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Microbial Assay

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Bioassays for Microorganism

Microbiological assays are used during production to work out the potency and internal control. These are wont to determine the pharmacokinetics of medicine in animal and human. In antimicrobial chemotherapy to watch, in managing, controlling the chemotherapeutic agents.

• Microorganisms have found widespread uses within the performance of bioassays for:

• Determining the concentration of certain compounds (e.g., amino-acids, vitamins and a few antibiotics) in complex chemical mixtures or in body fluids.

• Diagnosing certain diseases.

• Testing chemicals for potential mutagenicity or carcinogenicity.

• Monitoring purposes involving the utilization of immobilized enzymes.

• Sterility testing of antibiotics.

• Microbiological assay of antibiotics

• The potency of an antibiotic is estimated by comparing the inhibition of growth of sensitive micro-organisms produced by known concentrations of the antibiotic to be examined and a reference substance.

• The reference substances utilized in the assays are substances whose activity has been precisely determined with regard to the corresponding international standard or international reference preparation.

• The assay must be designed during a way which will permit examination of the validity of the mathematical model on which the potency equation is predicated.

• If a parallel-line model is chosen, the two log dose-response lines of the preparation to be examined and therefore the reference preparation must be parallel, they need to be linear over the range of doses utilized in the calculation.

• These conditions must be verified by validity tests for a given probability, usually P=0.05.

• Other mathematical models, like the slope ratio model, could also be used as long as proof of validity is demonstrated.

• Unless otherwise stated within the monograph, the arrogance limits (P=0.95) of the assay for potency aren't but 95 percent and less than 105 percent of the estimated potency.

Methods of microbiological assay of antibiotics

Following methods are used for microbiological assays:

Disc diffusion method (cylindrical cup plate method): Use petri dishes or rectangular trays filled to a depth of 3-4 mm with a medium that has previously been inoculated with an appropriate inoculum of a susceptible test organism prepared as described below. The agar could also be composed of two separate layers of which only the upper one could also be inoculated. The concentration of the inoculum should be so selected that the sharpest zones of inhibition and suitable dose-response at different concentrations of the quality are obtained. When using the inoculum, an inoculated medium containing 1 ml of inoculum per 100 ml of the medium is typically suitable. When the inoculum consists of a suspension of vegetative organisms, the temperature of the molten agar medium must not exceed 48-50°C at the time of inoculation. The dishes or trays should be especially selected with flat bottoms. During the filling they ought to be placed on a flat, level so on make sure that the layer of the medium are going to be of uniform thickness. With some test organisms, the procedure may be improved if the inoculated plates are allowed to dry for half-hour at temperature before use, or refrigerated at 4oC for several hours. For the appliance of the test solution, small sterile cylinders of uniform size, approximately 10 mm high and having an indoor diameter of roughly 5mm, made from suitable material like glass, porcelain, or chrome steel , are placed on the surface of the inoculated medium. Instead of cylinders, holes 8-10 mm in diameter could also be bored in medium with a previously sterilized borer. Other methods of application of the test solution can also be used. The arrangement on the plate should be such overlapping of zones is avoided. Solutions of the reference material of known concentration and corresponding dilutions of the test substance, presumed to be of roughly an equivalent concentration, are prepared during a sterile buffer of an appropriate pH value. To assess the validity of the assay a minimum of 3 different doses of the reference material should be used alongside an equal number of doses of the test substance which has an equivalent presumed activity because the solutions of the reference material. The dose levels used should be in progression, for instance, by preparing a series of dilutions within the ratio 2:1. Once the connection between the logarithm of concentration of the antibiotic and therefore the diameter of the zone of inhibition has been shown to be approximately rectilinear for the system used, routine assays could also be administered using only 2 concentrations of the reference material and a couple of dilutions of the test substance. Where a monograph gives directions for the initial preparation of an answer of the substance, this solution is then diluted as necessary with the acceptable sterile buffer. The solutions of the reference material and therefore the test substance are preferably arranged within the sort of a Latin square when rectangular trays are employed. When petri dishes are used, the solutions are arranged on each dish in order that the solutions of the reference material and people of the test substance alternate round the dish and are placed in such a fashion that the very best concentrations of the reference material and of the test substance aren't adjacent. he solutions are placed within the cylinders or holes by means of a pipette that delivers a consistent volume of liquid. When the holes are used, the delivered volume should be sufficient to fill them almost completely. The plates are incubated at an appropriate temperature, the chosen temperature being controlled at 0.5°C, for about 16 hours, and therefore the diameters or areas of the zones of inhibition produced by the numerous concentrations of the quality and of the test substance are measured accurately, preferably to the closest 0.1 mm of the particular zone size, by employing a suitable measuring instrument. From the results, the potency of the tested substance is calculated.

Turbidometric method: Inoculate an appropriate medium with a suspension of the chosen micro-organism having sensitivity to the antibiotic to be examined

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such a sufficiently large inhibition of microbial growth occurs within the conditions of the test. Use a known quantity of the suspension chosen so on obtain a readily measurable opacity after an time period of about 4 h. Use the inoculated medium immediately after its preparation. Using the solvent and therefore the solution indicated prepare solutions of the reference substance and of the antibiotic to be examined having known concentrations presumed to be an equal activity. In order that the validity of the assay could also be assesses, use not fewer than 3 doses of the reference substance and three doses of the antibiotic to be examined which has an equivalent presumed activity because the doses of the reference substance. It is preferable to use a series of doses in progression. So as to get the specified linearity, it's going to be necessary to pick from an outsized number 3 consecutive doses for the reference substance and therefore the antibiotic to be examined. Distribute an equal volume of every of the solutions into identical test-tubes and increase each tube an equal volume of inoculated medium (for example, 1 ml of the answer and 9 ml of the medium). For the assay of tyrothricin, add 0.1 ml of the answer to 9.9 ml of inoculated medium. Prepare at an equivalent time 2 control tubes without antibiotic, both containing the inoculated medium and to at least one of which is added immediately 0.5 ml of formaldehyde R. These tubes are wont to set the optical apparatus wont to measure the expansion. Place all the tubes, randomly distributed or during a Latin square or randomized block arrangement, during a water-bath or suitable apparatus fitted with a way of bringing all the tubes rapidly to the acceptable incubation temperature. Maintain them at that temperature for 3 h to 4 h, taking precautions to make sure uniformity of temperature and identical incubation time. After incubation, stop the expansion of the micro-organisms by adding 0.5 ml of formaldehyde R to every tube or by heat treatment and measure the opacity of three significant figures using suitable optical apparatus. Alternatively use a way which allows the opacity of every tube to be measured after precisely the same period of incubation. Calculate the potency using appropriate statistical methods. Linearity of the dose-response relationship, transformed or untransformed, is usually obtained only over a really limited range. It is this range which must be utilized in calculating the activity and it must include a minimum of 3 consecutive doses so as to allow linearity to be verified. In routine assays when the linearity of the system has been demonstrated over an adequate number of experiments employing a three-point assay, a two-point assay could also be sufficient, subject to agreement by the competent authority. However, altogether cases of dispute, a three-point assay must be applied. Use in each assay the amount of replications per dose sufficient to make sure the specified precision. The assay could also be repeated and therefore the results combined statistically to get the specified precision and to determine whether the potency of the antibiotic to be examined isn't but the minimum required.

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