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Pyrogens

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ABSTRACT

Bacterial Endotoxin

Bacterial endotoxin can be introduced to the human&animal body by medical devices , biological and pharmaceutical products through direct or indirect contact with blood, lymph nodes, and with cerebral spinal or brainstem contact if they are not properly manufactured or cleaned. Bacterial Endotoxin Testing is also referred to as the *Limulus* Amebocyte Lysate (LAL) Test. Bacterial endotoxin contamination can present high fever and potentially cause death among humans.

Since Gram negative bacteria, which produce endotoxin, are dangerous to the human body in either a dead or living state, it is imperative medical device manufacturers conduct bacterial endotoxin testing on their medical devices in the finished form. Testing may be performed before or after the sterilization process. We recommend testing pre-sterile for those devices that undergo radiation sterilization—to ensure minimal delay in getting the device shipped to market. The validation study should reflect whether the devices evaluated were pre or post the sterilization process. Traditional industrial sterilization generally does not reduce the bacterial endotoxin content on or in product.

[1]Endotoxins are fragments of Gram-negative bacterial cell walls that can produce fever and chills and possibly a fatal reaction when introduced into the circulation of humans and animals. Since even sterilized products can contain residual bacterial fragments, each batch of injectible drugs and some medical devices must be tested for the presence of bacterial endotoxins. This testing is called pyrogenicity testing.

Endotoxin does not have an effect on the body when taken orally, the testing is only necessary on devices that will come in contact with blood, lymph nodes, and cerebral spinal or brainstem contact. The FDA also mandates that manufacturers to test devices that come in contact with the vitreous fluid of the eye.

An endotoxin test is a lab test to check for the presence of endotoxins like lipopolysaccharide in a sample. Endotoxins are chemical compounds, made primarily by Gram negative bacteria. They can be very dangerous to humans, leading to health conditions like septic shock if they enter the bloodstream. Manufacturers of drugs and

medical devices use endotoxin testing in quality control to make sure their products are pure and safe for use. This testing can also be useful for labs concerned about contamination of samples used in research.

A laboratory doctors can run a variety of types of test using this compound to see if there are endotoxins in a sample and to determine their concentrations.

When medications or devices are contaminated with endotoxins, this can expose patients to risk. People who are already sick may be less able to metabolize the toxin, and could become seriously ill if they take contaminated drugs or use contaminated devices. Manufacturers and other facilities perform regular endotoxin testing to look for signs of contamination. If the endotoxin test reveals a problem, the manufacturer can halt distribution of a lot, or recall it in cases where it is already on the market. The testing takes place on a regular basis along with other quality control measures for safety.

ESTABLISHMENT OF ENDOTOXIN LIMITS

The endotoxin limit for parenteral drugs, defined on the basis of dose, is equal to K/M, [4] where K is the threshold human pyrogenic dose of endotoxin per kg of body weight, and M is equal to the maximum recommended human dose of product per kg of body weight in a single hour period.

The endotoxin limit for parenteral drugs is specified in individual monographs in units such as EU/mL, EU/mg, or EU/Unit of biological activity.

The US Pharmacopeia (USP) and others recognize the Limulus Amebocyte Lysate (LAL) method for testing drug products for the presence of Gram-negative bacterial endotoxins (lipopolysaccharides). This test was developed several decades ago; it is based on an enzyme cascade in LAL that occurs in the presence of an endotoxin and results in coagulation and gel formation. The rabbit pyrogen test may be used" if a product is incompatible with the LAL test." The US Center for Veterinary Medicine (CVM) and possibly other agencies, however, require that the first three batches manufactured be tested using both the LAL and RPT to determine whether other types of pyrogenic substances are present.

The recommended sample size is 3-10 devices to be pooled from each batch. Alternatives to batch testing may be defined and justified, in accordance with guidance given in AAMI ST72. If in the test there are failures (endotoxin is present) then manufacturers should look at their manufacturing process for endotoxin contributors such as raw materials, humans, water (which is the main source of Gram negative bacteria), and the environment.

Pyrogen testing of any pharmaceutical,biological products and medical device for parenteral application is therefore imperative.

Effects of Endotoxin

- **Binds to specific receptors on macrophages, B lymphocytes and other cells**
- **Fever** (Pyrogenicity):

Any elevation of the body temperature above the normal; functions to speed up immune reactions and to limit/slow bacterial growth and multiplication

Activation of alternative complement pathway: C3a; C5a

- **Circulatory system effects**:

Leukopenia followed by leukocytosis:

Leukopenia: an abnormal reduction in the number (-penia) of leukocytes inthe blood, (specifically a count of 5000 or less per cubic millimeter)

Leukocytosis: an abnormal increase in the number (-cytosis) of leukocytes in the blood, as during hemorrhage, infection, inflammation, or fever (specifically a count of 12,000 or more per cubic millimeter), respectively

Increased vascular permeability (vasodilation)

Decreased peripheral circulation

Decreased perfusion (blood flow) of blood to major organs

Capillary leakage; microhemorrhage; formation of petechiae (round, purple lesions caused by intradermal or submucosal microvascular hemorrhaging)

Hypotension (low blood pressure)

Effects on blood coagulation:

(DIC) Disseminated intravascular coagulation:

Disorder characterized by a reduction in the elements involved in blood coagulation due to their utilization in widespread blood clotting within the vessels; Late stages marked by profuse hemorrhaging

Activation of clotting pathway

Thrombosis: Formation of blood clot (thrombus) in heart or blood vessel

Thrombocytopenia: Abnormally low numbers of blood platelets

Effects on metabolic and liver functions

- **Decreased iron availability**
- **Hypoglycemia: Abnormally low glucose levels**
- **Cellular death (cytotoxicity)**
- **Organ necrosis:**

Sum of morphological changes indicative of cell death and caused by the progressive degradative action of enzymes

- **Shock:**
  - Characterized by failure of the circulatory system to maintain adequate blood flow to the vital organs
  - Symptoms include: Hypotension; Weak pulse; Rapid and shallow breathing; Low body temperature; CNS (central nervous system) effects (e.g., nausea)
- **Death**

Different methods using different protocols are in use.

*In vivo Rabbit Test

* Limulus Amoebocyte Lysate Test (LAL): This test measures the coagulation of the amoebocytes of the horseshoe crab, initiated by cell wall components (LPS) of gram-negative bacteria

C. Purity (FDA)

Product purity is defined as relative freedom from extraneous material in the finished product, whether or not harmful to the recipient or deleterious to the product (21 CFR 600.3(r)). Purity testing includes assays for pyrogenicity/endotoxin (see below), residual proteins or peptides used to stimulate or pulse cells, reagents/components used during manufacture, such as cytokines, growth factors, antibodies, and serum, and unintended cellular phenotypes.

2. Pyrogenicity/Endotoxin

The rabbit pyrogen test method is the currently required method for testing biological products for pyrogenic substances (21 CFR 610.13). Although the pyrogenicity test is required, there may be specific cases where this test method can not be performed for release due to properties of the cellular product (i.e., short product shelf life, toxicity of product in rabbits). Under these circumstances, a test method such as the Limulus Amebocyte Lysate test method (LAL) may be used as an alternative method, but prior to licensure must be shown to provide equal or greater assurances of safety, purity, and potency (see 21 CFR 610.9). The 1987 FDA Guideline on Validation of the Limulus Amebocyte Lysate (LAL) Test as End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices sets forth acceptable conditions for use of LAL.

For any parenteral drug, except those administered intrathecally, we recommend that the upper limit of acceptance criterion for endotoxin be 5 EU/kg body weight/hour. For intrathecally-administered drugs, we recommend an upper limit of acceptance criterion of 0.2 EU/kg body weight/hour. You should describe in your IND the pyrogenicity/endotoxin testing you conduct, and your acceptance criterion for release.

Note to FDA Reviewers: Document in your review the specification for pyrogenicity/endotoxin testing and verify that testing is on the final product and that results are available prior to lot release.

REFERENCES

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