LIMULUS AMEBOCYTE LYSATE
ENDOSAFE® - PTS CARTRIDGES MULTI-PACK
US License No. 1197

SINGLE-TEST, DISPOSABLE CARTRIDGES FOR ENDOTOXIN DETECTION

FOR USE WITH ENDOSAFE® CARTRIDGE READER

INTENDED USE

Disposable test cartridges contain Limulus Amebocyte Lysate (LAL), endotoxin, and synthetic color-producing substrate. They are intended for use with Endosafe® cartridge reader to perform quantitative detection of endotoxins by kinetic chromogenic methods.

WARNING

This product is intended as an in vitro end-product endotoxin test for human and animal parenteral drugs, biological products, and medical devices. This product is not intended for the detection of endotoxin in clinical samples or as an aid in the diagnosis of human disease.

BACKGROUND AND SUMMARY

Frederick Bang observed that bacteria causes intravascular coagulation in the American Horseshoe Crab, *Limulus polyphemus*.[1] In collaboration, Levin and Bang found that the agent responsible for the clotting phenomena resides in the crab's amebocytes, or circulating blood cells, and that pyrogens (bacterial endotoxin) triggered the enzymes involved in the clotting cascade.[5]

The LAL test is the most sensitive and specific means available to detect and measure bacterial endotoxin, a fever-producing byproduct of gram-negative bacteria, commonly known as pyrogen. The basis of the test is that the endotoxin produces changes in the appearance of LAL that are easily measured.[1-4,8] The simplicity and overall economy of the LAL Test encourages the testing of in-process solutions and raw materials as well as end-product drugs, devices, and biologics.[8] The USP Bacterial Endotoxins Test <85> provides standard methods for validating the LAL Test as a replacement for the rabbit pyrogen test.[7]

BIOLOGICAL PRINCIPLES

In this assay, bacterial endotoxins initiate the activation of a cascading series of serine proteases in LAL. The last activated enzyme in this series, the pro-clotting enzyme, cleaves a peptide from an endogenous substrate called coagulogen. The modified substrate produces an opacity and gelatin in LAL that is easily detected. A synthetic analog to coagulogen can also be used to quantitatively measure the endotoxin mediated activation of the LAL pro-clotting enzyme. This synthetic substrate undergoes cleavage, resulting in the release of the chromophore, p-nitroaniline (pNA). pNA is a yellow color that is measured photometrically at 385-410 nm. With the aid of a spectrophotometer, a kinetic colorimetric assay may be done, in which the early onset of color can be detected and precisely measured.

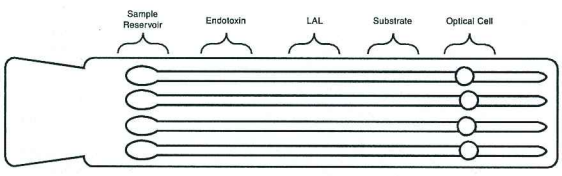
$$\text{Proenzyme} \xrightarrow{\text{Endotoxin}} \text{Enzyme}$$

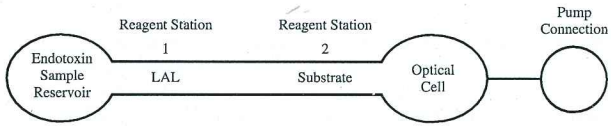
$$\text{Substrate} \xrightarrow{\text{Enzyme}} \text{Peptide + p-nitroaniline}$$

The Endosafe® - PTS cartridge and its interface with the reader have been designed to mimic currently licensed Endpoint Chromogenic and Kinetic Chromogenic Methods by measuring color intensity directly related to the endotoxin concentration in a sample. Each cartridge contains precise amounts of FDA licensed LAL formulations, chromogenic substrate, and Control Standard Endotoxin (CSE)

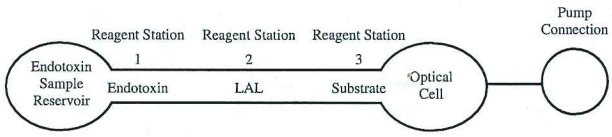
CARTRIDGE REAGENTS

Each Endosafe® - PTS cartridge contains four channels to which LAL reagent and a chromogenic substrate have been applied. Two of the four channels also contain an endotoxin spike, and serve as the positive product controls. The use of two Sample Channels and two Spike Channels is consistent with current USP <85> photometric Limulus Amebocyte Lysate (LAL) methods. The reader can be used to detect endotoxin in variety of ranges.





Sample Channel Zoom



PPC (Spike) Channel Zoom

STORAGE CONDITIONS AND PRECAUTIONS

PTS cartridges are relatively heat stable and should be stored at 2 - 25°C. Allow the cartridges to come to room temperature before opening the pouch and testing. Prolonged exposure to temperatures above 25°C should be avoided. All cartridges in the Multi-Cartridge pouch must be used within two hours of breaking the seal on the pouch or stored in an alternate validated condition as determined at the end users facility. Cartridges are for single-test use only.

REAGENTS REQUIRED BUT NOT SUPPLIED

LAL Reagent Grade Water must be used during the initial qualification of each lot of PTS cartridges.

MATERIALS REQUIRED BUT NOT SUPPLIED

Pipettor (Endosafe® PTS 400 or equivalent) and sterile tips.

Disposable, endotoxin-free glass dilution tubes or sterile, disposable polystyrene tubes (Endosafe® T300 or equivalent) for sample collection or dilution if necessary.

Vortex-Type Mixer (if necessary).

EQUIPMENT REQUIRED BUT NOT SUPPLIED

Endosafe® Portable Test System (PTS) Reader: The reader is a dedicated instrument that accepts the cartridge and runs the PTS LAL test. The reader consists of an incubating chamber, a sample pump, four LEDs and four detectors, a display, a keypad interface, and a microprocessor. The reader operates using standard AC power or an internal rechargeable battery. Battery power also acts as automatic back-up power in case of AC power failure.

Endosafe® Multi-Cartridge System (MCS): This reader is a dedicated instrument that is equipped with five independent cartridge readers. Each MCS instrument can test up to five cartridges. The cartridges can be run simultaneously or in a random access fashion. Each MCS cartridge reader consists of an incubating chamber, a sample pump, four LEDs, and four detectors.

Internally, the reader measures the reaction time in each channel. An archived standard curve specific for each lot of cartridges is constructed using the log of the reaction time vs. the log of the concentration. The sample and spike values are calculated by interpolation of the standard curve using the reaction times. See Certificate of Analysis for the lot specific standard curve.

SPECIMEN COLLECTION AND PREPARATION

Specimen for testing must be collected and prepared using depyrogenated materials and endotoxin-free reagents. Glassware must be depyrogenated by validated conditions, such as 30 minutes exposure at 250°C.[7] It is prudent to test for endotoxin those materials that cannot be heat sterilized or those which are sold without an endotoxin-free label. Use aseptic technique at all times.

Assay Suitability Requirements

Acceptance criteria for a valid assay consists of a positive product control (PPC) recovery value of 50-200%, and a coefficient of variation (%CV) of less than 25% on reaction times for both sample and PPC channels. Note: Samples should be tested (following the USP BET recommendation) at a dilution (less than the MVD) necessary to consistently eliminate interfering factors such as pH, ionic strength, and high background endotoxin.[7]

TYPES OF ASSAYS

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%).

Inhibition/ Enhancement: The absence of interference in a kinetic LAL assay is demonstrated by achieving an acceptable Positive Product Control spike recovery of 50-200% on a given sample preparation. The spike recovery requirement is applicable for routine testing and for three lot product validations. It is recommended that a pH measurement of the sample / LAL mixture be made during the three lot product validation. This can be accomplished at the conclusion of the successful cartridge test by tipping the cartridge towards the samples well reservoirs and recovering 50 to 100 µL volume using the same pipettor and pipette tips. A micro pH probe or pH paper can be used to measure the pH.

NOTE: Historically a validation defined by the Guideline on Validation of the Limulus Amebocyte Lysate Test as an End-Product Endotoxin test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices, U.S. Dept. of Health and Human Services, FDA, December required three lots of product to be tested at the same dilution.

Inhibition is usually concentration dependent and can be overcome by dilution with LAL Reagent Water. The most common sources of inhibition are: 1) conditions that interfere with the enzyme activity due to ionic strength and/or pH; and 2) those that alter the dispersion of the endotoxin (positive) control.[6] If the positive product control fails and a pH related problem is suspected, the pH of the test specimen should be measured to assure a pH within the range of 6 - 8. Use an endotoxin-free TRIS buffer (Endosafe® BT101, BT103 or equivalent) if pH adjustment is necessary. Do not arbitrarily adjust the pH of unbuffered solutions.

Maximum Valid Dilution: USP <85> has listed endotoxin limits of 5 EU/kg for intravenous drugs and 0.2 EU/kg for intrathecal drugs. Specific limits for compendial items have been adopted.[7] These limits may be used to determine the extent of dilution that may be used to overcome an interference problem without exceeding the limit endotoxin concentration.[9]

The Maximum Valid Dilution (MVD) is calculated by formulae presented in the previously mentioned documents and other pharmacopeia.[7]

For drug products that have a published limit, the MVD may be calculated by the following formula:

$$MVD = \frac{\text{Endotoxin Limit X Product Potency}}{\text{Lambda}}$$

EL = K/M, where K= 5 EU/kg and
M= Maximum Dose per kg of body weight administered per hour

Product Potency = concentration of product

Lambda = sensitivity (lowest point on the archived curve) of test cartridge

For example, the compendial limit for Sterile Water for Irrigation (SWI) is 0.25 EU/mL. If a test cartridge with an archived standard curve containing the lowest level of 0.05 EU/mL of endotoxin is used to test this product, where the potency is 1 mL/mL, the MVD equals 1:5. Thus, SWI may be diluted up to 1:5 to resolve potential inhibition (one part to a total of five parts LRW).

Routine Tests with the Endosafe® - PTS cartridges:
See the User's Guide supplied with the Endosafe® - PTS or the Endosafe® MCS reader for complete operations, procedures, and guidelines.

BIBLIOGRAPHY

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PATENT INFORMATION

U.S. Patent No: US D472,324 S
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Other patents pending.

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