STANDARD OPERATING PROCEDURE

Title: Endotoxin Determination Using LAL Gel Clot Method

Effective Date: _____

Approvals (Signature and Date):

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

1.0 PURPOSE

1.1 To specify the procedure for detecting endotoxin levels in aqueous solutions using *Limulus* Amebocyte Lysate (LAL) gel clot method.

2.0 SCOPE

2.1 This procedure is to be used for determination of endotoxin levels in raw materials, subassemblies and final assemblies.

3.0 RESPONSIBILITY

3.1 It is the responsibility of the QC Microbiology department to perform endotoxin testing. It is the responsibility of department supervisor to ensure that all persons performing endotoxin testing by the LAL gel clot method have been properly trained in aseptic technique and the use of gel clot lysate.

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 09-0093-SOP-1.0, Material and Technician Qualification on 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 Pyrogen Free 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 required information and test results on the Endotoxin Test Data Sheet. Store all completed and approved Data Sheets in the Endotoxin Notebook in the QC Micro Lab. Completed notebooks should be submitted to Document Control for archiving.

8.0 PROCEDURE

8.1 Proper aseptic technique is essential when collecting and handling test samples. The LAL test is extremely time, temperature and vibration sensitive. Careful attention must be paid to the limits of this test.

8.2 Sampling Handling

8.2.1 The number of units sampled per lot is specified in each manufacturing batch record and PMS. Samples received for testing should be at least 1 mL for liquids and a whole assembly for non-liquids.

8.2.2 All samples must be stored at 2-8°C until tested, and must be tested within 24 hours of receipt.

8.3 Endotoxin Controls

8.3.1 Control Standard Endotoxin (CSE) is purified lippopolysaccharide prepared from the E. coli strain O:55 B5. It is used to confirm the sensitivity of the lysate, and to check for inhibition or enhancement in test samples. Each vial contains a measured weight of lyophilized endotoxin. All CSE potency is validated by its manufacturer against the USP Reference Standard Endotoxin (RSE), so that CSE can be considered the equivalent of the USP RSE. Each vial of Control Standard Endotoxin (CSE) contains 500 ηg of endotoxin. Reconstitute by adding 5.0 mL of pyrogen free water (LAL Reagent Water or LRW) to the vial, and vortexing for at least five minutes. Vortex for at least one minute before each use.

8.3.2 Each assay must include a set of serial two-fold dilution's of the Control Standard Endotoxin that brackets the labeled lysate sensitivity from 2λ to 0.25λ. Each vial of Pyrotell® *Limulus* Amebocyte Lysate is labeled with the lysate sensitivity and is also expressed in EUs. For