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Gentamicin Injection Job Aid to Assist with Laboratory Testing

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usp®

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The Promoting the Quality of Medicines Plus (PQM+) Program is a 5-year cooperative agreement between USAID and USP to sustainably strengthen medical product quality assurance systems in low- and middle-income countries. The program works to improve medical product quality through cross-sectoral and systems strengthening approaches and the application of international quality assurance standards across the pharmaceutical system. By sharing scientific expertise and providing technical support and leadership, PQM+ helps create resilient and robust local health systems that address diseases such as HIV/AIDS, tuberculosis, malaria, and neglected tropical diseases, as well as improve maternal, newborn, and child health.

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Acronyms

FDA	U.S. Food and Drug Administration
PQM+	Promoting the Quality of Medicines Plus program
PIR	product information report
USP	U.S. Pharmacopeial Convention

Overview

This document is a job aid to enable quick access to technical information for gentamicin testing. The information consists of Identification by thin layer chromatography, assay, pH, bacterial endotoxin test, sterility, particulate matter in injection, extractable volume, and packaging and storage controls. The information herein is compiled and adapted from the Promoting the Quality of Medicines (PQM+) gentamicin product information report (PIR) (2022–2023) and should be used to facilitate testing of gentamicin injection.

Aid for Gentamicin Injection: USP Tests/In-process testing from the PIR

Table 1. Tests for identification, assay, bacterial toxins, pH, appearance, color, melting point, density, optical activity, and solubility

Tests	Tests and Specifications
Identification by Thin Layer Chromatography <621>	**TEST SOLUTION PREPARATION:** Pipette 1mL of gentamicin injection in 200 ml volumetric flask, add 100ml water, mix well, dilute to volume, and mix again. From the resulting solution, pipette 1mL into another 200mL volumetric flask, add 100mL water, mix well, and dilute to volume. This corresponds to 0.001mg/ml of gentamicin sulfate. **STANDARD PREPARATION:** Weigh approximately 40mg of gentamicin sulfate reference standard (RS) into a 200mL, add 100mL of distilled water, mix, and dilute to volume. From the resulting solution, pipette 1mL of the solution into 200mL volumetric flask, mix and dilute volume, then mix well. This corresponds to 0.001mg/ml of gentamicin sulfate. **Chromatographic Conditions:** Chromatographic plate coated with 0.25mm layer of chromatographic silica gel having an average pore size of 6nm. **Mobile phase:** mixture of chloroform, methanol, and ammonium hydroxide (20:13:10). 20µl of the solution (using a calibrated syringe) is placed on the plate 1cm from the mobile phase and developed until the solvent moves three-fourth of the length of the plate. The plate is removed, air dried, and exposed to vapors of iodine in a detection jar containing iodine crystals. **Confirmation:** The intensity and the retention factor value of the three principal spots of the test solution corresponds to those obtained from the standard solution.
Assay	**Notes:** For substances like gentamicin, which are not easily quantified by chemical or physical means, it is still necessary to express quantities of biological activity in units of biological potency, each defined by an authoritative reference standard. The potency of the antibiotic is designated in either units (U) or µg of activity. Two general techniques are employed: the cylinder-plate (or plate) assay and the turbidimetric (or tube) assay. The cylinder-plate technique is used for gentamicin. **Method:** Cylinder-Plate Assay The cylinder-plate assay depends on diffusion of the antibiotic from a vertical cylinder through a solidified agar layer in a petri dish or plate. The growth of the specific microorganisms inoculated into the agar is prevented in a circular area or "zone" around the cylinder containing the solution of the antibiotic.
Bacterial Endotoxins Test <85>	Contains not more than 0.7 USP endotoxin units per mg of gentamicin.
pH <791>	Between 3.0 and 5.5.
Appearance	British Pharmacopeia and European Pharmacopeia: White or almost white, hygroscopic powder (2020). International Pharmacopeia: A white to cream-colored odorless powder.

Tests	Tests and Specifications
Color	White
Melting point	Melts with decomposition between 218ºC and 237ºC
Density	1.000 g/cm3
Optical Activity	International Pharmacopeia: 0.10 g/mL sample solution, with reference to the anhydrous substance: $[\alpha]_D^{20\,°C}$ = +107° to +121° (2020). USP: For 10 mg/mL sample solution, analyzed as per USP General Chapter <781> OPTICAL ROTATION: +107° to +121° (USP, n.d.) British Pharmacopeia: Test done as per Appendix V F (Determination of Optical Rotation and Specific Optical Rotation), (European Pharmacopeia. method 2.2.7): + 107° to + 121° (anhydrous basis) (Gentamicin Sulfate - British Pharmacopeia, n.d.) Merck| Sigma-Aldrich: $[\alpha]^{25}{}_{d}$ = 102° (water)
Solubility	Soluble 50 mg/mL

Aid for Gentamicin Injection: USP Tests / In-process testing from the PIR

Table 2. Tests for particulate matter in injection, other requirements, sterility test, extractable volume test, package leak test, and microbial assay

Tests	Tests and Specifications
Particulate Matter in Injection <788>	Perform per USP <788>. Must meet the requirements for small-volume injections.
Other Requirements	Meets requirements under injections and Implanted Drug Products <1>.
Sterility Tests <71>	The sterility test must be carried/performed under aseptic conditions (Sterile). (Note): To achieve sterility, the test environment must be adapted to the way in which the sterility test is performed.
Extractable Volume <1>	Must comply as per USP <1>.
Package Leak Test <1207.2>	Perform per USP <1207.2> Package Integrity Leak Test Technologies.
Assays <81>	Proceed as directed under Antibiotics-Microbial Assay <81> Use an accurately measured volume of injection diluted quantitatively and stepwise with Buffer B.3 to yield a test dilution having a concentration assumed to be equal to the median dose level of the standard (0.1µg of gentamicin per mL)

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