LIMULUS AMEBOCYTE LYSATE ENDOSE® 
U.S. License No. 1197

INTENDED USE:
Limulus amebocyte lysate (LAL) derived from Limulus polyphemus amebocyte lysate. It is included as the qualitative detection of gram- negative bacilli endotoxins by the Gel-clot method.

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 in vitro diagnostic tool.

SUMMARY AND GENERAL INFORMATION
The Gel-clot LAL test is a simple, reproducible test that is being conducted by mixing Endosafe® LAL reagent and test specimen and promptly incubating the mixture undisturbed for 60 minutes at 37°C. A positive response on the Gel-clot test indicates there is an amount of endotoxin in the sample which equals or exceeds the reagent's labeled sensitivity, represented by the symbol λ.

REAGENT PREPARATION
A fresh CSE control dilution series should be prepared from a stock solution in a two-fold dilution series that brackets the labeled sensitivity (λ) of the Endosafe® LAL reagent. A 4-point series is usually made with two endotoxin dilutions above and below. Add 0.2 mL of each concentration of endotoxin directly to the assay tube. Mix and incubate as described above.

BACTERIAL ENDOTOXIN TESTING FOR END-PRODUCT RELEASE
A LAL reagent is released by the U.S. Food and Drug Administration in 1987 to inform manufacturers of human drugs and biologicals, animal drugs, and medical devices of procedures the Agency considers necessary to validate the use of LAL as an end-product test.

GENERAL PRECAUTIONS
Endosafe® LAL is intended for in vitro diagnostic purposes only. It is not intended for use as a direct detection reagent for endotoxins. Direct contact with LAL because its toxicity is not known.

Correct application of this test requires strict adherence to all items in the endotoxin-testing protocols to detect and measure endotoxin, a live producing byproduct of gram-negative bacteria commonly associated with animal and plant products. The test requires that the endotoxin produces an opaquely gel in LAL that is readily recognized. The simplicity and economy of LAL makes it the preferred method of choice in preclinical and clinical studies. The test is performed by mixing the test sample with a reagent and incubating at 37°C.

The test for a suitable pyrogen test for nephrotoxicity/hemolysis. Use enough of each sample to test at least six different dilutions. Set up a control standard endotoxin dilution tubes. Timers are useful in measuring incubation times and endotoxin mixing periods.

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The LAL sensitivity is determined by calculating the geometric mean of the endpoint. Each endpoint of the quadruplicate assay is converted to log. The individual log values are averaged and the LAL sensitivity is taken as the antilog of this average log value.

**INITIAL QUALITY CONTROL PROCEDURE FOR A TESTING LABORATORY**

The variability of a test laboratory and its analysts should be assessed before any official tests are done. Each analyst, using a single lot of LAL and a single lot of endotoxin (CEP or RE), should correctly and satisfactorily complete the test for confirmation of labeled LAL sensitivity. Acceptable variation is one half (0.5) to twice (2) labeled sensitivity (S).

**TEST FOR CONFIRMATION OF LABELLED LAL REAGENT SENSITIVITY**

The labelled sensitivity must be confirmed before a new LAL lot is introduced into a test laboratory. This test is performed by assaying the lot of endotoxin (CEP or RE) by testing in quadruplicate (see Table I). The geometric mean of the endpoint must be within the limits of labeled claim, as defined and illustrated above.

**DETERMINATION OF ENDOXOIN IN AN UNKNOWN**

To determine the endotoxin concentration in an unknown, test serial two-fold dilutions of the specimen until an endpoint is reached. The endotoxin concentration in the unknown is calculated by multiplying the LAL labelled sensitivity by the reciprocal of the dilution representing the endpoint. For example, product aliquot was diluted by preparing a series of two-fold dilutions with LAL Reagent Water. A test of each product dilution yielded an endpoint at the 1:8 dilution when tested with LAL Reagent having a labeled sensitivity (λ) equal to 0.25 EU/mL. The endotoxin titer was determined to contain at least 0.5 EU/mL or λ = 0.125 EU/mL in the unknown.

**PRODUCT INHIBITION**

Before routine LAL testing is started, the potential for product inhibition must be excluded. Inhibition is usually concentration dependent, and is easily overcome by dilution with LAL Reagent Water. Common sources of inhibition include conditions that interfere with the enzyme-mediated clot formation, and 2) after the dispersion of the endotoxin control.

**LIMITATIONS**

Samples may be tested by LAL methods provided that no inhibition or enhancement conditions are present that cannot be eliminated by an acceptable dilution (refer to MVD calculation) or sample pretreatment, such as buffering. If the LAL method cannot be validated at a concentration within the maximum valid dilution, the LAL test cannot be substituted for the USP Bacterial Endotoxin Test.

The error of the Gel-clot method is plus or minus one two-fold dilution of the endpoint of the assay.

**SIGNIFICANT TEXTS ON LAL TESTING**


