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Summary

USP BET <85> and EP BET <2.6.14> provide guidelines and requirements for the detection or quantification of endotoxins from Gram-negative bacteria. This tech sheet provides information on how Endosafe® cartridge technology meets, and in some cases exceeds, these harmonized guidelines.

MICROBIAL SOLUTIONS

Endosafe® nexgen-PTS™ and Regulatory Requirements: USP BET<85> or EP BET<2.6.14>

The LAL (Limulus amoebocyte lysate) test is used to detect and/or quantify endotoxins from gram-negative bacterial origin. Three techniques are commercially available for this test: gel-clot test (available as a limit or semi-quantitative method), kinetic turbidimetric test, and chromogenic test (available as an end-point or kinetic method).

Charles River's licensed endotoxin detection system, the Endosafe® nexgen-PTS™, is a rapid point of use test system which, together with the Endosafe® nexgen-MCS™, provides quantitative kinetic turbidimetric test results in less than 15 minutes. The technology utilizes licensed LAL reagents in a disposable cartridge using the nexgen-PTS™ and nexgen-MCS™ for a completely contained, real-time endotoxin assay.

The flexibility of the nexgen-PTS™ allows it to be used in conventional quality control settings or as a point-of-use test to support the drug development process. Our cartridges are licensed by the FDA and accepted by USP/EP, allowing users to test raw materials, in process samples as well as final products.

BET Requirements for an USP/EP LAL Test:

- Methods
- Reagents
- Assurance of criteria for standard curve
- Test for interfering factors

In February 2008, the EDQM confirmed that no BET changes are required for the nexgen-PTS™.

Traditional LAL Methods Considered by USP/EP:

- Gel-clot limits test
- Gel-clot quantitative test
- Turbidimetric kinetic
- Chromogenic kinetic
- Chromogenic end-point
- Turbidimetric end-point

The Endosafe® nexgen-PTS™ uses cartridges containing FDA-licensed chromogenic kinetic reagents.

How the Endosafe® nexgen-PTS™ meets USP/EP requirements:

	USP/EP Requirements	Endosafe® nexgen-PTS™
Standard Curve	Minimum requirement: 3 concentrations, 3 replicates.	The nexgen-PTS™ has 3 concentrations and 10 replicates.
R of Standard Curve	|R value| ≥ 0.980	The nexgen-PTS™ system's R value is >0.990.
Water Negative Controls	Minimum requirement of 2 replicates: "The purpose of the negative control is to verify the absence of a detectable concentration of endotoxin in water for BET."	This can be achieved with the nexgen-PTS™ by running a cartridge before or after testing samples with the LRW used for any dilutions.
Test for Interfering Factors	Unspiked samples and spiked samples in duplicates.	The nexgen-PTS™ is set up to run in duplicate unspiked and spiked samples.
Routine Test	Standard curve with 3 concentrations in duplicate. It also requires |R value| ≥ 0.980 and spike recovery 50 to 200%.	The nexgen-PTS™ meets or exceeds these requirements.

How the Endosafe® nexgen-PTS™ meets USP/EP requirements:

Reference Paragraph	USP/EP Requirements	Endosafe® nexgen-PTS™
Bacterial Endotoxins Test	• There are two types of techniques...the photometric techniques...the latter include... a chromogenic method, which is based on the development of color after cleavage of a synthetic peptide-chromgen complex. • These tests require the establishment of a standard regression curve; the endotoxin content of the test material is determined by interpolation from the curve. • In the colormetric kinetic assay the absorbance is measured throughout the reaction period and rate values are determined from those readings.	• nexgen-PTS™ cartridges use a synthetic peptide-chromogen substrate. • The archived standard curve utilizes linear regression, and endotoxin values for samples containing endotoxin are interpolated from this curve. • The software measures change in optical density over time.
Preparation of the Standard Endotoxin Stock Solution and Standard Solutions	• The standard endotoxin stock solution is prepared from an endotoxin reference standard that has been calibrated against the WHO International Standard...Follow the specifications in the package leaflet and on the label for preparation...	• The RSE is prepared accordingly and used to determine the onset times for the archived standard curve data points.
Photometric Techniques	• The chromogenic method measures the chromophore released from a suitable chromogenic peptide by the reaction of endotoxins with the LAL Reagent...the test principle employed is classified as kinetic-chromogenic. • The kinetic-chromogenic technique is a method to measure either the onset time needed to reach a predetermined absorbance of the reaction mixture or the rate of color development. • All photometric tests are carried out at the incubation temperature recommended by the... manufacturer...usually 37°C.	• nexgen-PTS™ cartridges are kinetic-chromogenic reagents. • The nexgen-PTS™ and nexgen-MCS™ spectrophotometers measure the time to reach a predetermined change in absorbance and testing is done at 37+1°C.

Reference Paragraph	USP/EP Requirements	Endosafe® nexgen-PTS™
Preparatory Testing for the Photometric Techniques	• To assure the precision or validity of the... chromogenic techniques, preparatory tests are conducted to verify that the criteria for the standard curve are valid and that the sample solutions does not inhibit or enhance the reactions.	• Verification of the standard curve is done prior to batch release. • Sample validation/interference testing is performed by the end user.
Preparatory Testing for the Photometric Techniques – Verifiation of Criteria for the Standard Curve	• Using the Standard Endotoxin Solution, prepare at least three endotoxin concentrations to generate the standard curve. Perform the test using at least three replicates of each standard endotoxin concentration...The absolute value of the correlation coefficient, $\lvert r \rvert$, must be greater than or equal to 0.980.	• To determine the archived standard curve, 10 replicates are used for each of the 3 RSE concentrations. • Absolute value for the correlation coefficient must be $\geq$ 0.990 in order meet release criteria.
Preparatory Testing for Photometric Techniques – Interfering Factors Test for Photometric Techniques	• Select an endotoxin concentration at or near the middle of the endotoxin standard curve." This concentration is the concentration at which PPC is prepared in sample solution according to the table listing. The table also denotes that samples, spiked samples, standards, and negative water controls are run in duplicate at a minimum. • Also used to demonstrate initial qualification: Reagent Qualification, Technician Qualification, Laboratory Qualification. • In order to be considered free of interfering factors under the conditions of the test, the measured concentration of endotoxin added to the sample solution must be within 50% to 200% of the known added endotoxin concentration after subtraction of any endotoxin detected in the solution without added endotoxin.	• Pre-made spiked channels target the mid-point of the archived standard curve. • The sample channels and spiked channels are in duplicate as required. • The archived standard curve is generated from 10 replicates each from 3 concentrations. • The negative water controls are tested against 30 replicates during potency testing when developing the calibration code. • Our product insert, which is an FDA-approved document, states the following: Initial Qualification: Each new lot of cartridges must be qualified upon receipt. The initial qualification testing requires one cartridge with LAL reagent water as a sample. The evaluation must demonstrate no detectable endotoxin and acceptable spike recovery (50-200%). • The percentage of spike recovery calculation for nexgen-PTS™ spectrophotometers, as well as nexgen-MCS™, subtracts any endotoxin in the unspiked sample solution before calculating the spike recovery.

Reference Paragraph	USP/EP Requirements	Endosafe® nexgen-PTS™
Photometric Techniques – Calculation for the Photometric Techniques	• Calculate the endotoxin concentration of each of the replicates of test solution A using the standard curve generated by positive control series C ... (1) the results of the control series C comply with the requirements for validation defined under Verification of Criteria for the Standard Curve under Preparatory testing. (2) the endotoxin recovery calculated from concentration found in Solution B after subtracting the endotoxin Concentration found in solution A is within 50-200%; and (3) the result of the negative control series D does not exceed the limit of the blank value required in the description of the LAL reagent used. - Solution A: Duplicate Sample - Solution B: Duplicate Sample Spiked at Midpoint - Solution C: Standard Curve (duplicate replicates) - Solution D: Negative Water Control (duplicated)	• The positive control series C is the archived standard curve. • The USP/EP does not state that the use of an archived standard curve is not allowed or that the standard curve has to be prepared in the customer's laboratory. It simply states that the curve must be run with a certain number of replicates and must meet linearity requirements. In fact, the archived curve meets stricter linearity requirements, as the release criteria for a standard curve is the correlation coefficient and the absolute value for the archived curve must be $\geq$ 0.990. • The calculation for positive product controls percentage recovery subtracts endotoxin in the sample for the calculation. For a valid recovery our package inserts state you must recover 50-200%. • The negative water controls are performed during the potency testing of each batch using the water to prepare the standard curve. This meets the criteria of no reaction prior to the completion of the test (onset time for NWC > onset time for lambda). • The use of LRW to reconstitute CSE or LAL is not necessary for the nexgen-PTS™ and nexgen-MCS™; however, some customers choose to run an additional negative water control sample each day of testing using the LAL Reagent Water used to prepare sample dilutions.
BET International Harmonization	• In June 2007, Charles River submitted a request to the Pharmacopoeia Commission for inclusion of pre-calibrated single-use LAL cartridges used, exclusively, in conjunction with an archived standard curve to be added to BET text.	• EDQM confirmed that no elaboration in BET text was considered necessary for a "demonstrably suitable technique." • At the Nov. 2011, PMF Bacterial Endotoxin Summit, the FDA confirmed nexgen-PTS™ compliance with BET.