

Microbiological Assay of Antibiotics

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Microbiological Assay of Antibiotics

- The microbiological assay of an antibiotic is based upon a comparison of the inhibition of growth of micro-organisms by measured concentrations of the antibiotics under examination with that produced by known concentration of a standard preparation of the antibiotic having a known activity.

Types of Microbiological Assay

- The cylinder-plate(or cup-plate) method-

The cylinder-plate method (method A) depends upon diffusion of the antibiotic from a vertical cylinder through a solidified agar layer in the petri dish or plate to and extend such that growth of the added micro-organism is prevented entirely in a zone around the cylinder containing a solution of antibiotic

Types of Microbiological Assay

- The turbidimetric method-

This method depends upon the inhibition of growth of a microbial culture in a uniform solution of the antibiotic in a fluid medium that is favourable to its rapid growth in the absence of the antibiotic.

- The Diffusion Method

Preparation of the test

- **Culture media-**

Media has to be prepared for the specified test organism for the ingredients listed in IP according to the individual requirement of the test organism.

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Preparation of the test

- **Standard preparation and units of activity-**

A Standard Preparation is an authentic sample of the appropriate antibiotic for which the potency has been precisely determined by reference to the appropriate international standard. The Potency of the standard preparation may be expressed in International Units or in μg per mg of the pure antibiotic

Preparation of the test

- Preparation of the standard solution

The stock solution is prepared by dissolving a quantity of the Standard Preparation of a given antibiotic as per IP

Preparation of the test

- Preparation of the sample solution

From the information available for the substance under examination, a stock solution is prepared as specified in IP

Preparation of the test

- Test organism

The test organism for each antibiotic is listed in Table, together with its identification number in the American Type Culture Collection (ATCC)

Antibiotic	Test Organism	ATCC1 No.
Amikacin	<i>Staphylococcus aureus</i>	29737
Amphotericin B	<i>Saccharomyces cerevisiae</i>	9763
Bacitracin	<i>Micrococcus luteus</i>	10240
Bleomycin	<i>Mycobacterium smegmatis</i>	607
Carbenicillin	<i>Pseudomonas aeruginosa</i>	25619
Chlortetracycline	<i>Bacillus pumilus</i>	14884
Erythromycin	<i>Micrococcus luteus</i>	9341
Framycetin	<i>Bacillus pumilus</i>	14884
	<i>Bacillus subtilis</i>	6633
Gentamicin	<i>Staphylococcus epidermidis</i>	12228
Kanamycin sulphate	<i>Bacillus pumilus</i>	14884
	<i>Staphylococcus aureus</i>	29737
Neomycin	<i>Staphylococcus epidermidis</i>	12228
Novobiocin	<i>Staphylococcus epidermidis</i>	12228
Nystatin	<i>Saccharomyces cerevisiae</i>	2601
Oxytetracycline	<i>Bacillus cereus var, mycoides</i>	11778
	<i>Staphylococcus aureus</i>	29737
Polymyxin B	<i>Bordetella bronchiseptica</i>	4617
Spiramycin	<i>Bacillus pumilus</i>	6633
Streptomycin	<i>Bacillus subtilis</i>	6633
	<i>Klebsiella pneumoniae</i>	10031
Tetracycline	<i>Bacillus cereus</i>	11778
	<i>Staphylococcus aureus</i>	29737
Tobramycin	<i>Staphylococcus aureus</i>	29737
Tylosin	<i>Staphylococcus aureus</i>	9144

Preparation of the test

- Temperature control-

Thermostatic control is required at several stages of microbial assay when culturing a micro organism and preparing its inoculum and during incubation in a plate assay. Closer control of the temperature is required during incubation in a tube assay.

Preparation of the test

- Spectrophotometer –

Measuring transmittance within a fairly narrow frequency band requires a suitable spectrophotometer in which the wavelength of the light source can be varied or restricted by the use of a 580nm filter for preparing inocula of the required density or with a 530 nm filter for reading an absorbance in a tube assay

Methods of Microbiological Assay

A. Cylinder plate or cup plate method

- A previously liquefied medium with the required quantity of microbial suspension is inoculated
- The suspension is added to the medium at a temperature between 40-50 degree and inoculated medium is immediately poured

Methods of Microbiological Assay

- The solution are applied to the surface of the solid medium in sterile cylinder or in agar cavities
- They are incubated for about 18 hours at the temperature indicated
- The quantities estimation of antibiotic is done by accurately measuring the diameter or areas of the circular inhibition zones .

Methods of Microbiological Assay

B. Turbidimetric or Tube Assay method

- Advantage- shorter incubation period for the growth of the test organism(usually 3 to 4 hrs)
- Disadvantage- The presence of solvent residues inhibitory substances affects mor.
- Not recommended for cloudy or turbid preparation.

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Methods of Microbiological Assay

- Five different concentration of the standard solution are prepared for preparing the standard curve.
- 1ml of each concentration of the standard solution of the sample solution are placed in each of the tubes in duplicate at 9 ml of nutrients medium previously seeded with the appropriate test organism at to each other

Three control tubes are prepared

- One containing the inoculated culture medium(culture control)
- Another one treated immediately with 0.5 ml of dilute formaldehyde solution(blank)
- Third containing uninoculated culture medium.

Three control tubes are prepared

- All the tubes are placed in an incubator and maintain at the specified temperature for 3 to 4 hour.
- The growth of the test organism is measured by determining the absorbance at 530 nm of its against the blank.
- The standard calibration card is prepared and the absorbance obtained for the sample is plotted on it to obtain the concentration of the test antibiotic