Lal Test

parenteral preparations.
Contents

Aim

LAL test aim is to measure quantitatively the amount of bacterial endotoxins in a given sample of parenteral.
Principle

The solution of endotoxins containing preparation is added to the lysate derived from heamolymph cells of horseshoe crab (limulus polymhemus). The result of the reaction is turbidity or precipitation or gelation of the mixture. This is used as a quantitative measure to estimate the endotoxin content. The rate of reaction depends upon conc. of endotoxins, pH, temperature and presence of clotting enzyme system and clottable proteins from lysate.
The quantities of endotoxins are expressed in defined Endotoxin Units (EU). Also 1 EU is equal to 1 IU.
The endotoxin limit for a given test preparation is calculated from the expression K/M; where M is maximum dose administered to adult per kg per hour. The value for K is 5.0 EU/Kg for pareneteral preparations, and it is 0.2 EU/Kg for intrathecal preparations.
Reagents:

1. Endotoxin reference standard (ERS): It is the freeze dried purified endotoxin of Escherichia coli, which is calibrated in Endotoxin units (EU) by comparison with the International standard.
2. Control Standard Endotoxin (CSE):CSE is suitably standardized against the ERS which is used for routine bacterial endotoxin testing.
3. Water BET: Water that gives a negative result under the conditions prescribed in the test for bacterial endotoxins on the preparation under examination.

4. 0.1 M HCl BET: Prepared from hydrochloric acid using BET with pH adjusted to 6.0- 8.0 with o.1 M sodium hydroxide BET. It also gives a negative result under the conditions of the test.

5. 0.1 M NaOH BET:Prepared from sodium hydroxide using water BET with pH adjusted to 6.0- 8.0 with 0.1 M hydrochloric acid BET. It gives a negative result under conditions of the test.

6. Lysate: Lysate is the reconstituted lysate of amoebocytes of either of the species of horseshoe crabs; Limulus polyphemus or Tachypleus tridentatus.

GEL CLOT METHODS:

Both method A and B depend upon formation of firm gel formed from reaction of bacterial endotoxin with Lysate on incubation. Method A is a limit test wherein both the replicate solutions of preparation under examination must contain endotoxin in the concentration less than the endotoxin limit concentration specified in monograph. Method B determines the endotoxin concentration semi quantitatively in the preparation under examination.

Sensitivity of Lysate:

Sensitivity of the lysate is determined for every batch of lysate used. One vial from the batch of lysate must be used to evaluate the sensitivity. For the test, prepare a series of dilutions of CSE as 2l, l, 0.5l, 0.25 l; where l is the labeled sensitivity in EU per ml. Perform the test as per method in duplicate. Include a negative control using water BET. At least one dilution in each series must give a negative result. Calculate the average of the logarithm of the lowest concentration of endotoxin in each series of the dilutions for which positive results is found. The geometric mean end point concentration is measured sensitivity of the lysate in EU per ml which is calculated as:

Geometric mean end point concentration = antilog ($\sum e/f$)

Method A:

Prepare a series of test solutions with water BET. Adjust the pH to 6.0-8.0 using sterile 0.1 M HCl BET and 0.1 M NaOH BET.

Water BET is used as negative control and two positive controls are to be used.

One positive control is CSE at 2 λ concentration.

Second positive control is test solution spiked with CSE to give 2 λ concentrations (PPC)

Method: Procedure is same as under test for interfering factors. Perform in duplicate.

Interpretation of results: The given sample complies with endotoxin test if the positive product control gives positive result and the negative as well as the test solution gives negative result.

The test is not valid if the product positive control is negative or the negative control is positive or both of these conditions occur.

Retest: If the positive test is found for one of the test in the duplicate, the test is repeated as above.

Method B:

Prepare a series of test solutions in the concentrations of MVD, 0.5 MVD, and 0.25 MVD. Additionally prepare series of test solutions spiked with 2 λ CSE each (PPC)

Method: Procedure is same as under test for interfering factors. Perform in duplicate.

Calculation and interpretation of result: For the series of test solutions, determine the lowest concentration or the highest dilution giving positive reaction. Multiply this factor with lambda to obtain endotoxin concentration of the product.

E.g.: If the MVD = 8 and positive reaction is obtained at 0.25 MVD and l = 0.125 EU per ml,

Endotoxin concentration = 8x0.25x0.125

= 0.25 EU per ml.

The product meets the requirements of the test if the endotoxin concentration is less than the limit prescribed in the given monograph.

What are the advantages of LAL Test over Rabbits Test ?

1. LAL test is used to quantify the endotoxin count where as rabbit test is done to detect the presence of pyrogens.

2. Less time consuming (within 1 hr)

3. Simple and Economic.

Reference

Indian Pharmacopeia, 2007, Volume 1, Published by The Indian Pharmacopeia Commission, Ghaziabad; Tests for pyrogens 2.2.3