SOP: LAL ASSAY - Gel Clot Method

Approvals:

Preparer: Dr. Maggie Bryans — Date: 09JAN14
Reviewer: Jason McMillan — Date: 10JAN14

1. **Purpose:**
   1.1. To perform the LAL Gel Clot Assay
2. **Scope:**
   2.1. To perform the LAL Gel Clot assay on various samples such as raw materials, in process materials and the final product for determination of endotoxin concentration.
3. **Responsibility:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. LAL pack instructions
   4.2. water bath SOP
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. LRW (LAL reagent water)
   7.2. LAL with a label sensitivity of 0.06EU/mL or 0.03EU/mL
   7.3. 5-10mL syringe and needle
   7.4. de-pyrogenated soda lime test tubes with screw caps
   7.5. 100µl micropipette and sterile pipet tips
   7.6. test tube rack
   7.7. 37°C heating block
8. **Procedure:**
   8.1. **Prepare the LAL Reagent**
      8.1.1. Reconstitute the LAL by adding LAL grade reagent water (LRW). Swirl occasionally until completely dissolved (about 3 minutes).
   8.2. **Dilute the Sample**
      8.2.1. Set up a row of 7 de-pyrogenated test tubes and label the tubes as: Undiluted, 1:2, 1:4, 1:8, 1:16, 1:32, Negative Control.
      8.2.2. Add 100µL LRW to all tubes EXCEPT the "Undiluted" tube using the same pipet tip.
      8.2.3. Add 200µL of the sample to the "Undiluted" tube. Change pipet tip.
      8.2.4. Remove 100µL from the "Undiluted" tube. Add it to the 1:2 tube.
      8.2.5. Vortex the tube for 4 seconds. Change pipet tip.
      8.2.6. Remove 100µL from the 1:2 tube. Add it to the 1:4 tube.
      8.2.7. Vortex mix the 1:4 tube for 4 seconds. Change pipet tip.
      8.2.8. Remove 100µL from the 1:4 tube. Add it to the 1:8 tube.
      8.2.9. Vortex mix the 1:8 tube for 4 seconds. Change pipet tip.

SOP: LAL ASSAY - Gel Clot Method

8.2.10. Remove 100µL from the 1:8 tube. Add it to the 1:16 tube.
8.2.11. Vortex mix the 1:16 tube for 4 seconds. Change pipet tip.
8.2.12. Remove 100µL from the 1:16 tube. Add it to the 1:32 tube.
8.2.13. Vortex mix the 1:32 tube for 4 seconds. Change pipet tip.
8.2.14. Remove 100µL from the 1:32 tube, and DISCARD it.
8.2.15. Do not add sample to the negative control tube.
Note: All the tubes should have 100µL of liquid.

8.3. **Add the LAL Reagent**

8.3.1. Starting with the negative controls and proceeding from the lowest to the highest sample concentration, add 100µL LAL to each tube. Tips need to be changed after each addition.
Note: LAL must be added to all tubes within 2 minutes.
8.3.2. Shake the test tube rack vigorously for 30 seconds to mix the LAL and sample.

8.4. **Incubate the Tubes**

8.4.1. Place the screw caps on the tubes and CAREFULLY place the rack in the 37°C heating block (Do not disturb other racks). Record the temperature and time.
Note: Do not disturb the tubes during the incubation. Once a clot is broken, it will not re-form.
8.4.2. Incubate for approximately 60 minutes.

8.5. **Analyze the Tubes**

8.5.1. Remove the tubes one at a time from the heating block and invert them SLOWLY and SMOOTHLY. Score tubes as positive if a firm clot has formed. Score tubes as negative if a gel holds, but collapses after the tube is fully inverted.
8.5.2. Record data.
8.5.3. Determine the amount of endotoxin in the samples using the formula:
Endotoxin concentration < LAL label sensitivity x dilution factor of most concentrated sample NOT to clot.

9. Attachments:

9.1. Data Table

10. History:

Name	Date	Amendment
Deb Audino	2001	Initial Release
Deb Audino	2003	Added more detailed directions.
Deb Audino	02Feb05	Replaced CSE with a sample.
Deb Audino	10Oct05	Added undiluted sample, data table, and how to calculate endotoxin level.
Deb Audino	04Apr08	College name change

SOP: LAL ASSAY - Gel Clot Method

Jason McMillan	10JAN14	College name change
Jason McMillan	11FEB16	Changed water bath to heating block, replaced Para film with screw caps, and removed inverting tubes due to long tips

	Undiluted 1	1:2	1:4	1:8	1:16	1:32	Negative Control
Sample ID _____							
Sample ID _____							