ENDOSAFE® - LAL CARTRIDGES

I/E SCREENING, DISPOSABLE CARTRIDGES FOR INHIBITION/ENHANCEMENT DETECTION

FOR USE WITH ENDOSAFE® CARTRIDGE TECHNOLOGY INSTRUMENTS

INTENDED USE

Disposable test cartridges contain Limulus Amebocyte Lysate (LAL), endotoxin, and synthetic color-producing substrate. They are intended for use with Endosafe® cartridge technology instruments to perform the initial screening for Inhibition/Enhancement on samples by kinetic chromogenic methods.

CAUTION:

This product is not licensed by the FDA. The Endosafe® I/E cartridge is not to be used for end product release or formal validation testing that is associated with Pharmaceutical Drugs, Devices or Biologics that are regulated by the FDA. The I/E screening cartridge, however, is a tool that can be used to rapidly determine a suitable sample preparation technique for a subsequent and official LAL product validation.

BACKGROUND AND SUMMARY

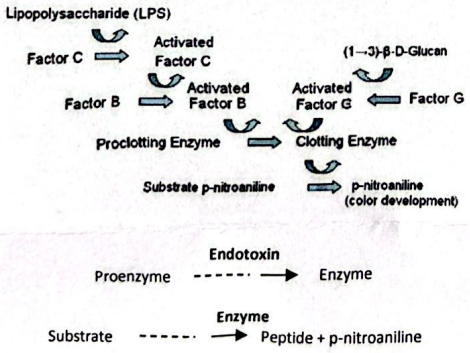
Frederick Bang observed that bacteria causes intravascular coagulation in the American Horseshoe Crab, *Limulus polyphemus*.[1] In collaboration, Levin and Bang[5] found that the agent responsible for the clotting phenomena resides in the crab's amebocytes, or circulating blood cells, and that pyrogens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

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.[1-5] 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 > and Ph. Eur. 2.6.14 provide 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.

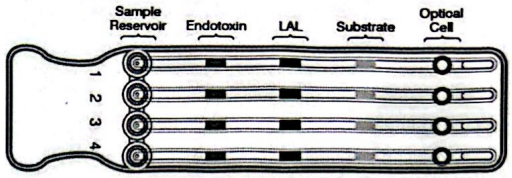
Limulus Enzyme Cascade



The Endosafe® LAL cartridge and its interface with the cartridge technology instruments 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® LAL cartridge contains four channels to which these LAL reagent, an endotoxin spike, and a chromogenic substrate have been applied. Four channels serve as positive product controls.



STORAGE CONDITIONS AND PRECAUTIONS

Endosafe® LAL cartridges are relatively heat stable and should be stored at 2 - 25°C.

Test Preparation Information: To minimize invalidities, cartridges should be allowed to come to room temperature (20-25°C) for a minimum of 8 hours prior to opening the pouch and testing. To minimize contamination of the sample wells, the cartridge should be used immediately once the foil pouch seal has been opened. 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 Endosafe® LAL cartridges.

MATERIALS REQUIRED BUT NOT SUPPLIED

Pipettor (Endosafe® PTS700 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® Cartridge Technology Instruments: This is a dedicated instrument that accepts the cartridge and runs the LAL test. The cartridge technology instrument consists of an incubating chamber, a sample pump, four LEDs and four detectors, and a display.

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.

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 acceptable spike recovery (50-200%) in all four channels.

Inhibition/ Enhancement: Absence of interference is demonstrated by achieving acceptable spike recovery (50-200%) on a given sample preparation. This cartridge is a tool that is designed to rapidly identify an appropriate test dilution/concentration and/or other sample preparatory technique for LAL testing. Once identified, the same sample preparatory technique can then be used in a formal 3 lot validation that utilizes the duplicate sample and duplicate spike cartridge.

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 > and Ph. Eur. 2.6.14 have 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= 5EU/Kg and
M= Maximum Dose per kg of body weight administered per hour

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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).

Inhibition/Enhancement Screening Tests: An Inhibition/Enhancement LAL assay is conducted by following the simple prompts on the Endosafe® cartridge technology instrument. Different dilutions of one sample can be placed in each test well of one test cartridge to find the non-interfering test dilution or different samples can be placed in each test well.

TEST RESULTS

When the test is complete, the Endosafe® cartridge technology instrument gives an audible notification that the assay is finished.

Data reporting is simple. At the conclusion of the test, the spike recovery criteria are displayed on the screen.

The instrument or software displays the following results:

- Spike #1 Recovery
- Spike #2 Recovery
- Spike #3 Recovery
- Spike #4 Recovery

BIBLIOGRAPHY

1. Bang, F.B., "A Bacterial Disease of Limulus Polyphemus", *Bulletin of Johns Hopkins Hospital*, Nr. 98, p. 325 (1956).

2. Cooper, J.F., Levin, J., and Wagner, H.N., "Endotoxins as a Cause of Aseptic Meningitis after Radionuclide Cisternography", *Journal of Nuclear Medicine*, Nr. 16, p. 809 (1976).

3. Cooper, J.F., Levin, J., and Wagner, H.N., "Quantitative Comparison of In Vitro and In Vivo Methods for the Detection of Endotoxin", *Journal of Laboratory and Clinical Medicine*, Nr. 78, p. 138 (1971).

4. Hochstein, H.D., "The LAL Test versus the Rabbit Pyrogen Test for Endotoxin Detection: Update '87", *Pharmaceutical Technology*, Nr. 11(6), p. 124 (1987).

5. Levin, J., and Bang, F.B., "Clottable Protein in Limulus: Its Localization and Kinetics of Its Coagulation by Endotoxin", *Thromb. Diath. Haemorrh.*, Nr. 19, p. 186 (1968).

6. Cooper, J.F., "Resolving LAL Test Interferences", *Journal of Parenteral Science and Technology*, Nr. 44:1, p. 13, (1990).

7. Bacterial endotoxins test (Chapter <85>). In: *United States Pharmacopeia and National Formulary*. Rockville, MD: United States Pharmacopeia Convention.

8. McCullough, K.Z. "Process Control: In-process and Raw Material Testing Using LAL." Pharmaceutical Technology, 12(5) p.40 (1988).

9. Weary, M.E. "Understanding and setting endotoxin limits." Journal of Parenteral Science and Technology, 44:1, p. 16 (1990).

10. Bacterial endotoxins (2.6.14). In: *European Pharmacopeia* (Ph. Eur.). Strasbourg, France: EDQM Council of Europe; 2001.

PATENT INFORMATION

U.S. Patent No: US D472,324 S
U.S. Patent No: US 7,329,538 B2
Other patents pending.

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