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Cancer Imaging Program

National Cancer Institute

Standard Operating Procedure

GEN-Q114A

Alternative Procedure for Bacterial Endotoxins by Limulus Amebocyte Lysate (LAL) Test Using the PTS (Portable Test System)

1. **PURPOSE:** To describe the alternate quality control assay, the PTS (Portable Test System) used for measuring bacterial endotoxin levels in the final product solution. This assay quantifies bacterial endotoxins in sample solutions by titration to an endpoint.

2. **SCOPE:** The PTS Limulus Amebocyte Lysate (LAL) Test will be performed at the sites manufacturing radiopharmaceuticals. This procedure describes how to test PET radiopharmaceuticals for endotoxin using the Endosafe®-PTS Portable Test System, which is based on a kinetic chromogenic BET, as described in the USP. This quality control procedure is one of the tests performed on the final batch of product. The results of this test must be obtained prior to releasing the batch for human administration.

3. **RESPONSIBILITIES:** To follow this procedure as written and to ensure that all results, control information, and raw data are documented as specified in dedicated Quality Control Laboratory Notebooks and recorded on the appropriate testing forms. Preliminary testing may be required prior to conducting the final bacterial endotoxin assay to qualify a new lot of PTS cartridges prior to running the product assay.

4. **REFERENCES**

4.1 USP 28 ‹821› Radioactivity
4.2 USP 28, ‹85› Bacterial Endotoxins Test
4.3 USP 28, ‹823› Radiopharmaceuticals for PET Compounding
4.4 Charles River Laboratory, User's Guide, Endosafe®-PTS Portable Test System
4.5 **Gen-Q015**, "Investigating Out of Specification QC Test Results"
4.6 FDA Draft Guidance PET Drug Products—Current Good Manufacturing Practice (CGMP)—September 2005 (CDER)

Printed Name	Signature	Date
Author:		
Regulatory Approval:		

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4.7 Guideline on Validation of the Limulus Amebocyte Lysate Test as an End-Product Endotoxin Test for Human and Animal Parental Drugs, Biological Products, and Medical Devices, Dec. 1987; CDER, CBER, CDRH, & CVM.

5. FORMS

- **Form FQC-###**, Corresponding "Final Product QC Test Results" Form
- **Form IOS-001**, "Investigating Out of Specification (OOS) QC Test Results Form"
- **Form Q114A-L**, "GEL-CLOT ASSAY: Bacterial Endotoxins LAL Test for PTS Form" (or dedicated PTS results laboratory notebook).

6. DEFINITIONS

6.1 Pyrogen: A fever producing substance.

6.2 Endotoxin: Toxins that can be extracted from the cell structure of a wide variety of gram-negative bacteria.

6.3 Limulus Amoebocyte Lysate: The cell-free reagent derived from the blood cells, amoebocytes, of the horseshoe crab, (LAL), that reacts to form a clot when exposed to endotoxins.

6.4 PTS cartridge: The portable test system cartridge from Charles River that is used to run the LAL assay.

6.5 Control Standard Endotoxin (**CSE**): A control standard endotoxin (CSE) is an endotoxin preparation other than the RSE that has been standardized against the RSE.

6.6 Reference Standard Endotoxin (**RSE**): This is the Reference Standard Endotoxin obtained from the USP (RSE), and it is the standard against which all LAL reagents are validated and qualified. It has a defined potency of 10, 000 USP Endotoxin Units (EU) per vial.

6.7 Lambda, λ = the lowest point of the endotoxin standard curve.

6.8 PPC is the positive product control for BET interference detection.

6.9 Spike is a known amount of endotoxin that is contained in the PPC.

6.10 MVD is the maximum valid dilution.

7. GUIDELINES/POLICIES

7.1 There are four basic LAL test methods commercially available and currently approved by the FDA for end-product release testing: (1) the gel-clot; (2) the turbidimetric (spectrophotometric); (3) the colorimetric (Lowry protein); and (4) the chromogenic assay. The LAL reagents used in these methods must be obtained from an FDA-licensed manufacturer and must be designed specifically for the method chosen. The method described in this SOP is for the PTS chromogenic method.

7.2 Some products interfere with the LAL endotoxin reaction. These factors may be chemical or physical. Chemical inhibitors cause chelation (*i.e.* EDTA), protein denaturation (*i.e.* fluorescein), or pH disruption (*i.e.* pH outside range of 6.0-7.5). Physical inhibitors include adsorption of endotoxin, or product

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viscosity. However, most of the inhibition can be overcome by dilution of the product. It is important to determine the non-interfering concentration for routine bacterial endotoxin testing of final product initially and also anytime when changing the formulation or manufacturing processes.

7.3 Each new lot of cartridges, or technician running the test, must be qualified upon receipt; one cartridge is required with LRW as a sample, as analyzed in steps below. Answer the prompt for product ID by entering date followed by "qual", then enter the lot No. for LAL reagent water. The evaluation must demonstrate no detectable endotoxin and acceptable spike recovery (50-200%) with a CB of <25% for both.

7.4 When the manufacturing process, the product formulation, or the source of a particular ingredient of the drug formulation is changed samples are required to be tested for inhibition and enhancement by spiking the sample with endotoxin and running the PTS analysis of this spiked sample in duplicate.

7.5 The administered dose of the final product solution should not contain more than 175 EU/V of the injection of the maximum administered total dose. The endotoxin limit for parenteral drugs is specified in units such as EU/mL, EU/mg, or EU/Unit of biological activity.

7.6 If an endotoxin test is repeated, due to a high sample endotoxin value, the repeat should consist of twice the initial number of replicates of the sample in question to examine the possibility that extrinsic contamination occurred in the initial assay procedure.

7.7 The Pass/Fail Cutoff equals 175 EU in the final injected dose, ie. 175 EU times the fractional dilution of the product dilution (1/40 or .025) used for the test and divided by the mL volume.

7.8 Use appropriate cautions when handling radioactive material.

7.9 **Storage Conditions and Precautions:** PTS cartridges should be stored at in the refrigerator at >2°C. Keep cartridges within the sealed pouch until ready for use.

7.10 Each new lot of cartridges and analyst must be qualified upon before use for radiopharmaceuticals.

8. MATERIALS AND EQUIPMENT

8.1 Endosafe®-PTS Reader

8.2 Endosafe®-PTS Cartridge(s), catalog #PTS2005F, Sensitivity 5 to 0.05 EU/mL with Certificate of Analysis (COA).

8.3 LAL Reagent Grade Water (LRW) or sterile water for injection (SWFI), USP, for sample dilution.

8.4 Disposable, sterile, disposable polystyrene tubes for sample collection or dilution, as needed.

8.5 Mechanical pipettor with sterile pipette tips for sample preparation (dilution) and loading a sample into the PTS.

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9. **PROCEDURES**

9.1 Allow a cartridge to reach room (ambient) temperature in the pouch before opening and initiating a test.

9.2 Initial Qualification of a new lot of cartridges or new analyst. Each new lot of cartridges must be qualified upon before use for radiopharmaceuticals. The qualification requires testing one cartridge with LRW or SWFI as a sample. The analysis procedure is presented in sections 9.3 and 9.5 to 9.8. Answer the prompt for product ID by entering date followed by "qual", then enter the lot No. for the water. The evaluation must demonstrate no detectable endotoxin and acceptable spike recovery (50-200%) with less than 25% CVs. Conduct the same procedure to qualify a new analyst (technician).

9.3 Instrument (Endosafe®-PTS Reader) Setup and Operation

9.3.1 Press the menu key on the PTS keypad to turn instrument on (Menu 5 turns it off). The reader then initiates a "SYSTEM SELF TEST" as it warms to 37 °C. Warming takes about 5 minutes.

9.3.2 The reader will display "SELF TEST OK" and then "INSERT CARTRIDGE."

9.4 Sample Preparation

9.4.1 Use aseptic technique and LRW or SWFI or Sterile water for irrigation, USP for all dilutions and use endotoxin-free containers for preparing and testing samples with PTS.

9.4.2 Before adding radioactivity, label the tube for dilution with the product batch number.

9.4.3 Make a sample dilution in accordance with validation information. All products must be diluted to 1:39 (1/40) prior to assay. Use pipettors with sterile tips and sterile pyrogen-free polystyrene tubes.

9.5 Sample Analysis

9.5.1 When the reader flashes "Insert Cartridge," remove a cartridge from its pouch and insert it into the PTS slot with the sample reservoirs facing up and to the front of the PTS reader. DO NOT TOUCH sample reservoirs or optical cells. Press the cartridge firmly into the slot.

9.5.2 Answer the prompts correctly for "Enter OID" and "Enter Cartridge Lot #." If using a new lot number for cartridges, also respond to the "Enter Calibration Code" prompt by entering the calibration code found on the Certificate of Analysis that came in the box of cartridges.

9.5.3 Under "Sample Lot," enter the radiopharmaceutical batch number (example: MBRFLTXXXX).

9.5.4 Under "Sample ID" enter any additional information you wish to enter or leave blank.

9.5.5 Under Dilution Factor accept default setting of 1, (the dilution will be accounted for after the test).

9.5.6 When the reader displays: "ADD SAMPLE PRESS ENTER." Use sterile, pipet tips to pipet exactly 25 µL each of the 1/40 diluted test sample into all 4 sample reservoirs of the inserted cartridge and press Enter on the reader keypad. Aseptic technique is critical in preventing

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contamination and erroneous results. Do not touch the reservoirs with the pipet tips.

9.5.7 The PTS test can take up to 15 minutes and the PTS reader will beep when the analysis is complete.

9.6 Reviewing and Recording Results

9.6.1 Review the results for endotoxin measurement and assay acceptance criteria that are displayed on the screen. Note that the reader display alternately flashes the following results: Sample EU/mL
Sample %CV
% Spike Recovery
Spike %CV
Remove Cartridge

9.6.2 Record above data in PTS logbook or on **Form Q114A-L**.

9.7 Acceptance Criteria

9.7.1 Validity. The test is valid if the CV (Coefficient of Variation) for the sample and spike are <25% and Spike Recovery is between 50 and 200%. If the test is not valid repeat the test.

9.7.2 Invalid Spike Recovery. If the invalid Recovery occurred because it was <50% and Spike %CV was valid (<25%), then product inhibition may exist. Repeat the test. If the repeated test has the same problem, repeat the test with a new dilution and / or new lot of cartridges. The cartridges may be losing potency. If the invalid spike recovery problem persists, contact the NCI contract person for advice.

9.7.3 Pass/Fail. The test passes if Sample Value is less than the 175 EU/final dose when corrected for the test sample dilution factor, 1/40 and the final product volume in mL. For example, if the final product dose was 10 mL then the test passes if it is less than 0.437 EU/mL, i.e. 175 EU/mL times 1/40 divided by 10 mL. (175 EU is the maximum endotoxin allowed per injection).

9.7.4 If the test is valid but fails, repeat the test in duplicate to make certain that the product was not contaminated. Run the repeat tests on a new dilution of the product. Record the results, pass or fail, on the QC results form for the radiopharmaceutical batch.

9.8 Data Storage and File Maintenance

The PTS unit will hold only 100 test files. Download and clear the files before 100 tests are run.

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