Microbiological assay Method of measuring compounds such as vitamins and amino acids, using micro-organisms. The principle is that the organism is inoculated into a medium containing all the growth factors needed except the one under examination; the rate of growth is then proportional to the amount of this nutrient added in the test substance.

Two types :-

1. Agar plate diffusion assay(Method A)- refere to **DISK DIFFUSION METHOD**
2. Rapid reliable –reproducible microbial assay.

Rapid-Reliable-Reproducible Microbial Assay Methods

It is worthwhile to mention here that the usual 'conventional agar-plate assays' not only require stipulated incubation for several hours but also are rather quite slow. Furthermore, reasonably judicious constant, rigorous, and honest attempts do prevail for the development of 'rapid-reliable reproducible
microbial assay methods' based on the exploitation of techniques that essentially measure definite cognizable variations in the pattern of growth-rate invariably after a short incubation. Nevertheless, these so called 'rapid methods' generally suffer from the similar critical problems usually encountered in the 'slow methods' namely :

- inadequate specificity, and
- lack of precision.

In actual practice there are two well-known techniques that provide rapid-reliable-reproducible microbial assay methods, namely :

(a) Urease Activity, and

(b) Luciferase Assay.

These two aforesaid techniques shall now be discussed briefly in the sections that follows :

Urease Activity

Urease refers to an enzyme that specifically catalyzes the hydrolysis of urea to ammonia (NH_3) and carbon dioxide (CO_2) ; it is a nickel protein of microbes and plants which is critically employed in
carrying out the clinical assays of plasma-urea concentration.

Importanlty, the microorganism Proteius mirabilis grows significanlty in a urea-containing culture medium, whereupon it particularly causes the hydrolysis of urea to ammonia, and thereby helpsto raise the pH of the medium. However, the actual production of urease is reasonably

inhibited by theso called ‘aminoglycoside antibiotics’,* such as : amikacin, gentamicin, kanamycin, neomycin,netilmicin, tobramycin, doxorubicin, cephalosporins, cephamycius, thienamycin, lincomycin, clindamycin, erythromycin, clarithromycin, azithromycin, oleandomycin, spramycins, and the like.

Methodology :

The various steps involved are as follows :

(1) Assay is performed with two series of tubes of urea-containing culture medium that have been duly incorporated with a range of calibrator solutions.

(2) First series of tubes in duly added a certain volume of the sample which is essentially equivalent to the volume of the calibrator.

(3) Second series of tubes is duly added exactly half the volume of the sample.

(4) Both ‘set of tubes’ are subsequently inoculated with P. mirabilis, and duly incubated for a duration of 60–70 minutes.

(5) pH of the resulting solution is measured accurately upto 0.01 pH units.

(6) In fact, it is possible to obtain two distinct ‘calibration curves’ by plotting pH Vs log10 i.e., the ensuing calibrator concentration for each of the two series.

(7) The ‘vertical distance’ existing between the two curves is found to be almost equal to the legarithm of 1/2 the concentration of ‘drug substance’ present in the sample.

Note : (1) In usual practice, it is rather difficult to obtain ‘reliable’ results by adopting the ‘Urease
Activity’ method.

(2) A standardized, senstitive, and reliable pH Meter is an absolute must for this particular assay.

Luciferase Assay

In the specific ‘Luciferase Assay’, the firefly luciferase** is made use of for the actual measurement
of small quantum of ATP*** duly present in a microbial culture, whereby the levels of ATP get proportionately reduced by the ensuing action of the aminoglycoside antibiotics (see Section 10.3.2.1).

Methodology : The various steps involved in the ‘Luciferase Assay’ are as enumerated under sequentially :

(1) Both test solutions (i.e., after preliminary heating provided the matrix is serum) along with calibrators are carefully added into the various tubes of the culture medium specifically containing a
growing microbial culture (i.e., organism).

(2) After adequate incubation for a 90 minute duration the cultures are duly treated with ‘apyrase’
so as to ensure the complete destruction of the extracellular ATP.
(3) The resulting solution is duly extracted with EDTA/sulphuric acid, and thus the intracellular ATP critically assayed with the firefly enzyme using a ‘Luminometer’.
(4) Finally, a ‘calibration curve’ is constructed meticulously by plotting the two vital components,
namely : (a) intracellular ATP content, and (b) log10 i.e., the calibrator concentration.
Note : As to date, the ‘Luciferase Assay’ has not yet accomplished a wide application ; however, it
may find its enormous usage in the near future with the advent of such ‘luciferase formulations’ that would turn out to be even much more active, reliable, and dependable.