

Standard Operating Procedure

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

Operator Verification, tubes 3, 4, 5, 6 and 7 should be used as standards and the test must be done in quadruplicate.

8. Performing the Qualitative LAL test

- 8.1. Prepare and label the 10 x 75 mm pyrogen-free test tubes as below.

NOTE: All solutions must be vortexed for 15 seconds prior to pipetting into tubes.

8.1.1. Standards

For 0.06 EU/mL Lysate sensitivity use standard tubes 4,5, 6 and 7 (as prepared in Section 7). Pipette 100 μ L into duplicate 10x75mm labelled test tubes.
For 0.125 EU/mL Lysate sensitivity, use standard tubes 3, 4, 5 and 6.

8.1.2. Negative control

Pipette 100 μ L of pyrogen-free WFI into duplicate 10x75mm labelled test tubes.

8.1.3. Finished Products and Raw Materials

Pipette 100 μ L of each test solution (as prepared in Section 3 – Finished products, Section 5 – Raw Materials) into duplicate 10x75mm labelled test tubes.

8.1.4. Positive control

Pipette 100 μ L of each of the positive product control solutions (as prepared in Section 4) into single 10x75mm labelled test tubes.

8.1.5. Water Samples

Pipette 100 μ L of each water sample into a single 10x75mm labelled test tube.

NOTE

If more than one heating block is used per session, a set of standards and negative controls must be used in each heating block.

9. Preparation of Pyrogent

- 9.1. Prepare Pyrogent as follows. (Each new lot number kit must be verified for its Lysate Sensitivity before use and step 7.3 for details of requirements)

9.1.1. Remove the metal cap and grey stopper from the Pyrogent 50 test vial. Record the date of reconstitution on the vial.

9.1.2. Pipette 5.2 mL of WFI into the Pyrogent vial and mix gently to dissolve Pyrogent. Avoid foaming.

9.1.3. Pipette 100 μ L of the reconstituted Pyrogent to each of the above test tubes. Swirl each tube gently to mix while transferring to heating block. Incubate undisturbed at 37°C \pm 0.5 C for 60 \pm 2 minutes.

9.1.4. Any remaining Pyrogent at the conclusion of the working day can be frozen for use in the next test session. It will remain stable for up to 1 month. Reconstituted Pyrogent can be frozen-thawed only once.

- 9.2. After incubation, gently remove the tubes from the block and visually check for gel formation by inverting tubes through 180 degrees.

- 9.3. Test Results Interpretation, Out-of-Specification and Test Failure Response