LAL Test Procedure

While the LAL test is a relatively simple procedure, especially when compared with the USP rabbit test, certain specific conditions must be met. These include the following:

- All materials that will come into contact with the LAL reagent or test sample must be thoroughly cleaned and depyrogenated.
- The reaction temperature cannot be outside the range of 36°C to 38°C.
- The pH of the reaction mixture must be within the range of pH 5–7.
- The reaction time should be no longer than one hour.
- Each test must be accompanied by positive and negative controls.

The basic procedure of the LAL test is the combination of 0.1 mL test sample with 0.1 mL LAL reagent. After one-hour incubation at 37°C, the mixture is analyzed for the presence of a gel clot. The LAL test is positive, indicating the presence of endotoxin, if the gel clot maintains its integrity after slow inversion of the test tube containing the mixture (Fig. 28-6).

Standards

For drugs, biological products, and medical devices, the endotoxin standard is called the United States Standard Endotoxin or the USP Reference Standard Endotoxin (RSE). The first RSE lot was designated as Lot EC-2 and had a defined activity of 1 Endotoxin Unit (EU)[2] in 0.2 nanograms (ng) of the standard (19). One vial of RSE contains 10,000 EUs.

When the USP selected the FDA endotoxin standard (purified LPS from *E. coli* 0113) as the new USP reference standard (with established potency in endotoxin units) this gave manufacturers the opportunity to standardize their own control endotoxin standard (CSE) against the USP RSE.

If a manufacturer chooses to use an endotoxin preparation (CSE) other than the U. S. RSE, the CSE will have to be standardized against the RSE. What this means is that the CSE reaction in the rabbit, its uniformity, its stability, and its interaction to a particular LAL lot all must be determined and related to these same characteristics of the RSE.

Validation of the LAL Test

To validate the use of the LAL test for any application requires two determinations: initial qualification of the laboratory and inhibition or enhancement properties of the product on the LAL–endotoxin interaction. Extensive details of LAL test validation requirements are found in the "Guideline on Validation of the Limulus Amebocyte Lysate Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices" (20).

Qualification of the laboratory simply involves using the selected test method (gel clot end point, chromogenic, and end point turbidimetric, or kinetic turbidimetric techniques) to determine its variability, to test new lots of lysate before use, and to qualify the ability of the analyst(s) to conduct the test. The LAL reagent used must have a confirmed potency (sensitivity). This is achieved by combining the particular reagent with a series of concentrations of RSE or CSE endotoxin bracketing the stated sensitivity (EU/mL) of the LAL reagent. Use four replicates per concentration of endotoxin. The series of endotoxin concentrations are prepared by twofold dilutions of the RSE or CSE endotoxin using LAL-negative water for injection. Following incubation and end point determination (manual or instrumental), the sensitivity of the LAL reagent will be confirmed if the test results are positive to within one twofold dilution of the state label potency.

[2] It has become accepted practice to use Endotoxin Units (EU) as the more desirable expression of endotoxin strength than weight or concentration terms. The use of EU will allow any endotoxin type or lot to be used as a reference lot because its activity can always be related to the original U.S. Reference Standard lot. This chapter will use the EU term as much as possible, but most literature references cited will use the weight or concentration terms as reported in the published articles. It is noted in the USP and EP that 1 EU = 1 IU (international unit).