PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Antibiotic Assay of Different Antibiotics	**Effective Date:**
Supersedes: Nil	**Review Date:**
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1.0 OBJECTIVE:

1.1 The objective of this SOP is to define the procedure for antibiotic assay of different antibiotics.

2.0 SCOPE:

2.1 This SOP is applicable for antibiotic assay of different antibiotics in Microbiology Section of Quality Control Department.

3.0 RESPONSIBILITY:

3.1 Microbiologist is responsible for antibiotic assay of different antibiotics.

4.0 ACCOUNTABILITY:

4.1 Head Microbiology.

5.0 EHS CONSIDERATIONS:

5.1 Always wear nose mask, sterile hand gloves during culture handling.
5.2 Do not inhale the cultures.

6.0 PROCEDURE:

6.1 General

6.1.1 Use the culture of required test organism for antimicrobial assay of product.
6.1.2 Slants are prepared for the maintenance of test organism.
6.1.3 Maintain the culture as per current SOP Maintenance of pure culture.

6.2 Preparation of Assay Medium

6.2.1 Prepare antibiotic Assay media as per current SOP of Media preparation

6.3 Preparation of Culture Suspension

6.3.1 Streak Culture on agar slants and incubated at 32.5 ± 2.5°C for 24-48 hrs. (In case of Bacillus incubated at 32.5 ± 2.5°C for 5days)
6.3.2 Next day wash the culture with normal Saline (0.9% w/v NaCl).
6.3.3 Use this suspension for assay.
6.3.4 1ml of suspension added to 100ml of assay medium cooled upto skin bearing temperature.

6.4 Preparation of dilution for the Assay.

6.4.1 **Prepare the Phosphate Buffer**-Prepare the Potassium Phosphate Buffer for the antibiotic assay as per USP. the buffer are sterilized after preparation and the p^H specified in each case is the p^H after sterilization
(Example for Vancomycin: Potassium Phosphate Buffer 0.1M, Dissolve13.61gm of monobasic Potassium Phosphate in 1000ml of water. adjust with 18N phosphoric acid or 10N potassium hydroxide to a p^H of 4.5±0.05.)

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6.4.2 Preparation of the Standard- To prepare a stock solution dissolve a quantity of the USP Reference Standard/Working Standard of given antibiotic, accurately weighed, or the entire contents of a vial of USP Reference Standard, where appropriate in the solvent specified in USP and then dilute to the required concentration as indicated. Store in a refrigerator and use within the period indicated. On the day of the assay, prepare from the stock solution five or more test dilution, the successive solution increasing stepwise in concentration, usually in the ratio of 1:1.25 for a cylinder plate assay. Use the final diluents specified. And a sequence such that the middle or median has the concentration designated.

6.4.3 Preparation of the sample- From the information available for the preparation to be assayed (the "unknown") assign to it an assumed potency per unit weight or volume and on this assumption prepare on the day of the assay a stock solution and test dilution as specified for each antibiotic but with the same final diluents as used for the USP Reference Standard. The assay with five level of the standard requires only one level of unknown at a concentration assumed equal to the median level of the standard.

6.5 Preparation of assay Plate for Antibiotic Assay (Cylinder-Plate Method.)

6.5.1 Cool the required amount of media up to skin bearing temperature.

6.5.2 Add necessary amount of culture to the medium, and swirl well to mix medium and culture.

6.5.3 Pour 25ml quantities aseptically with sterile measuring cylinder to sterile Petri dishes and allow the medium to solidify for 1 hour.

6.5.4 With the help of sterile borer, make 4 wells in each plate.

6.5.5 Use 4 Petri plates per sample.

6.6 Test procedure

6.6.1 Add 100 µl of each of the high and low concentration dilutions of standard and test solutions accurately using an auto pipette into the wells.

6.6.2 Allow solutions to diffuse through the medium for 1 - 4 hrs at room temperature.

6.6.3 Incubate the plates at 32.5 ± 2.5°C for 18hrs.

6.6.4 Diameters of zones of inhibition are measured using antibiotic zone reader or Vernier calipers.

6.6.5 Calculation

Percentage potency = antilog (2.0 + a log I)

Where,

I = ratio of high and low concentration

$$a = \frac{(T_H + T_L) - (S_H + S_L)}{(T_H - T_L) + (S_H - S_L)}$$

Where,

T_H = diameter of high concentration of test

T_L = diameter of low concentration of test

S_H = diameter of high concentration of Std.

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S_L = diameter of low concentration of Std.

6.6.6 From percentage potency and potency of standard used, potency of sample is determined.

6.6.7 Report prepared as per **Annexure**

7.0 DEFINITIONS AND ABBREVIATIONS

SOP : Standard operation procedure
QC : Quality control
USP : United States Pharmacopeia

8.0 REFERENCE

8.1 USP Chapter No 81.

9.0 ANNEXURES

9.1 Annexure I : Report of Antibiotic Assay

9.2 Annexure I : Antibiotic Assay Record

10.0 DISTRIBUTION DETAILS

10.1 Controlled copy of this SOP shall be distributed to Quality Assurance and Microbiology Department.

11.0 REVISION HISTORY

Supersedes SOP No.	Change Control No.	Reason for revision