Microbiological assay of antibiotic

INTRODUCTION:

The microbiological assay is based upon a comparison of the inhibition of growth of bacteria by measured concentration of the antibiotics to be examined with that reduced by know concentration of a standard preparation of the antibiotic having a known activity.

Any suitable change in the antibiotic molecule which may not be detected by chemical methods will be revealed by a reduction in the anti microbial activity and hence microbiological assay are very useful for resolving doubt regarding possible loss of potency of antibiotics and their preparation.

Assay Design: Two Level Assays

Assay Method: Cup Plate

Reagent and Media:

1.0 Antibiotic assay medium no. 11
2.0 Antibiotic assay medium no. 1
3.0 Phosphate buffer pH 8.0
4.0 Normal saline (0.9%) prepare by dissolving 9 gm of sodium chloride in 1 liter of purified 3.0 Water and sterilize at 121°C and 15 lbs pressure for 30 minute.
5.0 Methanol AR grade.
6.0 Bacillus pumilus ATCC 14881

PREPRATION OF ASSAY MEDIA AND TEST ORGANISM INOCULUM MEDIUM

3.0 Phosphate buffer pH 8.0

Take 16.73 gm of K2HPO4 (DI Potassium Hydrogen phosphate) and 0.523gm KH2PO4 (Potassium Di Hydrogen phosphate) in a 1000 ml capacity beaker. Add approximate 850 ml of water stir well to dissolve, adjust the ph to 8.0 ± 0.1 with 10 M potassium Hydroxide. Transfer the solution into a 1000 ml capacity volumetric flask and make volume up to mark with water.

10M Potassium Hydroxide Preparations

Weigh about 56.11 gm of Potassium hydroxide in a beaker. Add about 70 ml water stir well to dissolve and cool. Transfer the solution into a 100 ml, flask and make the volume up to mark with water

4.0 Test organism: Bacillus pumilus ATCC 14881

1.0 Preparation of inoculum:

Maintain the culture on slant of antibiotic medium no. 11 Use a 24 hrs. Old slant to prepare inoculum .using 3 ml sterile normal saline wash the growth from the slant and inoculate on the surface of roux bottle
Containing 250 ml of Antibiotic assay medium no. 1 incubate for 5to7 days at 35 °C to 37°C .after incubation wash the growth using sterile normal saline. Centrifuge and decant the supernatant liquid

resuspended the sediment with 50 ml of normal saline solution. Heat the suspension to 70°C for 30 minutes. Wash the suspension with sterile normal saline 3 times
Resuspend in 50 ml of sterile normal saline. Store the suspension under refrigerator for not more than 1 month.For the assay determine by trial the amount of suspension to be added to each 100 ml of medium that result in satisfactory demarcation of the zones of inhibition and giving a reproducible dose
On the day of the assay prepare the inoculum by adding the stock suspension to a sufficient amount of antibiotic medium no. 11 that has been cooled to 45 to 60°C and mixing well to attin a homogenous suspension.

Test procedure:

1.0 Standard preparation:

Preparation of stock solution- 1mg/ml
Accurately weigh a quantity of Azithromycin working standard equivalent to 100mg and transfer to 100 ml volumetric flask. Dissolve with 10ml methanol and make up the volume with phosphate buffer pH 8.0 store in refrigerator and use up to 7 days.

1.1 Preparation of standard high and low solution:
A. Standard high solution preparation (SH):
Take a 200 ml volumetric flask containing 50ml of phosphate buffer pH 8.0 and accurately pipette 1 ml of standard stock solution to the flask. Shake well and make up the volume with phosphate buffer pH 8.0.

B. Standard low preparation (SL)
Take 50 ml volumetric flask containing 10ml of phosphate buffer pH 8.0 and accurately pipette 25 ml of standard High solution to the flask. Shake well and make up the volume with phosphate buffer pH 8.0.

2.0 Preparation of test solution:
Weight and powder 20 tablets/ liquid accurately weigh a quantity of the sample equivalent to 100 mg of Azithromycin and transfer to a 100 ml volumetric flask. Dissolve in 50 ml methanol and make up the volume with phosphate buffer pH 8.0.

2.1 Preparation of test high and low solution:

A. Test high solution (UH) :
Take a 200 ml volumetric flask containing 50ml of phosphate buffer pH 8.0 and accurately pipette 1 ml of test stock solution to the flask. Shake well and make up the volume with phosphate buffer pH 8.0.

B. Test low solution (UL) :
Take 50 ml volumetric flask containing 10ml of phosphate buffer pH 8.0 and accurately pipette 25 ml of test High solution to the flask. Shake well and make up the volume with phosphate buffer pH 8.0.

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Preparation of plates:

Using sterile Petri dishes. Plate 21 ml of antibiotic assay medium no. 11 allow to solids. Add 4 ml of seed layer i.e. antibiotic medium no. 11 containing Bacillus pumilus inoculum. Spread the seed layer evenly over the base layer allow it to solids after solidification of the media in Petri dishes

Make four cups with the help of sterile stainless steel borer at equal distance prepare a set of 4 plates for each sample. Fill each cup with alternating standard and test dilution.

Keep the plates at room temperature for diffusion for 2 hrs. Incubate the plates at 37°C to 39°C in an incubator for 16 to 18 hours. Measure the diameter of the zone of inhibition.

Calculation:

Sum the diameter of the zones of inhibition of each dilution for the 4 plates and calculate the % potency of the sample from following equation.

% potency = Antilog (2.0 + a log I)

$$a = \frac{(UH + UL) - (SH + SL)}{(UH - UL) + (SH - SL)}$$

Where

UH = sum of the zone diameter of 4 plates with test high solution

UL = sum of the zone diameter of 4 plates with test low solution

SH = sum of the zone diameter of 4 plates with standard high solution

SL = sum of the zone diameter of 4 plates with standard low solution

I = Ratio of dilution

The potency of the sample in mg per tablet is given by the formula.

$$\frac{\%\ \text{potency} \times Ws \times P \times \text{Avg. wt.}}{100 \quad XW \times 1000}$$

Where:

Ws = Weight of working standard in mg

P = potency of working standard in µg/ mg (as IP)

W = weight of sample taken in mg

Avg. wt. == Average weight of a tablet in mg

NOTE: PROCEDURE FOR RETESTING:

For the purpose of confirming a doubtful result by any of the procedures

Proceed as directed under Procedure, but make allowance for the larger specimen size.