New Hampshire Community Technical College
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Portsmouth, NH 03801

Title: LAL ASSAY - Gel Clot Method SOP

Approvals

Preparer:____Deb Audino_____ Date _100605_____
Reviewer:___Bob O'Brien_____ Date _100605_____

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. Incubator SOP
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. LRW (LAL reagent water)
   7.2. LAL with a known label sensitivity
   7.3. Depyrogenated soda lime test tubes
   7.4. 100ul micropipetter and sterile pipet tips
   7.5. parafilm
   7.6. test tube rack
   7.7. 37°C water bath
8. **Process**
   8.1. **Prepare the LAL Reagent**
      8.1.1. Reconstitute the LAL by adding LRW. Swirl occasionally until completely dissolved (about 3 minutes)
   8.2. **Dilute the Sample**
      8.2.1. Set up a row of 7 depyrogenated test tubes and label the tubes as: undiluted, 1:2, 1:4, 1:8, 1:16, 1:32, neg control.
      NOTE: KEEP TUBES COVERED WITH PARAFILM WHEN NOT IN USE.
      8.2.2. Add 100ul LRW to all tubes EXCEPT THE "UNDILUTED" TUBE using the same pipet tip.

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8.2.3. Add 200ul of the sample to the "undiluted" tube.
8.2.4. Tip the tube so that the liquid reaches the lip of the tube and remove 100ul of the liquid. Add it to the 1:2 tube.
8.2.5. Vortex mix the tube for 4 seconds. Change pipet tip.
8.2.6. Tip the tube so that the liquid reaches the lip of the tube and remove 100ul of the liquid. 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. Tip the tube so that the liquid reaches the lip of the tube and remove 100ul of the liquid. Add it to the 1:8 tube.
8.2.9. Vortex mix the 1:8 tube for 4 seconds. Change pipet tip.
8.2.10. Tip the tube so that the liquid reaches the lip of the tube and remove 100ul of the liquid. 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. Tip the tube so that the liquid reaches the lip of the tube and remove 100ul of the liquid. 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. Tip the 1:32 tube, remove 100ul and DISCARD it.
8.2.15. Do not add sample to the negative control tube.
NOTE: ALL THE TUBES SHOULD HAVE 100ul 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 100ul LAL to each tube.
NOTE: LAL MUST BE ADDED TO ALL TUBES WITHIN 2 MINUTES. TIPS NEED TO BE CHANGED AFTER EACH ADDITION
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. Cover the tubes with parafilm and CAREFULLY place the rack in the water bath at about 37C (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 REFORM.
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 incubator 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.

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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:
10.1. Deb Audino 2001, Initial release
10.2. Deb Audino 2003
10.3. Deb Audino 2/2005, replaced CSE with a sample
10.4. Deb Audino 10/10/05, added undiluted sample, added data table, added how to calculate endotoxin level

Attachment:
Data Table

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