STANDARD OPERATING PROCEDURE

Title: Material and Technician Qualification on the LAL Gel-Clot Method

Effective Date: _____

Approvals (Signature and Date):

_____	_____	_____
Responsible Department Head	Technical Authority	QA/QC Manager

1.0 PURPOSE

1.1 To describe the procedure to qualify technicians for testing in, and materials for testing by the LAL Gel-Clot method for endotoxin determination.

2.0 SCOPE

2.1 This procedure applies to all persons performing endotoxin testing by LAL Gel-Clot, and all materials, reagents and products that require endotoxin determination by the LAL Gel-Clot method.

3.0 RESPONSIBILITY

3.1 It is the responsibility of QC Microbiology to qualify materials and products for testing by the LAL Gel-Clot method. It is the responsibility of the department supervisor to ensure that all persons performing endotoxin determination have been adequately trained in the Gel-Clot method and proper aseptic technique.

4.0 REFERENCES AND APPLICABLE DOCUMENTS

4.1 USP XXII Eighth Supplement, USP-NF <85> Bacterial Endotoxins Test.
4.2 LAL Update, Associates of Cape Cod, Inc.
4.3 Guideline for Validation of the *Limulus* Amebocyte Lysate Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices. FDA, August 15, 1987.
4.4 09-0032-SOP-1.0, Endotoxin Determination using the LAL Gel-Clot Method.

5.0 MATERIALS AND EQUIPMENT

5.1 50 - 200 µL pipet
5.2 200 - 1000 µL pipet
5.3 Sterile, pyrogen-free disposable pipet tips
5.4 37 ± 1° C water bath or dri-bath type heat block incubator
5.5 10 x 75 mm depyrogenated, borosillicate glass test tubes
5.6 Vortex mixer
5.7 Timer
5.8 *Limulus* Amebocyte Lysate in 50 test vials from an FDA OBRR approved manufacturer (such as Pyrotell® from Associates of Cape Cod)
5.9 LAL Reagent Water
5.10 Control Standard Endotoxin (CSE) from an FDA OBRR approved manufacturer
5.11 Test Tube Rack

5.12 Parafilm® M

6.0 HEALTH AND SAFETY CONSIDERATIONS

6.1 Endotoxin is pyrogenic. Use proper aseptic technique when handling Control Standard Endotoxin to avoid contaminating it or the environment.

7.0 DOCUMENTATION REQUIREMENTS

7.1 Record all information and test results in the appropriate spaces on the Technician Qualification form for technician qualification, and the Inhibition/Enhancement form for material inhibition/enhancement testing.

7.2 Store completed and approved Technician Qualification forms in technicians training file. Store completed and approved Inhibition/Enhancement Test form in the Material Qualification notebook in the QC Micro lab.

8.0 PROCEDURE

8.1 Analyst Qualification

8.1.1 Each person performing endotoxin determination by the LAL Gel-Clot method must be qualified by running a test for conformation of the labeled lysate sensitivity using a single lot of Control Standard Endotoxin and Lysate.

8.1.2 Each analyst must perform the test according to 09-0032-SOP-1.0 in quadruplicate on a series of serial two-fold dilutions of the CSE to give concentrations of 4λ, 2λ, 1λ, 0.5λ and 0.25λ, where λ is the labeled sensitivity of the lysate. The geometric mean endpoint concentration must be greater than or equal to 0.5λ and less than or equal to 2λ. Analysts must repeat the procedure until endotoxin recovery is between these two points. Record results on the Technician Qualification form.

8.2 Inhibition/Enhancement Test

8.2.1 The LAL test is limited by the capacity of the specimen to inhibit or enhance the gel-clot reaction. Every substance tested for endotoxin determination has the potential to either inhibit or enhance the clotting reaction, resulting in either artificially high or low endotoxin results. Initially, each type of material that will be tested, must be screened for inhibition/enhancement. Three separate lots must be tested for qualification to be complete.

8.2.2 To test for inhibition/enhancement prepare a set of serial two-fold dilutions of the CSE in LRW to give concentrations of 4λ, 2λ, 1λ, 0.5λ and 0.25λ, or 0.5, 0.25, 0.125, 0.06 and 0.03 EUs/mL for a 0.125 EUs/mL Lysate sensitivity as the control. Prepare an identical set of dilutions of the CSE but use the test sample as the diluent instead of LRW. The test sample is being spiked with endotoxin to compare it with the control standard results.

8.2.3 Run the controls in duplicate and the test sample in quadruplicate according to 09-0032-SOP-1.0. Compare the results of the inhibition/enhancement tubes to the control standards. Products are said to be free of inhibition/enhancement if the geometric mean endpoint of endotoxin in product is within 1/2 to 2 times the geometric mean endpoint of endotoxin in LRW. Record results on the Inhibition/Enhancement Test data sheet.