Endotoxin / LAL Testing

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Introduction

Endotoxins, also known as pyrogens or fever-causing toxins, are a concern for pharmaceutical and medical devices that are intended to come in contact with human blood or cerebro-spinal fluid. Even products that pass sterility testing are at risk for endotoxin presence. Although the product may have been sterilized, if gram-negative organisms were present prior to sterilization, the endotoxin of the organism still remains.

Bacterial endotoxin comes from an extra layer in the cell wall of gram-negative organisms made of lipopolysaccharide. The lipopolysaccharide toxin is not eliminated when the organism is killed by the sterilization process; instead, the release of the lipopolysaccharide takes place upon the death of the cell. Testing for this endotoxin in the finished product is an important part of ensuring the safety of the sterilized product.

The currently FDA accepted test used for batch release testing of product is the Limulus Amoebocyte Lysate (LAL) test. The standard method for pyrogen or endotoxin testing was previously the Rabbit Pyrogenicity Test (still used for establishing initial biocompatibility in devices).

In 1983, the FDA officially recognized the LAL test as a standard for bacterial endotoxin testing. In samples that are not at risk for chemical endotoxin, the LAL test is ideal. The USP established <85> with the Gel Clot test. The LAL Gel Clot test is more sensitive than the Rabbit Pyrogenicity Test in detecting bacterial endotoxin.

LAL is a clotting agent drawn from the blood of the horseshoe crab, found in the Atlantic Ocean off the coast of the New England states. The crabs are not harmed in the process, but are sampled and returned to the ocean, where they regenerate the blood taken. The clotting agent is uniquely able to identify the presence of endotoxin in a sample by clotting when exposed to the endotoxin-contaminated sample.

Compendial Testing

The most commonly requested LAL method from the compendia is the gel clot method. This test detects the presence or absence of endotoxin through the blood clot reaction between the endotoxin and the lysate. Clotting indicates the presence of endotoxin. If present, the endotoxin can be quantified, but the detection limit is not as sensitive as the LAL kinetic tests.

There are two kinetic methods from which to choose, Chromogenic and Turbidimetric. The Turbidimetric is the most sensitive of the two, with a detection limit of 0.001 Endotoxin Units (EU) when a tube reader is used. A standard plate reader yields a detection limit of 0.005 EU. The Endotoxin Unit (EU) is defined as the endotoxin activity of 0.2 ng of Reference Endotoxin Standard.

For the turbidimetric method, results are determined by the rate of increase in turbidity or the time taken to reach a particular level of turbidity. This technique is not recommended for already turbid samples, as this would interfere with detection.

The other kinetic test is the Chromogenic method. This method is also sensitive, with a detection limit of 0.005 EU. The method utilizes a colorless artificial peptide substrate that is co-lyophilized with the lysate. A color change detected during the test indicates the presence of endotoxin. The technique is not recommended for samples that have strong color that may interfere with detecting the color change.

Endotoxin/LAL Testing Options with BioScreen

- **Gel Clot-** Detects the presence or absence of endotoxin through the blood clot reaction between the endotoxin and the lysate. Clotting indicates the presence of endotoxin
- **Chromogenic-** Quantitative method, sensitivity to 0.005 EU. Utilizes a colorless artificial peptide substrate that is co-lyophilized with the lysate. Color change indicates the presence of endotoxin.
- **Turbidimetric-** Quantitative method, sensitivity to 0.001 EU, when the tube reader is used; 0005 EU with the plate reader. Results determined by the rate of increase in turbidity or the time taken to reach a particular level of turbidity.

Validation and Other Considerations

Test method validation is critical to ensure that the proper rinse level is reached for the device, and verifies that there is no interference in detecting and recovering endotoxin present. Proper validation involves inoculating the product with endotoxin, and verifying that comparable recovery may be achieved.

The most common source of endotoxin contamination is water that may be a part of the manufacturing process. Once contaminated, removing endotoxin from the product is difficult and sometimes it is not possible, depending on the material of which the product is made.

Common procedures for removing endotoxin, or depyrogenating, are heat treatment and removal by washing. Traditional sterilization methods, such as irradiation and ethylene oxide treatment are not reliable for removing endotoxin contamination. It is best to control the process and keep finished products relatively free of gram-negative organisms to reduce the risk of endotoxin contamination.

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