QUALIFICATION AND VALIDATION DOCUMENT PROTOCOL

Title: Method Validation of Endotoxin Recovery in Water Using the ThermoMax Microplate Reader

Issue Date: **System No.:**

APPROVAL SIGNATURES

Written by:	**QA Microbiologist**	**Date**
Reviewed and Approved by:	**QA/QC Manager**	**Date**
	QC Supervisor	**Date**
	Manufacturing Manager	**Date**
	VP of Development and Operations	**Date**

1.0 PURPOSE

1.1. The objective of this validation protocol is to define the requirements for completing the Method Validation (MV) of endotoxin recovery in water using the ThermoMax Microplate Reader and SoftMax Operating Software from Molecular Devices Corporation (system number 02-519) in Building 2. The specific objectives of the MV are to:

1.1.1 To verify the labeled sensitivity of the kinetic turbidimetric *Limulus* amebocyte lysate, and to verify that the pyrogen-free LAL Reagent Water (LRW) has no detectable level of endotoxin by comparison against a standard curve of certified Control Standard Endotoxin (CSE). This will determine the limit of detection and limit of quantitation of this assay by determining the lowest concentration of endotoxin detectable; the linearity by generating a standard curve; and the accuracy of the reagents by comparing the results to the CSE. This test will also fulfill the FDA requirement for initial qualification of the reagents and the analyst.

1.1.2 Determine the degree, if any, of inhibition or enhancement of the gel-clot reaction caused by samples from the Building 2 water system; and, if present, the dilution at which inhibition/enhancement is overcome. This will determine the selectivity of the assay and fulfill the FDA requirement for inhibition/enhancement validation.

1.1.3 To validate the procedure that will be used for routine endotoxin determination on the water systems in Building 2. This will determine the ruggedness and precision of the assay.

2.0 SYSTEM DESCRIPTION AND HISTORY

2.1 The system, #02-234, consists of a ThermoMax Microplate Reader from Molecular Devices Corporation, an IBM compatible computer and SoftMax™ controlling and analysis software from Molecular Devices Corporation.

2.2 The ThermoMax Microplate Reader system, located in room 100, Building 1, will be used to perform quantitative endotoxin analysis on incoming city water, DI water, water for production and clean steam condensate. In addition, the system will be used to perform quantitative endotoxin analysis on incoming raw materials, in-process production samples and final product samples; however the validation for these methods will be covered separately.

2.3 An Installation and Operational Qualification was performed on the system and is covered in 09-0004-IOP-1.0.

3.0 RESPONSIBILITY

3.1. Execution of the validation procedures in section 9.0 shall be performed by the Quality Assurance Microbiologist. The MV Validation Report shall be written by the Quality Assurance Microbiologist.

4.0 REFERENCES AND APPLICABLE DOCUMENTS

4.1 USP XXII Eighth Supplement, USP-NF <85> Bacterial Endotoxins Test

4.2 *Interim Guidance for Human and Veterinary Drug Products and Biologicals, Kinetic LAL Techniques*. Dept. of Health and Human Services, FDA, Rockville, MD. July 15, 1991.

4.3 21 CFR 210.13.b

4.4 *ThermoMax Users Manual.* Molecular Devices Corporation, Menlo Park, CA