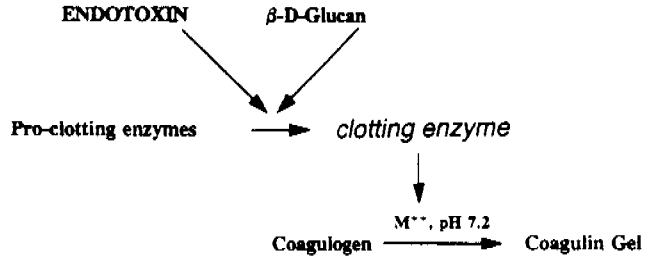
Validation of a LAL test method requires proving that there is no interference when testing a specific product under specified conditions. A common inhibition-screening technique calls for diluting a specific product with 2λ CSE solution until a positive result is obtained. Basically, the goal of validation is to document that endotoxin can be recovered in a specific diluted product as easily as it is recovered in water. The validation step requires that a CDS is made in the diluted product and compared to one made in LRW. Table 2 exemplifies a valid study where λ = 0.125 EU/mL. Validation means that endpoints of the two series are within one 2-fold dilution of the labeled sensitivity. The radiotracer concentration selected for validation may be anywhere from the lowest compatible dilution up to the MVD.

RECOGNIZING CONDITIONS THAT INTERFERE WITH BET

Sources of BET Interference

Ideal conditions for an LAL test include neutral pH and optimum levels of sodium ions and divalent cations (M^{++}).[7] It is the intent of the LAL manufacturer to incorporate the required conditions in the reagent formulation. However, the components of complex test materials can alter the LAL assay milieu to the extent that considerable dilution is required to reach a compatible test status. Introduction of the MVD by the FDA provided a realistic way to use simple dilution to solve interference problems and avoid excessively stringent test criteria. Most final dosage forms of parenteral products are in aqueous solution and near physiological pH and osmolarity. Simple dilution with LRW is usually all that is needed to reach LAL-compatible conditions. Raw materials often present unique inhibition problems that may be due to multiple interference mechanisms. The LAL-endotoxin reaction, as summarized in Figure 2, provides clues as to sources of product-related interference.

Figure 2. Clotting Reaction for Activated LAL



Note: M^{++} refers to divalent cations

The most common type of LAL test inhibition is sub-optimum pH conditions in the assay mixture.[8] The ideal LAL reagent provides substantial buffering so that minor pH disparity is satisfied either by the reagent or modest dilution of the test material before applying the test. Tris and HEPES are highly compatible buffers with the LAL enzymatic system, and they are found in LAL formulations. Potentially inhibiting pH conditions are identified by measuring the pH of a mixture of LAL and test material. The acceptable range is 6.5 to 8.0 but, ideally, the pH should be 7.3 ± 0.5. If the assay mixture is out of this range, and the interference controls fail, the first step is to apply dilution to the test sample within permissible limits (see MVD calculations). If simple dilution fails to resolve the problem, as may occur when testing raw materials, the first dilution step should be made with 0.1M Tris HCl buffer rather than LRW.

Another chemical interference is caused by depletion of divalent cations from the LAL formulation due to the presence of chelating agents. Examples of such chelaters are anticoagulants such as citrate and heparin. Since divalent cations are coenzymes in the LAL-endotoxin reaction, inhibition will occur if the chelaters are in sufficiently high concentration. The first step in avoiding this type of inhibition is to use permissible dilution. However, if dilution alone fails to resolve the inhibition, a $MgSO_4$/Tris buffer may be required to replace the sequestered divalent cations.

Although rare, soluble trivalent cations in a test material are highly inhibitory to the BET; some magnetic resonance imaging agents interfere by this mechanism. The way to resolve this type of inhibition is to sequester the unbound trivalent cation by adding magnesium citrate or calcium ethylenediaminetetraacetic acid (EDTA) to the first dilution of the test material.

Second only to pH problems, loss of endotoxin in the positive control is a critical source of interference. Endotoxin depletion is due to product-induced aggregation of endotoxin in the positive control. Further product dilution and more aggressive vortexing of the standards is quite helpful. Finally, the in-tube spiking method recommended herein is also helpful because it limits the exposure time of the test material to the endotoxin spike.

Enhancement is limited to the presence of β-D-glucan, a glucose polymer that is the only known non-specific activator of the LAL test. Products at risk of glucan-related LAL activation include those exposed to hollow-fiber membranes, unwashed cellulose acetate filters, cellulose derivatives or yeast-fermentation products.[13] Care is required to assure that the product is not exposed to cotton during processing or column purification.

LAL Test Parameters for Non-radioactive Drugs

Pharmacopeia generally have fixed limits for endotoxin concentration in medical-device extracts, large-volume parenterals, radiopharmaceuticals and pharmaceutical waters. LAL test reports for these types of products should indicate a **pass/fail** statement relative to the appropriate endotoxin limit concentration, such as < 0.25 EU/mL for Water for Injection.

Performing calculations and reporting LAL test results for pharmaceuticals is somewhat different from radiotracers because each drug has a product-specific EL based on the magnitude of the administered dose. Appendix E of the FDA LAL Test Guideline contains a list of drug entities with dose-dependent endotoxin limits reported as EU/mL, EU/mg or EU per designated weight or unit of product. All reports of release testing should reference either a published or calculated EL that is product specific.

To exemplify how LAL test parameters are determined for parenterals, let us consider an IND peptide which is compounded extemporaneously at 100 mg/mL in a hospital or pharmacy setting.

Since the drug is investigational, there is no published EL. The maximum adult dose is 10 mg/kg. The EL is calculated from the FDA's K/M formula where K is the general EL and M is the maximum human dose per kilogram. Therefore, the EL for the IND peptide is 0.5 EU/mg of peptide (5 EU/kg ÷ 10 mg/kg). From interference screening (LAL tests on 10-fold dilutions), the compatible range for testing the peptide with Endosafe® reagent was 1 mg/mL, and below. The Minimum Valid Concentration (MVC) for testing the peptide with a 0.03 EU/mL LAL sensitivity (λ) is 0.06 mg/mL. The permissible dilution for a 100 mg/mL potency is substantial (MVD equals 1:1667). If an analyst elects to test the peptide at a convenient dilution of 1:100, rather than the MVC, the LAL-test concentration of the peptide is 1 mg/mL.

The product specific sensitivity (PSS) expresses the sensitivity of an LAL test when lambda, endotoxin limit and test concentration (TC) of a product are considered. The PSS for an LAL test described above is λ divided by the TC, 0.03 EU/mL ÷ 1 mg/mL, or 0.03 EU/mg. This test is > 10 times more stringent than the specified EL of 0.5 EU/mg. The calculations below summarize all of the parameters for filing an official LAL-test report:

Test Product and Potency: IND peptide, 100 mg/mL
Endotoxin Limit: 5 EU/kg ÷ 10 mg/kg = 0.5 EU/mg (K÷M)
Licensed LAL reagent: 0.03 EU/mL (λ) Selected arbitrarily
MVC: 0.03 EU/mL÷0.5 EU/mg = 0.06 mg/mL (λ ÷ EL)
MVD: 100 mg/mL÷0.06 mg/mL = 1:1667 (Potency ÷ MVC)
MVD: (alternative) (0.5 EU/mg x 100 mg/mL) ÷ 0.03 EU/mL = 1:1667
Endosafe® Compatible Conc: 1 mg/mL and below, validated on 3 lots
Elected Dilution, Test Conc: 1:100 dilution gives a 1.0 mg/mL test concentration (TC)
PSS: 0.03 EU/mL÷1 mg/mL = 0.03 EU/mg (λ ÷ TC)

Acceptance Criteria

The peptide **passes** the BET when the controls for a validated test method are valid and the diluted test material is negative because the test meets the criteria of less endotoxin than the product-specific EL. Therefore, if the product has a negative result in a valid test, the results should be reported as < 0.03 EU/mg. Such a test **passes** the BET because any potential endotoxin is far less than the EL. If the product gave a positive (gel) result in a valid test, the product should either be retested at the MVD or an endpoint found by a two-fold dilution series. If the controls were not valid in the test, it should be designated as "invalid" and subjected to a new test rather than designating the process as a "retest."

Quantitation by Gel-clot Methods

If a product sample is positive at an undiluted level, it may be diluted by serial 2-fold or 8-fold dilutions until an endpoint is reached. Then, the highest positive dilution is multiplied times λ to estimate endotoxin concentration. For example, a raw material was tested undiluted (UND) and in serial two-fold dilutions until an endpoint was reached at 1:4 using a 0.125 EU/mL LAL reagent, as diagrammed below.

UND	1:2	1:4	1:8	1:16
⊕ ⊕	⊕ ⊕	⊕ ⊕	⊖ ⊖	⊖ ⊖

The endotoxin concentration (E) is calculated at 0.5 EU/mL which is safely within the EL.

$$(E) = (\lambda)\,(4/1) = (0.125\ \text{EU/mL})\,(4) = 0.5\ \text{EU/mL}$$

Quality Control Requirements

The FDA LAL Test Guideline requires an endotoxin assay before using a new set of LAL and CSE lots.[10] This task is called "confirmation of label claim" and entails an endotoxin assay, 2λ through $\frac{1}{4}\lambda$, tested in quadruplicate on one vial of LAL reagent. Also, new analysts must successfully complete the task called "analyst qualification" by conducting the same type of endotoxin assay. Only qualified analysts should be conducting official LAL testing. Label claim is verified or an analyst is qualified if the Geometric Mean (GM) of the endpoints confirms the label claim sensitivity (λ) of the LAL reagent (Table 4), that is, the GM must be greater than or equal to $\frac{1}{2}\lambda$ and less than or equal to 2.0λ.[10]

Table 4. Endotoxin Assay for Confirmation of Label Claim

Rep.	0.25 2λ	0.125 λ	0.06 $\frac{1}{2}\lambda$	0.03 $\frac{1}{4}\lambda$	Endpoint (EU/mL)	Endpoint ($\log_{10}$)
1	⊕	⊕	⊕	⊖	0.062	-1.222
2	⊕	⊕	⊖	⊖	0.125	-0.903
3	⊕	⊕	⊖	⊖	0.125	-0.903
4	⊕	⊕	⊕	⊖	0.062	-1.222

Geometric Mean = antilog $\Sigma e/f$ = antilog (-4.25/4) = 0.085 EU/mL

where, Σe = sum of $\log_{10}$ endpoints and f = # replicates, and λ = 0.125 EU/mL

Preparing and Testing Raw Materials

Depyrogenation of glassware, heat-stable salts and equipment requires destruction by either exposure to strong bases or oxidants or dry-heat treatment, such as exposure to 200°C for at least 3 hours.[9] All solutions should be clarified and rendered sterile as soon as possible after preparation. Commercial sources of sterile and endotoxin-free components can simplify preparation. For example, Sterile Water for Irrigation is economical and available in large volumes. Finally, critical solutions must be prepared aseptically and verified to produce microbiologically pure pharmaceuticals before initiating compounding.[14] Raw-materials testing may be complicated with solubilization or pH-related problems, so it is advantageous to use a low molarity HCl or NaOH to accomplish this task, when possible.

Quantitative LAL Methods

Kinetic turbidimetric or chromogenic LAL systems provide results quickly (< 30 minutes) and give excellent data management. Endotoxin-activation of LAL causes a sudden increase in Optical Density (OD) from turbidity, or color if a chromogenic substrate is present. The OD change in each tube is monitored by a computer-assisted spectrophotometer to determine the time required to reach a preset threshold OD. This onset time is plotted logarithmically versus endotoxin concentration to give an inverse relationship where the highest endotoxin level yields the shortest onset time. For this application, a standard curve is made from 50 to 0.5 EU/mL. In contrast to gel-clot, the mid-point concentration of the curve, 5 EU/mL, is used for the positive water and positive product controls. The endotoxin in the PPC must be recovered within ±50% of 5 EU/mL to assure absence of interference. A standard curve may be archived to minimize future analysis time and the number of controls. The ATi 320 (ATi Corp., Baltimore) and Toxinometer (Wako USA, Richmond) are tube-reading instruments, with endotoxin-specific software for kinetic-turbidimetric analysis (KTA), which are ideally suited for testing PET radiopharmaceuticals.

STERILITY TESTING

Sterility is the most important quality of injectables because these drugs bypass most of the body's defense mechanisms. Sterility Testing is described in ***USP 23*** and is well reviewed elsewhere.[1,15] This discussion summarizes key aspects for testing in the hospital or pharmacy setting.

Microbes vary greatly in their requirements for growth including temperature, food/nutrient source, pH and oxygen requirement. No single growth medium satisfies the optimum conditions for all microorganisms. The vast majority of microbial contamination found in pharmaceutical manufacturing operations are mesophiles (thrive at 10-40 °C), heterotrophs (require preformed organic compounds for metabolism) and aerobes (need oxygen for growth). The required media may be

purchased in ready-to-use tubes with a Certificate of CoA which assures that growth promotion and other *USP*-required properties were documented.

For sterility testing, two media are required which support the growth of human pathogens and a broad spectrum of microbes.[1] Fluid Thioglycollate Medium (FTM) is designed to support the growth of both aerobes and anaerobes in a single medium. A FTM tube has a yellow and pink (top) layer. The aerobic phase has an oxygen-sensitive dye that is pink when oxygen is present. The bottom yellow phase has residual oxygen scavenged by antioxidants. When incubated at 30-35 °C, FTM supports the growth of heterotrophic, facultative (grow with or without oxygen) or anaerobic microorganisms. Soybean-Casein Digest Medium (SCD) is devised to support heterotrophic facultative and aerobic organisms at 20-25 °C; its higher pH facilitates the growth of fungi, also. Media conditions are summarized in Table 5.

Successful sterility testing requires a controlled environment, such as a laminar-flow hood with clean-room apparel. The greatest risk of false-positive results arises in the sampling and transfer of the test aliquot from the vial to the media. The best approach is direct inoculation of 0.1 mL or greater aliquots from the test material into commercial media and incubation for 14 days. The media is observed after day 3, 7 and on the 14th day. Bacteria usually grow out quickly in the first few days, as evidenced by turbidity or precipitation at the bottom of the tube, but the 14-day incubation facilitates the detection of slow-growing microbes and germination of spores. The test procedure must delineate all test conditions and observations.

Table 5. Properties of Sterility Test Media

Media	pH	Phases	Temperature
FTM	7.1	2	30 - 35 °C
SCD	7.3	1	20 - 25 °C

SUMMARY

Preparing Parenterals Free of Microbial Contamination

Microbial contamination is difficult to control because of its ubiquity in nature. Removal of contamination from a process is costly and often incomplete. The best approach for making compounds free of microbiological contamination is to avoid its introduction.[14] The preparation of radiopharmaceuticals designed for positron emission tomography (PET) poses interesting challenges with respect to compounding and quality assurance because of their limited radioactive life. Emphasis on prevention of microbiological impurities must be directed toward the synthetic process. The compounding environment, selection of reagents, assembly of equipment and training of operators must unequivocally create a synthesis process that prevents or eliminates microbial contamination. Procurement of sterile, endotoxin-free starting materials and proper sterilization of other in-process materials and equipment are cornerstones to responsible compounding in the hospital or pharmacy setting. The procedures suggested herein for compounding and testing radiotracers are certainly applicable to any other type of prescriptions compounded in a pharmacy environment.

With emphasis on the compounding process, tests for the absence of microbial contamination become critical to documenting the success of preventive measures. The bacterial endotoxins test assesses the potential danger from the most important microbial byproduct, and its incubation period may be shortened, under properly controlled conditions, to provide a result prior to the administration of PET drugs. However, the sterility test provides information retrospectively for radiopharmaceuticals. Wherever possible, the sterility test for non-radioactive drugs should continue its course prior to patient administration. Validation of the compounding and quality control procedures prior to production activities provides the needed assurance that products will have the required quality attributes.

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GLOSSARY

BET	Bacterial Endotoxins Test
CBER	Center for Biologics Evaluation & Research
CDS	Controled Dilution Series
CSE	Controlled Standard Endotoxin
CoA	Certificate of Analysis
DP	Depyrogenated
EDTA	Ethylenediaminetetraacetic Acid
EL	Endotoxin Limit
EU	Endotoxin Units
FDA	Food and Drug Administration
FDG	Flurodeoxyglucose
FTM	Fluid Thioglycollate Medium
GM	Geometric Mean
GNB	Gram-Negative Bacteria
IND	Investigational New Drug
KTA	Kinetic Turbidometric Analysis
LAL	*Limulus* Amebocyte Lysate
LPS	Lipopolysaccharide
LRW	LAL Reagent Water
MVC	Minimum Valid Concentration
MVD	Maximum Valid Dilution
NC	Negative Control
OD	Optical Density
PET	Positron Emission Tomography
PPC	Positive Product Control
PSS	Product Specific Sensitivity
PWC	Psitive Water Control
RSE	Reference Standard Endotoxin
SCD	Soybean-Casein Digest
SOP	Standard Operating Procedure
STV	Single Test Vial
TC	Test Concentration
UND	Undiluted
USP	*U.S. Pharmacopeia*

APPENDIX A

LAL TEST STANDARD PROCEDURE FOR FINAL PRODUCTS/RAW MATERIALS

I. **Purpose.** Assure that the endotoxin limit is not exceeded using a procedure which is compatible with the FDA LAL Test Guideline.

II. **References.** *Guideline on Validation of the LAL Test as an End-Product Endotoxins Test. DHHS, FDA, December, 1987.

*Bacterial Endotoxins Test and Radiopharmaceutical monographs, ***U.S. Pharmacopeia 23***, 1995.

*Instructions for Use from the LAL Reagent Supplier.

TERMS.

A. **BET**. Bacterial Endotoxins Test, *USP 23*.
B. **CDS**. Control Dilution Series, 2 λ through 1/4λ.
C **CoA**. Certificate of Analysis matching the LAL Reagent and CSE.
D. **CSE**. Control Standard Endotoxin referenced in Endotoxin Units (EU).
E. **DP**. Depyrogenated, dry-heat sterilization by a validated cycle, or equivalent.
F. **Lambda, λ**. Labeled Reagent sensitivity in EU/mL.
G. **LRW**. LAL Reagent Water, non-reactive with LAL.
H. **MVD**. Maximum Valid Dilution.
I. **PPC**. Positive Product Control, 2 λ, for detection of inhibitory conditions.
J. **PWC**. Positive Water Control, 2 λ, used in place of CDS, where applicable.
K. **PSS**. Product Specific Sensitivity, Lambda x product dilution.

III. **Reagents, Materials and Equipment**

A. FDA-licensed LAL reagent, buffered;
CSE with CoA for RSE/CSE ratio;
LRW from LAL-reagent vendor or Sterile Water for Irrigation, *USP*

B. 10 x 75 mm flint-glass tubes, depyrogenated (DP) by valid cycle;
DP Borosilicate or Sterile polystyrene tubes for preparation of positive controls;
Sterile, disposable pipettes and pipette tips

C. Vortex mixer for CSE dilutions and controls
Calibrated mechanical pipetters, Eppendorf® Repeator with Combitips®, or equivalent;
Incubating device: heat block or water bath set at 37±1° C
Tube racks, timer and pH meter

IV. Preparation of Test Solutions and Controls

A. Reporting: Initiate BET form, Appendix B.

B. Test Solutions: Describe sampling, dilution and modifications for preparing test solutions from stock solutions, such as 1:4 dilution with LRW. Label all tubes.

C. Positive Water Controls (by FDA LAL Test Guideline): Make a CSE dilution series according to the CoA to give a 20λ tube and serial two-fold dilution of 4λ, 2λ, λ, 1/2λ and 1/4λ which brackets label claim. Test in duplicate a control series, 2λ through 1/4λ with the day's first set of tests; otherwise, test only a 2λ CSE level if there is no change in test parameters.

D. Preparation of the Positive Product Control (PPC): Add 10 μL of a 20λ CSE solution directly to the PPC tubes OR mix equal parts of a double-strength test solution and a 4λ endotoxin spiking solution to yield interference controls containing 2λ CSE.

E. Preparation and Storage of LAL reagent: Rehydrate and store LAL according to supplier's Package Insert. Store rehydrated LAL on a cold surface or at 2-8° C during intermittent use; otherwise, store frozen below -20° C up to 4 weeks after reconstitution. LAL may be frozen/thawed once.

V. Routine LAL Testing of Radiopharmaceuticals or Raw Materials

A. Precautions: Use aseptic technique and use endotoxin-free containers for preparing and testing samples in duplicate.

B. Test Solution: Aseptically withdraw 0.2 mL or greater from the dosage form, and dilute, if necessary, to a validated test concentration with LRW. Dilution may be needed to generate the necessary volume for LAL controls and samples.

C. Negative Control: Test the LAL reagent water (LRW) used for endotoxin and sample dilutions, and LAL hydrations, as the Negative control.

D. Positive Water Control: Test either a 2λ control or a CSE standard series, 2λ through 1/4λ, as in Part IV.

E. Positive Product Control: Test the positive product control prepared as described in Part IV (Fig. 1).

F. 20-min. endotoxins test: Test an additional test-solution tube and 10 EU/mL PPC inhibition control tube.

VI. Incubation Procedure

A. Rehydrate or thaw out frozen LAL just before testing. Test **in duplicate** a set of samples and controls.

B. Inoculate 0.1 mL of test specimen and controls (Part IV) into depyrogenated 10x75 mm flint assay tubes, beginning with the negative control and ending with the greatest endotoxin concentration; test all products in duplicate.

C. In the same order, add 0.1 ml of LAL to each assay tube, mix gently, and promptly incubate the reaction tubes at $37 \pm 1°$ C for 60 minutes, $\pm$ 2 minutes.

D. After incubation remove and invert the tubes; record as positive a gel that remains intact after 180° rotation and negative a non-gel or one that breaks on rotation.

VII. Interpretation of Results

A. VALIDITY: A valid gel-clot test requires a positive (gel) in the positive product controls (2λ spike), a valid positive water control, and no gel in the negative control.

INVALIDITY: If the controls do not perform as expected, void the test and initiate a NEW TEST, and investigate the source of invalidity.

C. A negative result is no gel in the product/sample tubes. Under valid conditions (above), a negative result means that the product is within limits. Record as PASSES.

D. In case of a positive result in the test sample, retest at the Maximum Valid Dilution. The product PASSES if there is no gel at the MVD because endotoxin levels are less than the endotoxin limit. The product FAILS and must be discarded if there is a gel at the MVD and the test conditions are valid.

E. A valid 20-minute endotoxins test requires a negative sample tube whereas the 10 EU/mL PPC has a positive gel.

F. REPORTING: The results should reflect the PSS by reporting in EU/mL or EU/mg. For radiotracers, the PSS = lambda times the product dilution. For other test materials, PSS = lambda divided by the test concentration.

APPENDIX B

Sample Form for Reporting Results: LAL Clot-Testing for Endotoxins by FDA Guidelines

CHARLES RIVER ENDOSAFE a division of Charles River Laboratories, Inc. 1023 Wappoo Road, Suite 43-B, Charleston, SC 29407	Phone: 803-766-7575 Fax: 803-766-7576

LAL GEL-CLOT TESTING FOR ENDOTOXINS BY FDA GUIDELINE

CUSTOMER:_____ **Units Received:**_____ **CT#:**_____

PRODUCT:_____ **Potency:**_____ **Lot#:**_____

Test Dilution: _____ **Endotoxin Limit:** _____ **MVC/MVD** _____

REAGENT INFORMATION:

REAGENT	MFG./LOT#	REHYDRATION DATE	EXPIRY
LAL _0._____ EU/mL	ENDOSAFE/		
CONTROL STD. ENDOTOXIN			
LAL REAGENT WATER		N/A	
Other:			

SAMPLE PREPARATION:_____

TEST DATA and RESULTS: **TEST DATE:**_____

TEMPERATURE: **TIME:**

_____START _____START

_____END _____END

CONTROL	STANDARD	SERIES	SAMPLE DILUTIONS		
2 λ			DILUTION TESTED	NPC	PPC
λ					
½ λ					
¼ λ					
NWC					

NOTE: The error of the gel clot method is ± one two-fold dilution.

COMMENTS:

INTERPRETATION:

TEST DATA AND RESULTS INDICATE _____ EU/_____.

(CIRCLE ONE) PASS FAIL

ANALYST/DATE:_____ **REVIEWED BY/DATE:**_____

REFERENCE SOP:_____

QUESTIONS

1. The first group of drugs given endotoxin limits by LAL testing in the *USP* was:

   a. antibiotics
   b. intrathecally-administered drugs
   c. large-volume parenterals
   d. radiopharmaceuticals

2. The Greek symbol representing the labeled sensitivity of a LAL reagent is:

   a. alpha
   b. delta
   c. lambda
   d. rho

3. The most common type of LAL method found in the hospital setting is the:

   a. endpoint chromogenic
   b. gel clot
   c. kinetic chromogenic
   d. kinetic turbidimetric

4. The most sensitive LAL reagent has the following descriptive information:

   a. 0.125 EU/mL label
   b. 0.03 EU/mL label
   c. multi-test fill
   d. STV container

5. Direct host-response reactions to endotoxin include which of the following:

   a. fever
   b. hypotension
   c. vascular shock
   d. all of the above

6. Endotoxin is most commonly found in:

   a. fungal cell wall
   b. gram-negative rods
   c. gram-positive cocci
   d. all of the above

7. The best way to depyrogenate glassware is by:

   a. dry-heat oven cycle at 150° for 3 hours
   b. dry heat sterilization at 200° for 3 hours
   c. soak in acid for 1 hour
   d. steam sterilization cycle for 1 hour

8. The most relevant LAL-Test procedure is found in the:

   a. Code of Federal Regulations
   b. European Pharmacopeia
   c. FDA LAL-Test Guideline
   d. US Pharmacopeia

9. Required LAL-test components include:

   a. negative controls
   b. positive controls
   c. temperature requirement
   d. all of the above

10. It is standard industry practice to dilute radiopharmaceuticals prior to a BET to:

    a. avoid interference
    b. minimize hazardous waste
    c. reduce sensitivity
    d all of the above

11. The 20-minute LAL test is ideally suited for testing:

    a. Fludeoxyglucose F-18 Injection
    b. Iodinated I-131 Albumin Injection
    c. Gallium Ga-67 Citrate Injection
    d. Technetium Tc-99m Medronate Injection

12. The unique feature in the 20-minute LAL test is the:

    a. high endotoxin limit
    b. high concentration positive product control
    c. high dilution of the test material
    d. high temperature incubation

13. Which of the following conditions are required in order to meet the criteria for a valid BET and thus release a radiotracer for clinical use?

    a. product samples produce no gel
    b. the PPC samples are positive
    c. the negative control is negative
    d. all of the above

14. Optimum reaction conditions for LAL testing include which of the following?

    a. neutral pH conditions
    b. isotonic levels of sodium
    c. presence of divalent cations
    d. all of the above

15. The endotoxin limit for Sterile Water for Injection, USP, is:

a. 0.03 EU/mL
b. 0.25 EU/mL
c. 0.5 EU/mL
d. 5.0 EU/mL

16. Which of the following cations are necessary for optimum LAL activity:

a. calcium ion
b. magnesium ion
c. sodium ion
d. all of the above

17. An eight-fold dilution of a parenteral is negative when tested with 0.03 EU/mL reagent. The results are reported as less than:

a. 0.03 EU/mL
b. 0.24 EU/mL
c. 0.5 EU/mL
d. 8.0 EU/mL

18. The permissible dilution for a LAL-inhibitory drug is equal to the:

a. endotoxin limit
b. MVC
c. MVD
d. PSS

19. A radiopharmaceutical fails the BET if the:

a. concentration is greater than 0.03 EU/mL
b. dose exceeds 175 EU
c. positive product control gives a gel
d. volume exceeds 20 EU/mL

20. An investigational drug gave a positive at a 10-fold dilution and a negative result at a 20-fold dilution when tested with a 0.06 EU/mL LAL reagent. The concentration of endotoxin present in the parenteral is approximately:

a. 0.03 EU/mL
b. 0.6 EU/mL
c. 1.0 EU/mL
d. 20 EU/mL

21. Factors needed for calculating the Product Specific Sensitivity are:

a. lambda and test concentration
b. MVC and endotoxin limit
c. MVC and MVD
d. MVD and test concentration

22. Validation of a BET means that a product-specific test-method was proven to be:

a. conducted without interference
b. tested without dilution
c. tested above the endotoxin limit
d. all of the above

23. The official sterility test medium most likely to reveal fungal contamination is:

a. Fluid Sabouraud Medium
b. Fluid Thioglycollate Medium
c. Soybean Casein Digest Medium
d. Sporulating Agar Medium

24. The sterility test medium designed to detect both aerobic and anaerobic organisms is:

a. Blood Agar Medium
b. Fluid Thioglycollate Medium
c. Soybean-Casein Digest Medium
d. Sporulating Agar Medium

25. For sterility testing, samples are incubated in media for which of the following time periods?

a. 1 day
b. 3 days
c. 7 days
d. 14 days