STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Microbiological Assay for Antibiotic & Vitamin B_{12}	**Effective Date:**
Supersedes: Nil	**Review Date:**
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1.0 OBJECTIVE

To lay down a procedure for operation for Microbial assay.

2.0 RESPONSIBILITY

Microbiologist/QC Executive

3.0 ACCOUNTABILITY

Quality Control Manager

4.0 PROCEDURE

4.1 INTRODUCTION

4.1.1 The inhibition of microbial growth under standardized condition may be utilized for demonstrating the therapeutic efficacy of antibiotics. Any such changes in the antibiotics molecules which may not be defected by chemicals methods will be revealed by a change in the antimicrobial activity and microbiological assays are very useful for resolving doubts regarding possible change in potency of antibiotics and their preparation .The microbial assay is bases upon a comparison of the inhibition of growth of micro-organisms by known concentration of a standard preparation of antibiotics having known activity.

4.2 METHOD: CUP PLATE METHOD

4.2.1 The Cup-Plate methods depends upon diffusion of antibiotics from a vertical cylinder through a solidified agar layer in a petri-dish or plate to an extent such that growth of the added microorganisms is prevented entirely in a zone around the cylinder containing solution of antibiotics.

4.3 REQUIRMENTS

4.3.1 Given Antibiotics to be assayed, working standard, dehydrated media, Buffer solution, Initial solvents, Test Organism, Spectrophotometer, sterile petri-dish, sterile volumetric flask& cylinder (25 ml).

4.4 PREPARATION OF STANDARD SOLUTION

4.4.1 To prepare a stock solution, dissolve a quantity of the standard preparation of a given antibiotics, accurately weight; indicated in the table–3 (Appendix 9.1/Indian Pharmacopoeia, Volume –2) in the solvent specified in the table, and then dilute to the required concentration as indicated.

4.5 PREPARATION OF THE SAMPLE SOLUTION

4.5.1 From the information available for the substances being examined at (the unknown) assign to it and 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 antibiotics in table 3 (Appendix 9.1/Indian Pharmacopoeia Volume –2). But with the name final diluent as used for the standard preparation.

4.6 TEST ORGANISMS.

4.6.1 The test organism for each antibiotics is listed in Table 4 (Appendix 9.1/Indian Pharmacopoeia, Volume –2) together with it identification number (ATCC Number).Maintain a culture on slant of medium and under the incubation condition specified in Table 5 (Appendix 9.1/Indian Pharmacopoeia, Volume –2) and transfer weekly to fresh slants.

4.7 PREPARATION OF INNOCULUM

4.7.1 Take the test organism maintained on the slant, and scrap its growth with Nichrome wire loop with 25 ml sterile 0.9% Saline. Incubate for 30 minutes and determine the dilution factor which will give 25% light transmission at about 530 nm. Determine the amount of suspension to be added to each 100 ml agar.

4.8 PREPARATION OF PLATE

4.8.1 Inoculate the previously liquidified medium appropriate to the assay with require quantity of suspension of micro-organism, add the suspension to the medium at a temperature between 40 to 50°C and immediately pour the inoculated medium into petri-dishes to give a depth of 3-4 mm. Ensure that the layers of medium are uniform in thickness, by placing the petri-dishes on a level surface.

4.8.2 The prepared dishes must be stored in a manner so as to ensure that no significant growth or death of the organism occurs before the petri-dishes are used for Assay.

4.8.3 Using the appropriate buffer solution indicated in table 2 & 3 (Appendix 9.1/Indian Pharmacopoeia, Volume –2). Prepare solutions

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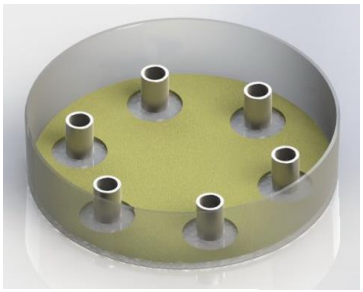
of known concentration of the standard preparation and solutions of the corresponding assumed concentration of the antibiotic to be examined .Apply the solutions to the surface of the solid medium in well (Well in the agar surface should be made by punching into the agar surface with the help of SS316L borer).The volume of the solution added to each well must be uniform and sufficient almost fill the wells (i.e.100 µl).



Pipetting dilution

4.8.4 When the petri-dishes are used, arrange the solutions of the standard preparation and the antibiotic to be examined on each dish so that they alternate around the dish and so that the highest concentration of the standard and test preparation are not adjacent.

4.8.5 Leave the petri-dishes are used, arrange the solutions of the standard preparation and the antibiotics to be examined on each dish so that they alternate around the dish and so that the highest concentration of the standard and test preparation are not adjacent. Leave the petri-dishes standing for about 1 hrs. for pre-incubation diffusion. Incubate the petri-dishes at 37°C for 18-24 hours.

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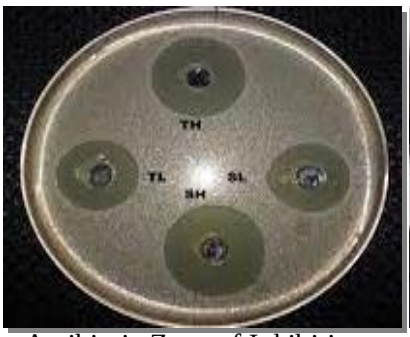
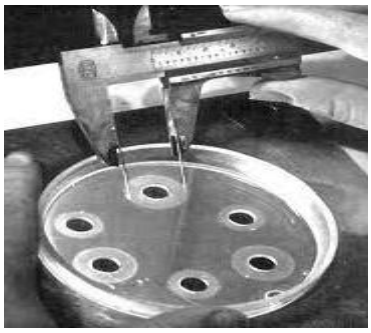
4.8.6 After the incubation period, accurately measure the diameters of zones of inhibition and calculate the results.



Antibiotic Zone of Inhibition


Measuring Antibiotic Zone


Vitamin B_{12} Zone of Exhibition

4.9 ESTIMATION OF POTENCY

$$a = \frac{(TH + TL) - (SH + SL)}{(TH + TL) - (SH + SL)}$$

$M = a \times I$

Potency of Sample =potency ratio x dilution factor x units per mg of standard

SH	-	Standard high Concentration
SL	-	Standard Low Concentration
TH	-	Test high Concentration
TL	-	Test Low Concentration
M	-	log Potency ratio = a x I
I	-	log Ratio of dosage
Potency Ratio	-	Antilog M

5.0 REASON FOR REVISION

New SOP.

7.0 DISTRIBUTION:

Certified Copy No. 1 : Head of Department – Quality Control
Certified Copy No. 2 : Microbiology Dept
Certified Copy No. 3 : For Record file
Original Copy : Head – Quality Assurance.

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8.0 ANNEXURES:

Annexure-I : Format for Microbial Assay for Vancomycin Hydrochloride

Annexure-II : Format for Microbial Assay for Vitamin B_{12}

9.0 REFERENCES:

Pharmaceutical Microbiology Manual", FDA, 2014
Indian Pharmacopoeia

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ANNEXURE-I

MICROBIOLOGICAL ASSAY FOR VANCOMYCIN HYDROCHLORIDE STERILE USP (BY CUP PLATE METHOD)

Product/Item Name:	
Batch No. :	**Medium: Antibiotic Medium No. 8 M 041**
Batch Size:	**Test Organisms:** *Bacillus subtilis* **(ATCC 6633)**
Mfg Date:	**Sampled On:**
Exp Date:	**Sampled By:**
Date of testing:	**Released On:**

Testing Details:

Initial diluent: Distilled water Final diluent: Sterile Phosphate Buffer No.4 (pH: 4.5)

Potency of the Std. = _____mcg/mg

Standard Weight: _____mg/100 ml → 10 ml/50 ml → 2 ml/25 ml → 2 ml/100 ml.

Sample Weight: _____mg/100 ml → 10 ml/50 ml → 2 ml/25 ml → 2 ml/100 ml.

Plate No.	TH	TL	SH	SL
01				
02				
03				
04				
Avg.(mm)				

Key: **TH**: Test high dose, **TL**: Test low dose **SH**: Std. High dose, **SL**: Std. low dose

CALCULATION OF POTENCY:

$$a = \frac{(TH+TL) - (SH+SL)}{(TH-TL) + (SH-SL)} = \text{----------} = \text{----------} =$$

I = Log of doses: 0.6021

M= Log Potency ratio = a x 0.6021 = ---------- =

Potency ratio = Antilog M = ----------

Potency of sample = Potency ration x dilution factor x Units/ ml. of std.

= ----------mcg/mg. on as is basis.

ANALYSED BY/DATE **CHECKED BY/DATE**

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ANNEXURE-II

MICROBIOLOGICAL ASSAY FOR VITAMIN B_{12} (BY CUP PLATE METHOD)

Product/Item Name:	
Batch No. :	**Medium B_{12} Culture agar (E. coli Maintenance Medium) (E. coli Mutant Culture Agar)**
Batch Size :	**Test Organisms:** *Escherichia coli*, **(ATCC 11105)**
Mfg Date :	**Sampled on :**
Exp Date :	**Sampled By :**
Date of testing :	**Released on :**

Testing Details:

Diluent: Distilled water (Sterile)
Potency of the Std. = _____mcg /mg

Std Wt.: _____ mg/50 ml →5 ml/100 ml.→ 10 /100 ml → 10/100 ml ----→ 5 ml/25 ml.
(SH)(0.25conc.) (SL) 0.05 conc.
Test wt. taken: _____ mcg/50 ml ----→ 5 ml/25 ml

(TH) 0.25conc. (TL) 0.5conc

Plate	TH	TL	SH	SL
01				
02				
03				
04				
Avg.(mm)				

Key : **TH** : Test high dose, **TL** : Test low dose **SH** : Std. High dose, **SL** : Std. low dose

CALCULATION OF POTENCY:

$$a = \frac{(TH+TL) - (SH+SL)}{(TH-TL) + (SH-SL)} = \text{---------- = ---------- =}$$

I = Log of doses: 0.699 (fixed value)

M= Log Potency ratio = a x **I** = ---------- =

Potency ratio = **Antilog M** = ----------

Potency of sample = Potency ration x dilution factor x Units/ ml. of std.

ANALYSED BY/DATE **CHECKED BY/DATE**