Standard Operating Procedure

Title: Bacterial Endotoxin Testing (LAL) - Gel-Clot Method

The reagents used for testing and all the disposable equipment must be disposed of into the Biohazard Bin.

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GEL-CLOT TEST METHOD

1. General

1.1. The gel-clot method for bacterial endotoxin testing described in this SOP is based on the fact that Limulus Amoebocyte Lysate (LAL) will form a firm gel in the presence of bacterial endotoxin.

1.2. New operators must be adequately trained by a competent staff member, prior to performing routine testing. Routine testing may be performed once all training requirements are met and have been deemed to be satisfactory following review.

1.3. The reagents referred to in the Test Method i.e. "Pyrogent" Lysate, "Pyrosperse" dispersing agent and *Escherichia coli* Endotoxin are manufactured by Bio Whittaker Inc. Pyrogent and Endotoxin are ideally can be purchased in a 200 test kit form “Pyrogent plus” Cat. No. N284. Reagents of another manufacturer may not be substituted in this test method unless prior validation of their suitability has been carried out.

1.4. Bacterial Endotoxin testing is to be conducted on all WFI samples and batches of product for injection or irrigation where required as part of product specifications.

1.4.1. The testing regime for the gel-clot test is for the individual testing of the samples taken from the beginning, middle and end of the batch. Therefore a total of three LAL gel-clot tests are conducted for each commercial batch.

1.5. Documentation of LAL gel clot session should be recorded on **Form 685** and stored in the “LAL Gel-Clot Test Session Results” File. This includes the date of test session, Operator’s

3.1.4. **PYROSPERSE - MUST** be at room temperature before use. Add 200µL Pyrosperse to each of the 10mL test samples to form a 2% concentration. Vortex well. Some precipitation of Marcain and Naropin products is to be expected.

3.1.5. Transfer an amount of the prepared test solution into a fresh pyrogen free test tube to be used for section 4 as the PPC. The remaining amount is the test solution. This will depend on the Lysate sensitivity for accurate volume – see Appendix 2.

4. Preparing Positive Product control

4.1. Prepare a positive product control for each test sample, prepared in section 3.1, by spiking with Endotoxin of concentration twice the Lysate sensitivity, e.g. When using a Lysate of sensitivity **0.06 EU/mL** pipette 50µL of a 10 EU/mL Endotoxin standard into the tube containing 4mL of appropriately pH adjusted and diluted test sample. Vortex well. This will give an Endotoxin concentration in the Positive Product control of **0.125 EU/mL**. See Appendix 2 for calculations using other concentrations of Endotoxin standard.

5. Preparation of Raw materials

5.1 To prepare Raw Materials (Sodium Chloride, Dextrose Anhydrous & Sodium Acetate) for bacterial Endotoxin testing see Raw Material Specifications.

5.2. Prepare Raw Material Solution for testing using the same method as finished product (sections 3.1.2 to 3.1.4 – test solution and section 4.1 – positive product control).

6. Testing of Process Water

6.1. See **MICLAB 055** for the method and frequency of sampling process water outlets for pyrogen testing. The autoclaved 100mL vial of sample water will be used as the test solution.

7. Preparation of Working Endotoxin Standards

7.1. Prepare a two fold dilution series of the vortexed Endotoxin in pyrogen free WFI to obtain final Endotoxin concentrations of 1.0, 0.5, 0.25, 0.125, 0.06, 0.03, 0.015 EU/mL.

7.2. When the concentration of Endotoxin is 10 EU/mL, the following dilution series will provide the necessary concentrations:

Tube 1	200µL 10 EU/mL Endotoxin + 1.8mL pyrogen free WFI	= 1 EU/mL
Tube 2	1mL of Tube 1 + 1mL pyrogen free WFI	= 0.5 EU/mL
Tube 3	1mL of Tube 2 + 1mL pyrogen free WFI	= 0.25 EU/mL
Tube 4	1mL of Tube 3 + 1mL pyrogen free WFI	= 0.125 EU mL
Tube 5	1mL of Tube 4 + 1mL pyrogen free WFI	= 0.06 EU/mL
Tube 6	1mL of Tube 5 + 1mL pyrogen free WFI	= 0.03 EU/mL
Tube 7	1mL of Tube 5 + 1mL pyrogen free WFI	= 0.015 EU/mL

See Appendix 1 for initial dilution of Tube 1 using other Endotoxin concentrations. Tubes 2-6 remain the same.

7.3. Mix each dilution Tube for 30 seconds on the vortex mixer before transfer. For routine product testing, tubes 4, 5, 6 and 7 are used for standards. For Lysate Verification and

12. Appendix 1 - Initial dilution of Tube 1 using other Endotoxin concentrations

1. If Endotoxin = 10 EU/mL
   Tube 1 = 200µL + 1.8mL WFI = 1 EU/mL

2. If Endotoxin = 12 EU/mL
   Tube 1 = 200µL + 2.2mL WFI = 1 EU/mL

3. If Endotoxin = 14 EU/mL
   Tube 1 = 200µL + 2.6mL WFI = 1 EU/mL

4. If Endotoxin = 16 EU/mL
   Tube 1 = 200µL + 3.0mL WFI = 1 EU/mL

5. If Endotoxin = 18 EU/mL
   Tube 1 = 200µL + 3.4mL WFI = 1 EU/mL

6. If Endotoxin = 20 EU/mL
   Tube 1 = 200µL + 3.8mL WFI = 1 EU/mL

13. Appendix 2 - Positive Product Controls

Spike with an Endotoxin concentration of twice the Lysate sensitivity.

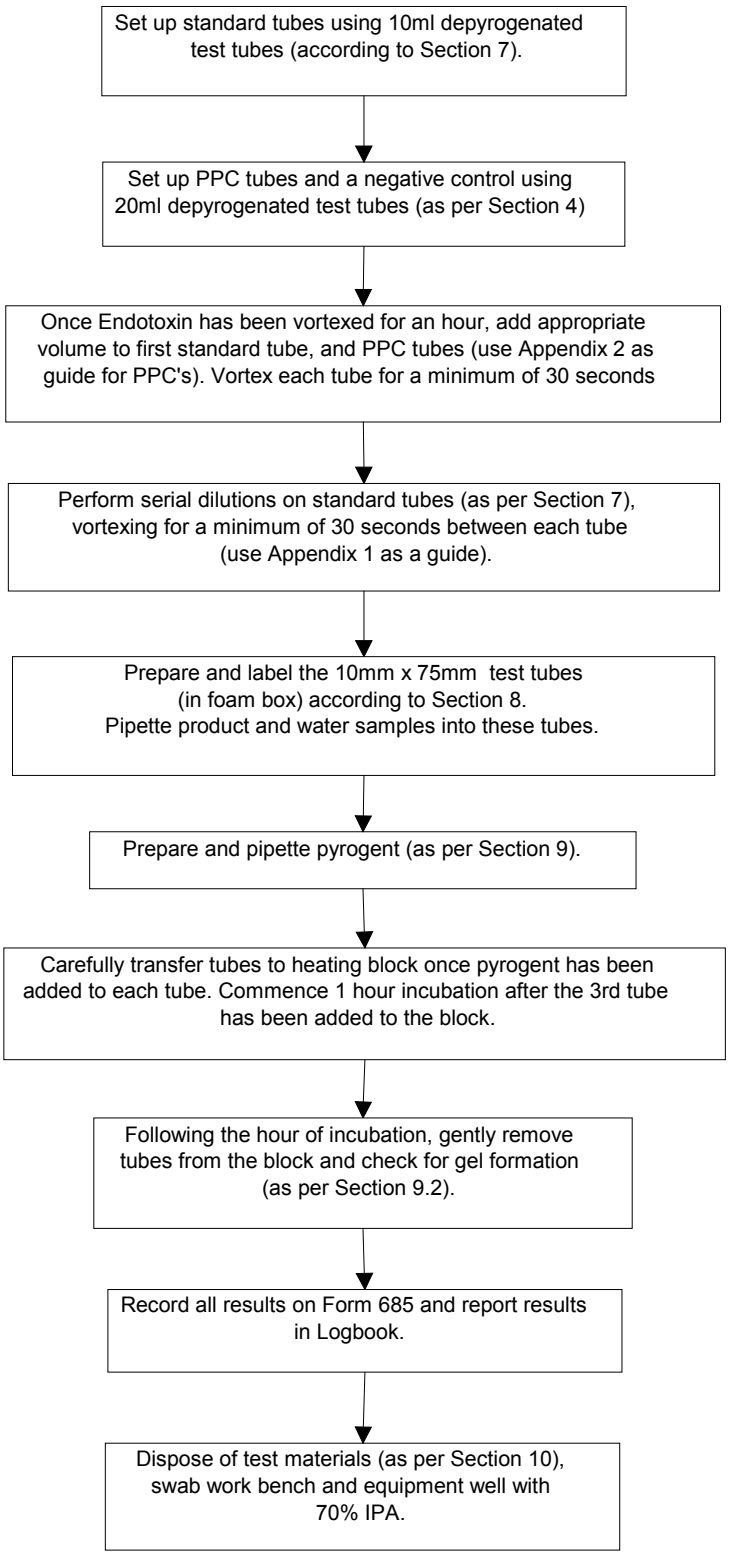
1. For 10 EU/mL Endotoxin
   a) If Lysate = 0.06 EU/mL then need to spike with 0.125 EU/mL
      4.0mL product + 50µL of 10 EU/mL Endotoxin.
   b) If Lysate = 0.125 EU/mL then need to spike with 0.25 EU/mL
      3.9mL product + 100µL of 10 EU/mL Endotoxin.

2. For 12 EU/mL Endotoxin
   a) If Lysate = 0.06 EU/mL then need to spike with 0.125 EU/mL
      4.8mL product + 50µL of 12 EU/mL Endotoxin.
   b) If Lysate = 0.125 EU/mL then need to spike with 0.25 EU/mL
      4.7mL product + 100µL of 12 EU/mL Endotoxin.

3. For 14 EU/mL Endotoxin
   a) If Lysate = 0.06 EU/mL then need to spike with 0.125 EU/mL
      5.6mL product + 50µL of 14 EU/mL Endotoxin.
   b) If Lysate = 0.125 EU/mL then need to spike with 0.25 EU/mL
      5.5mL product + 100µL of 14 EU/mL Endotoxin.

4. For 16 EU/mL Endotoxin

continued



LAL Gel-Clot Test Session Results

(Ref. MICLAB 080, MICLAB 015)

Assay Date: _____

Analyst: _____

TEST SESSION STANDARDS - RESULTS

Key: (+) firm gel, (-) no gel or viscous gel.

Replicate Assay No.	Positive Endotoxin Controls Concentration (EU/ml)						Endpoint (EU/ml)
	0.5	0.25	0.125	0.06	0.03	0.015	
1							
2							
3							
4							

NEGATIVE CONTROLS

Key: (+) firm gel, (-) no gel or viscous gel.

Replicate Assay No.	Control Results
1	
2	

TEST REAGENTS

Reagents	Lot No.	Reconstitution Date	Expiry date	
Pyrogent				EU/mL sensitivity
Endotoxin				EU/mL potency
Pyrosperse		NA		2% working concentration
Test kit		NA		

L.A.L, Endotoxin & Endotoxin Working Standards diluent.

W.F.I. (Tested to be L.A.L. negative) Batch No.: _____

Comments about Session: _____

SIGNATURE _____

Date: _____

