concentration in the test sample is determined with the help of standard concentration-response curve.

Microbiological Assay of Niacin (Niacinamide)

The organism to be selected must be able to utilise free niacin, niacinamide, nicotinuric acid, cozymase and niacinamide nucleoside. *Lactobacillus plantarum* fully satisfies this requirement. This acid forming organism is unable to synthesise niacin for its metabolic processes. This organism is also non-pathogenic, easy to culture and least affected by the presence of other stimulatory or inhibitory substances normally found in pharmaceutical preparations containing niacin. It is grown on a simple stab culture containing gelatin, yeast extract and glucose. For assay it is cultured in the assay tubes by transferring to the liquid medium consisting of the basic medium containing an optimum amount of added niacin. The range of 0.05 to 0.5 μg of niacin per tube can be used to obtain a measurable response.

For the preparation of test solution of niacin, the sample can be extracted either in acid or alkali medium by addition of alkali or acid such that the final volume contains 0.1 g of niacin per ml.

Reagents

Standard niacin stock solution I contains 100 μg/ml of U.S.P. Niacin RS.

Standard niacin stock solution II contains 10 μg/ml of U.S.P. Niacin RS and is prepared by diluting Solution I with water.

Standard niacin solution contains between 10 ng to 40 ng of niacin per ml and is prepared from Solution II by suitable dilution with water.

Basal medium stock solution : It contains—

Acid-hydrolysed casein solution	25 ml
Cystine-tryptophane solution	25 ml
Dextrose, anhydrous	10 g
Sodium acetate, anhydrous	5 g
Adenine-guanine-uracil solution	5 ml
Riboflavin-thiamine hydrochloride-biotin solution	5 ml
Aminobenzoic acid-calcium pantothenate-pyridoxine hydrochloride solution	5 ml
Salt Solution A	5 ml
Salt Solution B	5 ml

The medium is prepared by dissolving anhydrous dextrose and sodium acetate in the previously mixed solutions, adjusting the pH to 6.8

with 1 N sodium hydroxide, and finally, making up the volume to 250 ml with water.

Culture medium : To each of a series of test tubes containing 5 ml of the Basal Medium Stock Solution 5 ml of water containing 1.0 µg of niacin are added. These tubes are plugged with cotton, sterilised for 15 minutes in an autoclave at 121°C, and cooled.

Inoculum : From the stock culture of *Lactobacillus plantarum* cells are transferred to a sterile tube containing 10 ml of culture medium. This culture is incubated for 16 to 24 hours at any constant temperature between 30° and 37°C. This cell suspension is the inoculum.

Procedure : First of all the spectrophotometer is calibrated as recommended in the Pharmacopœia.

Standard niacin tubes are prepared by taking in duplicate 0.0, 0.5, 1.0, 1.5 2.0 .... 5.0 ml respectively of Standard Niacin Solution, adding to each tube 5.0 ml of Basal Medium Stock Solution and water to make 10.0 ml.

Next, the tubes containing the material to be assayed are prepared by taking in duplicate, 1.0, 2.0, 3.0 and 4.0 ml, respectively of the test solution of the material to be assayed, adding 5 ml of Basal Medium Stock Solution and water to make 10.0 ml. After mixing, the tubes are plugged with cotton and sterilised by autoclaving. After cooling, each tube is aseptically inoculated with 1 drop of inoculum and incubated for 16 to 24 hours at any constant temperature between 30° and 37°C.

With the transmittance set at 1.0 for the uninoculated blank, the transmittance of the inoculated blank is read. Then with the transmittance of the inoculated blank set at 1.0 the transmittance for each of the remaining tubes is also measured.

Calculation : A standard curve of the niacin standard transmittances for each level of Standard Niacin Solution is plotted against µg of niacin contained in the respective tubes. From this standard curve, the niacin content of the test solution in each tube is determined by interpolation. The niacin content of the test material is calculated from the average values obtained from not less than six tubes that do not vary by more than ± 10% from the average. If the results are to be expressed as niacinamide then the values obtained are multiplied by 0.992.

Microbiological Assay of Vitamin B_{12} (Cyanocobalamin)

The basic medium used for the assay of vitamin B_{12} activity is quite complex and contains a variety of essential components as a mixture in

solution. One set of tubes contains measured amounts of a standard cyanocobalamin solution and graded volumes of the test sample (unknown) are added to another corresponding set of tubes. All the tubes are inoculated with a small amount of culture of *Lactobacillus leichmannii* and then incubated. The extent of growth is determined by measuring light transmittance in a spectrophotometer. Transmittance values (response) for different concentrations (dose) of standard cyanocobalamin solution are used to obtain the concentration-response curve. The amount of vitamin B_{12} present in the test sample is calculated from the standard curve by interpolation.

Conclusion

The principles of microbiological assays were developed in 1920s. Today microbes are exploited as analytical tools in an analytical laboratory for the estimation of some vitamins, amino acids and trace elements. The microbiological assays are as accurate as chemical methods, they are simple, inexpensive and convenient, need small quantity of the material and do not require elaborate instrumentation. At the same time these assays require continuous checks for consistency, specificity and interferences and are time consuming in comparison with some of the modern methods of instrumental analysis. However automation of microbiological assay can overcome all the present limitations. The accuracy of observations as well as sampling handling capacity of the laboratory can be increased through automation of microbiological assay. For example, in case of automatic systems of photometric assay; the instrument automatically measures the amount of antibiotic present in a solution, adds inoculum and nutrient medium, incubates the mixture for 100 minutes, and transfers the incubated mixture to a photometer where the results are automatically read and recorded.

MICROBIAL ASSAY OF AMINO ACIDS

There are 20 different types of amino acid present which help in the synthesis of proteins. Various types of microorganisms (bacteria and fungi) are used for the assay of amino acids. For example, *Leuconostoc citrovorum* was used for alanine, and *Streptococcus faecalis* for arginine, methionine, thronine and tryptophan. For all other amino acids *Leuconostoc mesenteroides* was used. These included aspartic acids, cystine, glycine, histidine, phenylalanine, proline and serine.

Microorganisms are grown on their specific media and add a freshly prepared amino acid solution on the same day a standard solution containing 200 µg per mL. With an appropriately diluted solution, duplicate tubes were prepared using from 0 to 1.0 mL per tube in gradations of 0.1 mL. Two tubes were also prepared using 1.0 mL of the original at levels of 0.2, 0.4, 0.6, 0.8, and 1.0 mL of amino acids.

Add the amino acid into the media and autoclave it. When the media becomes cool, inoculate all the test tubes with microorganisms and leave some tubes for blank. Incubate all test tubes at 37° C for 15-20 hours. After incubation compare all the test tubes with blanks having no microorganisms. Finally determine the potency of the amino acid.