ENDOSAFE® - PTS/MCS CARTRIDGES

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

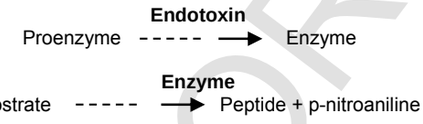
BACKGROUND AND SUMMARY

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 > provides standard methods for validating the LAL Test as a replacement for the rabbit pyrogen test.[7]

BIOLOGICAL PRINCIPLES

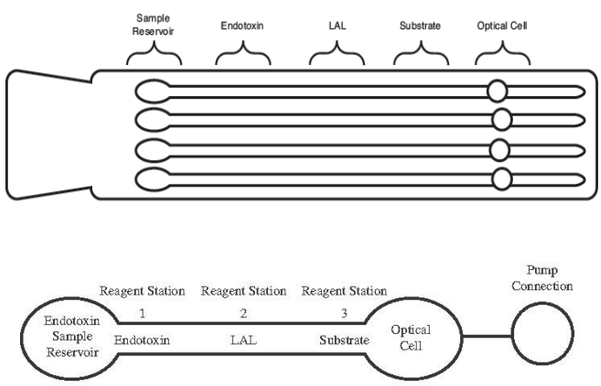
In this assay, bacterial endotoxins initiate the activation of a cascading series of serine proteases in LAL. The last activated enzyme in this series, the pro-clotting enzyme, cleaves a peptide from an endogenous substrate called coagulogen. The modified substrate produces an opacity and gelatin in 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.



The Endosafe® - PTS/MCS 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/MCS 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.



Spike Channel Zoom

STORAGE CONDITIONS AND PRECAUTIONS

PTS/MCS cartridges are relatively heat stable and should be stored at 2 - 25ºC. Allow the cartridges to come to room temperature before opening the pouch and testing. Prolonged exposure to temperatures above 25ºC should be avoided. To minimize contamination of the sample wells, the cartridge should be used immediately once the foil pouched 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 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® Cartridge Reader: The reader is a dedicated instrument that accepts the cartridge and runs the PTS/MCS 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 backup power in case of AC power failure.

See the *User's Guide* supplied with the Endosafe® cartridge reader for complete operations, procedures and guidelines.

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.

PERFORMANCE CHARACTERISTICS

Acceptance criteria for a valid assay is an archived curve correlation coefficient of ≤ -0.980 and a positive product control (spike) recovery of 50-200%.

Internally, the Endosafe® cartridge reader measures the reaction time in each channel. An archived standard curve is constructed using the log of the reaction time vs. the log of the concentration. The spike values are calculated by interpolation of the standard curve using the reaction 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 > 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= 5EU/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).

Inhibition/Enhancement Screening Tests: An Inhibition/Enhancement PTS/MCS LAL assay is conducted by following the simple prompts on the Endosafe® cartridge reader. 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. The following represents a typical assay procedure:

1. Instrument Operation
   - Press the menu key on the Endosafe® cartridge reader keypad to turn instrument on (Menu 5 turns instrument off).
   - The reader then initiates a "SYSTEM SELF TEST" as it heats up to 37°C – this takes approximately 5 minutes.
   - The reader displays "SELF TEST OK" and then "INSERT CARTRIDGE".

   Note: Allow the cartridge to come to room temperature inside the pouch.

2. Insert the Cartridge
   Allow the cartridge to reach room temperature. Remove the cartridge from the pouch and insert with the sample reservoirs facing up into slot at the front of the Endosafe® cartridge reader. Do not touch the sample reservoirs or optical cells.

   Press cartridge gently but firmly into slot.

3. Enter Required Information
   Once the cartridge has been firmly inserted into the reader, the reader prompts the user to enter the following information:

   * *Enter OID* (Operator ID or User Name)
   * *Enter Cartridge Lot #*
   * *Enter Calibration Code*

   (See the Certificate of Analysis for the Calibration Code. If the Calibration Code for the particular lot number has already been entered, the reader does not prompt for the code again.)

   * *Lot #*
   Cancel or Enter

   (This prompt is to confirm the cartridge lot number entered. Pressing cancel will return user to cartridge lot **#** prompt.)

   * *Enter Sample 1 Lot #*
   Enter Sample 2 Lot#
   Enter Sample 3 Lot#
   Enter Sample 4 Lot#

   * *Enter Sample 1 ID*
   Enter Sample 2 ID
   Enter Sample 3 ID
   Enter Sample 4 ID

   * *Enter Dilution #1 Factor*
   Enter Dilution #2 Factor
   Enter Dilution #3 Factor
   Enter Dilution #4 Factor

   Note: All LRW used to make product dilutions should be tested for detectable endotoxin.

   While the above information is being entered into the reader, the cartridge is being pre-warmed for a minimum of 30 seconds.

4. Dispense the sample
   Once all test information is entered, the reader displays:

   * *"ADD SAMPLE PRESS ENTER"*.
   * Pipette 25 µL of sample into all four sample reservoirs of the inserted cartridge and press **Enter** on the reader keypad.
   * The test will begin and takes about 15-20 minutes to produce results.

TEST RESULTS

When the test is complete, the Endosafe® cartridge reader 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 reader display alternates between the following results:

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

The reader or screen continues to display the assay results until the cartridge is removed. Once the assay is complete and the results are noted, remove the cartridge promptly from the reader.

Note: After suitable sample preparation is determined, continue in-process or product validation using the appropriate Endosafe® LAL cartridges with duplicate negative and positive channels.

Retrieving Results Options:

- Use EndoScan-V to download results or save data reports.
- Download results directly to your PC and retrieve from the designated location file.
- Use the Seiko® DPU-414 Printer or Epson TM-U220D (available from Charles River Endosafe) to print the last test result, all results from a particular date, or up to a maximum of one hundred stored test results.

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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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