

Standard Operating Procedure

Title: Bacterial Endotoxin Testing - KCA Method

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4. LAL grade WFI and Magnesium Chloride for dilutions
 5. Micropipettes 10-100 μ L, 100-1000 μ L with Pyrogen free micropipette tips
 6. Multichannel micropipette 200 μ L with Pyrogen free micropipette tips
 7. pH meter with 0.1M pyrogen-free HCl and NaOH for pH adjustments
 8. Certified KCA reagent kit (for example the brands "Charles River Endosafe" or "Cambrex (BioWhittaker)) stored at 2-8°C.
 9. Pyrogen free reagent wells
 10. Pyrogen free microtitre plates (96 well)
 11. BioWhittaker KQCL (brand) reader with software.

3. Verification Assays

Before any testing can be done using KCA, all reagents, equipment and operators must be verified. Results for all verification assays are to be kept in the "KCA Initial Qualification File". KCA Reagent qualification and equipment verification assays can be used as annual operator verification assays. Copies should be filed in both sections of the "KCA Initial Qualification File".

3.1. Reagent Verification

- 3.1.1. KCA Reagent Verification: for every new lot number of KCA kits from the supplier, a verification assay must be run to ensure that reagent activity conforms to specifications. This assay consists of running 4 replicates of the 5 concentration standard range with an LAL grade WFI blank (section 5.1).
- 3.1.2. Diluent Verification: for every new lot number of Water for Injection or Magnesium Chloride for dilutions, a verification assay must be run against the manufacturer's process LAL water to ensure that the diluents are pyrogen free and are acceptable to use for KCA Bacterial endotoxin testing. This assay consists of running duplicates of the 5 concentration standard range with a LAL grade WFI blank, (section 5.1) and duplicate samples of the new diluent and manufacturer's LAL process water including Positive Product Controls (spike concentration of 5 EU/mL). When entering the assay details, use the "Diluents" template.

3.2. Equipment Verification

- 3.2.1 96 well Microtitre plate Verification: for every new lot number of 96 well microtitre plates, a verification assay must be run to ensure that the plates are pyrogen free and free of "hot wells". This assay consists of running 4 replicates of the 5 concentration standard range with an LAL grade WFI blank, (section 5.1).

3.3. Operator Verification

- 3.3.1 Each new operator of the BioWhittaker KQCL reader must perform an initial qualification before performing routine testing. Also existing operators must perform a qualification on an annual basis. This assay consists of running 4 replicates of the 5 concentration standard range with an LAL grade WFI blank (section 5.1).

4. KCA Product Validation

Before routine testing can be performed on any product, it must be validated to ensure maximum endotoxin sensitivity and control spike recovery. If multiple concentrations of a type of product are to be tested, all concentrations must be validated separately as different concentrations may exhibit varying levels of inhibition or enhancement. For Validation, three (3) batches of a product must be assayed with one brand of reagent. To cover against reagent unavailability, a second brand of reagent may be validated using one (1) batch of product. The reagent brands to be used for KCA product validation should be available in all inclusive kit form. To complete the validation file, three (3) batches of the product must be routinely tested. The steps for product validation are as follows: