RAPID LAL SINGLE-TEST VIALS

ENDOSAFE®

Single-use vial for the detection of Endotoxins (Pyrogens) in Research Samples, Renal Dialysis Process Water, and Dialysate Solutions.

INTENDED USE

The Rapid Limulus Amebocyte Lysate (LAL) Single-Test Vial is derived from *Limulus polyphemus* amebocytes and is intended for use in the quantitative detection of Gram-negative bacterial endotoxins by the Gel-clot method.

CAUTION: This product is not licensed by the FDA. The Endosafe® Rapid LAL Single-Test Vial is not to be used for end-product release of Pharmaceutical Drugs, Devices, or Biologics that are regulated by the FDA.

PURPOSE

The Rapid LAL Single-Test Vial is designed primarily to monitor dialysis water, dialysate solutions, and research samples for levels of bacterial endotoxin, also known as pyrogen. Gram-negative bacteria (GNB) that are found in water purification systems produce endotoxin. LAL is a biological reagent that is the most sensitive and specific means to detect bacterial endotoxin. The test is based on the capacity of LAL reagent to develop an opaque gel in the presence of sufficient levels of endotoxin.

SOURCES OF ENDOTOXIN

Reverse osmosis (RO) systems and other water treatment components are used to minimize chemical impurities, bacteria and their toxic byproduct, and bacterial endotoxins in dialysis and research settings. Although RO removes most bacteria, some escape to downstream sites and begin to proliferate. GNB found in purified water systems include *Pseudomonas, Flavobacterium, Enterobacter* and *Alcaligenes*. All parts of a water system may become fouled with microbes in the form of biofilm, described as a collection of bacteria trapped in a gelatinous matrix that is mostly extracellular and secreted by the bacteria. Biofilm adheres tenaciously to surfaces (membranes, pipes, etc.) and is difficult to eradicate, once established. If sanitization is absent or inadequate, GNB and endotoxin will reach high levels. The only way to achieve low endotoxin concentrations on a consistent basis is to apply periodic sanitization to prevent or limit the proliferation of a biofilm inside the water system. The Rapid LAL Single-Test Vial is used to test water from specific points of use to determine the adequacy of the RO purification and sanitization processes.

NATURE OF ENDOTOXIN (PYROGEN)

Endotoxin, also known as pyrogen, is a large molecular complex that makes up the cell wall of GNB. It is constantly shed into the environment and is present in municipal water in the range of approximately 5-to-50 EU/mL. Unfortunately, it is stable, difficult to destroy by sanitizing agents, and it passes through sterilizing membrane filters. Only ultrafiltration consistently removes endotoxin from water.

A pyrogenic reaction is characterized by chills and fever, and may be accompanied by a fall in blood pressure and respiratory distress; it is mediated by cytokines, such as interleukin-1. Rapid increase in symptoms begins 1-to-3 hours after endotoxin exposure with peak illness occurring 3-to-6 hours afterwards.[1,2] Conditions such as β_2-microglobulin-associated amyloidosis and general life-shortening associated with cardiovascular disease and malnutrition are linked to chronic exposure to endotoxin.[3,4]

NATURE OF THE LAL TEST

The Gel-clot LAL test method is a simple, reproducible test that is conducted by mixing LAL reagent and a sample of dialysis fluid or water, followed by prompt incubation of the mixture undisturbed at 37°C for a specified amount of time. A positive response in a Gel-clot test indicates that there is a concentration of endotoxin in the test sample that is equal to or exceeds the reagent's labeled sensitivity, represented by the symbol lambda, λ. The LAL reaction with endotoxin requires a neutral pH and is time and concentration dependent. The test is limited to aqueous solutions. LAL test interference is often caused by high salt concentrations that are overcome by dilution with endotoxin-free water, known as LAL Reagent Water.

HEMODIALYSIS USE

Voluntary Standards for Hemodialysis Water

An endotoxin unit referenced to an international standard is used to express endotoxin potency. An international endotoxin unit in the European Pharmacopoeia (EP) is expressed as IU/mL whereas the U. S. Pharmacopoeia (USP) uses EU/mL. Note that 1 IU equals 1 EU because both are related to the same international reference standard. The EP has adopted a bioburden limit of 100 CFU/mL and an endotoxin limit of 0.25 IU/mL for water used to dilute concentrated hemodialysis fluids. This is non-mandatory requirement in the EP that serves as a useful guide to endotoxin levels in water. Renal care organizations in many European countries have similar microbial and endotoxin limits that apply locally.[5]

Frequency of Testing

The European Dialysis and Transplant Nurses Association and European Renal Care Association (EDTNA/ERCA) recommends that the frequency of microbiological testing should be established and based on historical data and maintenance procedures for the water system. For example, if the disinfection interval for a system is short and test results confirm efficacy of the water quality program, a monthly test interval may be sufficient. However, if the disinfection interval is long and there is a history of elevated endotoxin levels or no existing history, then a weekly test interval is needed to assure safety. Another important aspect is that the guideline recommends having in-house capability for conducting an endotoxin test so that results of regularly scheduled tests are known quickly. In-house test capability also allows immediate investigation of solutions associated with a patient's reaction so that the cause of unsafe conditions can be identified and corrected. This guideline also recommends a low-nutrient agar for monitoring bioburden.

RESEARCH USE

The Rapid LAL Single-Test Vial can also be used to detect endotoxin in research samples when there is no requirement to use an FDA licensed LAL reagent.

CONTENTS

Each box of Rapid LAL Single-Test Vials (green capped) and the Positive Product Control (PPC) Single-Test Vials (red capped) contains 50 vials. The LAL reagent in these vials is buffered and stabilized with monovalent and divalent cations. The PPC vials contain buffered LAL that is co-lyophilized with a known amount of bacterial endotoxin. The PPC tubes are used to confirm the absence of inhibitory test conditions that might produce false-negative results.

Lyophilized LAL and PPC vials should be stored at 2-8°C; avoid exposure to temperatures above 25°C. The tube should be discarded if there is any yellow discoloration.

MATERIALS NOT PROVIDED

- A water bath or heating block is required to incubate the assay mixture at a temperature of 37° C +/- 1° C.

REAGENT PREPARATION

Rapid LAL

Caution: The green capped Single-Test Vials and the red capped Singe-Test Vials must be incubated immediately after rehydration with test sample or control. Each LAL vial and each PPC vial is reconstituted by addition, directly into the test vial, a total volume of 0.2 mL of the sample to be tested.

SPECIMEN COLLECTION AND PREPARATION

Process water and dialysate specimens for testing with the Rapid LAL Single-Test Vial must be collected and prepared using depyrogenated materials and endotoxin-free materials. Sterile, non-pyrogenic syringes may by used to transfer aliquots of each specimen to the LAL and PPC reaction tubes.

TEST PROCEDURE

The green capped Rapid LAL Single-Test Vials serve as the test container. The red capped PPC serve as a control against LAL inhibition. Before use, collect the LAL and PPC Single-Test Vials contents by gently tapping the bottom of each vial on a hard surface.

Aseptically collect process water and dialysate specimens into sterile, non-pyrogenic containers.

Aseptically transfer 0.2 mL of the test specimen to a single green capped LAL assay tube. Transfer 0.2 mL specimen volume to a single red capped PPC tube. Immediately place the reaction tubes in a 37°C water or dry bath heater for times indicated on the vial label (+ 2 minutes) - depending on the sensitivity desired. Timing of the reaction of ENDOSAFE® LAL with endotoxin is critical.

Since the reaction of the Rapid LAL Single-Test Vials are temperature sensitive, the incubator must be monitored carefully. Also, the gel-forming reaction is delicate and may be irreversibly altered if the tubes are disturbed during the incubation period.

INTERPRETATION OF RESULTS
Each tube in the Gel-clot method is interpreted as either positive or negative. A positive result is defined as the formation of a firm gel capable of maintaining its integrity when the test tube is inverted 180°. A negative test is characterized by the absence of gel or by the formation of a viscous mass, which does not hold when the tube is inverted. Test results are only valid when the PPC exhibits gelation at the interval selected.

EXPECTED VALUES
Rapid LAL Single-Test Vials are standardized against the U.S. Reference Endotoxin, so that the sensitivity is expressed in Endotoxin Units per milliliter (EU/mL). Note: The Expert Committee on Biological Standardization of WHO has assigned a potency of the IS as 10,000 IU / vial of IS, so that 1 IU = 1 EU.

PRODUCT INHIBITION
Since the LAL test is enzymatic and since LAL enzyme activity may be inhibited by any number of chemical agents or physical test conditions, the potential for product inhibition must be excluded with every test. The red capped PPC Single-Test Vials serves as a measure of potential LAL inhibition.

If during the course of routine testing, the red capped PPC fails to produce a gel, the pH of the test specimen should be examined. The pH of the test specimen and LAL mixture should be within the range of pH 6.0 to 8.0. If pH adjustment is necessary, use endotoxin-free HCl or NaOH at a suitable concentration (generally 0.1N or less), or a Tris buffer from Charles River Endosafe. Do not arbitrarily adjust the pH of unbuffered solutions.

LAL chemical inhibition is usually concentration dependent, and is easily overcome by dilution with LAL Reagent Water. Common sources of inhibition include conditions that 1) interferes with the enzyme-mediated gelation reaction, and 2) alter 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.

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