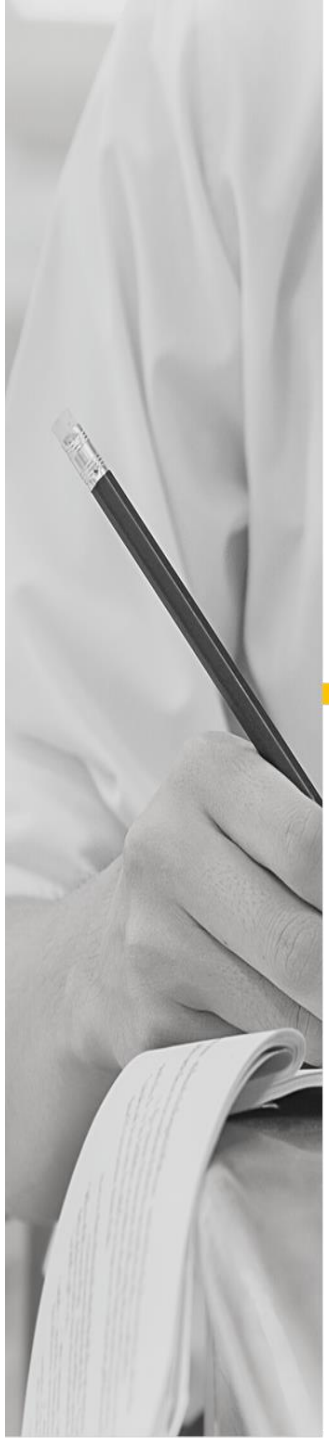

Tikrit University
College of Veterinary Medicine

Subject name:DRUG EVALUATION

Subject year: MSc - PHARMA

Lecturer name: MICRO. ASSAY

Academic Email:Sbc.s4@tu.edu.iq

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Subject name: DRUG EVALUATION

Subject year: MSc - PHARMA

LECTURER : Prof Dr Husamuldeen Alnajar

Lecture name: MICROBIO. ASSAY.

Academic Email:Sbc.s4@tu.edu.iq

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

PHAMACOLOGY MASTER COURSE – DRUG EVALUATION

أ د حسام الدين النجار

MICROBIOLOGICAL ASSAY

Qualitative and quantitative determination of any chemical compound with the use of micro organisms.

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 inhibition of growth under standardized conditions may be utilized for demonstrating the therapeutic efficacy of antibiotics.

- Microbiological assay is a technique in which the potency or concentration of a compound is assessed by determining its effect on micro-organisms.
- The principles are discussed by Roberts and Boyce. Microbiological assay is a legal QC requirement for the assay of a number of antibiotics, in both the British Pharmacopoeia (BP) and United States Pharmacopoeia (USP).
- Bioassay compares a reference standard and an unknown sample, the two preparations being measured simultaneously.

Reference standard and units of activity

The potency (activity) of an antibiotic product is expressed as **the ratio of the dose that inhibits the growth of a suitable susceptible microorganism to the dose of an International Biological Standard**, an International Biological Reference Preparation, or an International Chemical Reference Substance of that antibiotic that produces similar inhibition.

Potency of antibiotic can be expressed in “unit” or “µg” of activity per mg of dried material, as stated in Pharmacopeia.

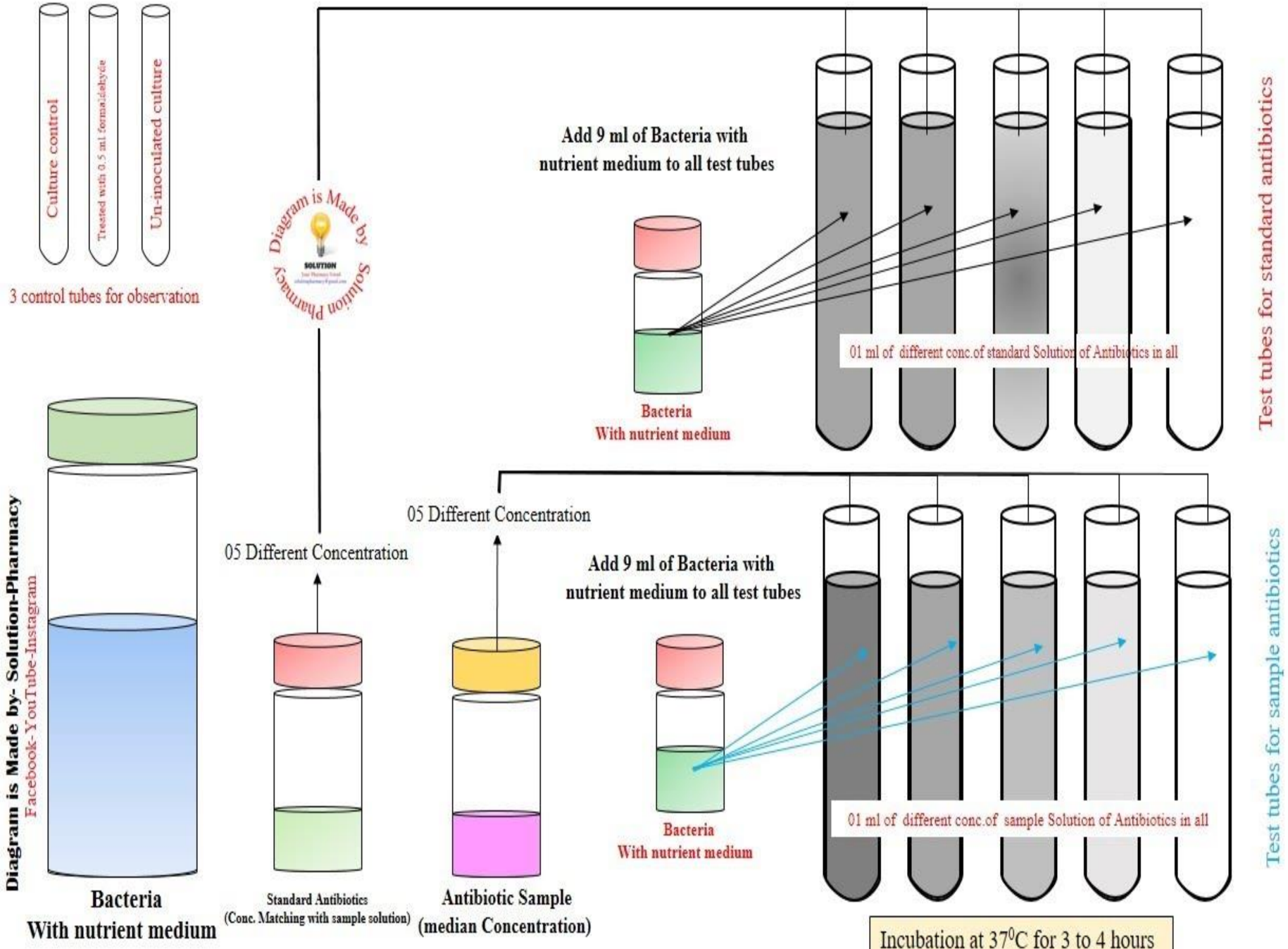
“µg” of activity is based on single active ingredient.

“unit” is used when there are more than one active ingredient in the antibiotic .

for resolving doubts regarding possible change in potency of antibiotics and their preparations.

PRINCIPLE

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


Culture control
Treated with 0.5 ml formaldehyde
Un-inoculated culture
3 control tubes for observation
Diagram is Made by Solution Pharmacy
Add 9 ml of Bacteria with nutrient medium to all test tubes
Bacteria With nutrient medium
01 ml of different conc.of standard Solution of Antibiotics in all
Test tubes for standard antibiotics
05 Different Concentration
05 Different Concentration
Add 9 ml of Bacteria with nutrient medium to all test tubes
Bacteria With nutrient medium
01 ml of different conc.of sample Solution of Antibiotics in all
Test tubes for sample antibiotics
Diagram is Made by- Solution-Pharmacy
Facebook-YouTube-Instagram
Bacteria With nutrient medium
Standard Antibiotics (Conc. Matching with sample solution)
Antibiotic Sample (median Concentration)
Incubation at 37^{0}C for 3 to 4 hours

PREPARATION OF MEDIA: The Media required for the preparation of test organism are made from the ingredients.

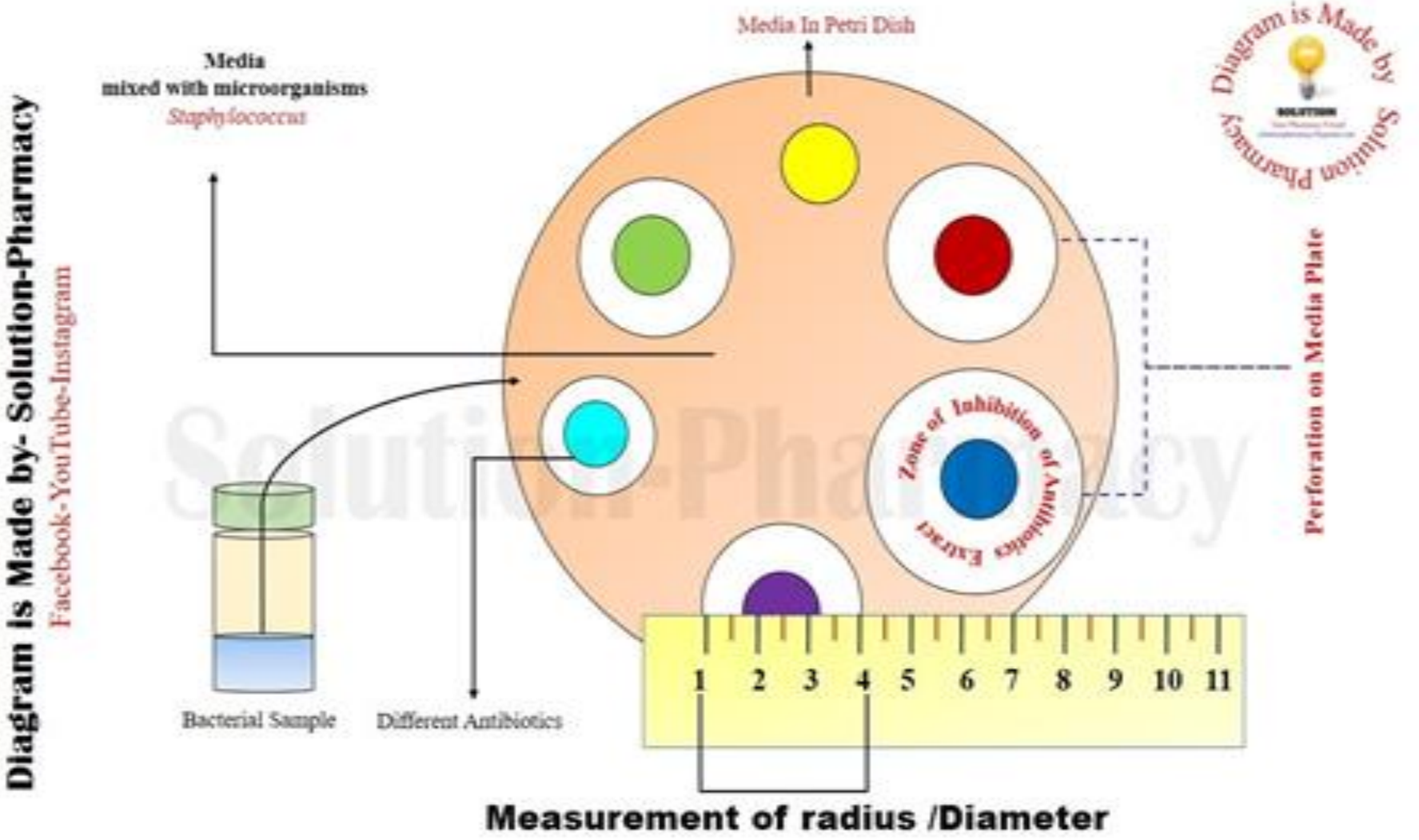
Minor modifications of the individual ingredients may be made, or reconstituted dehydrated media may be used provided the resulting media have equal or better growth-promoting properties and give a similar standard curve response.

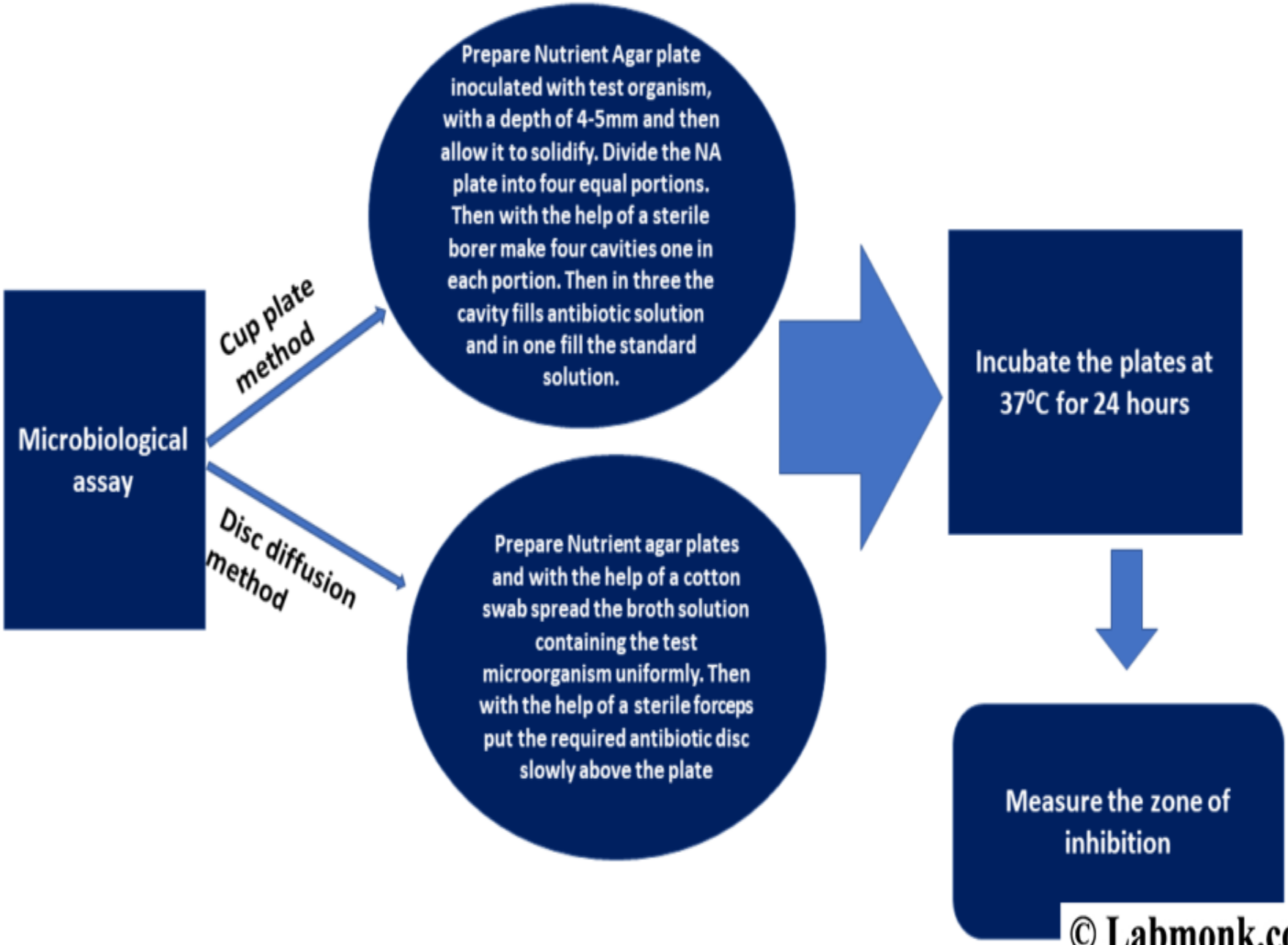
ASSAY METHODS Two general method are usually employed:-

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

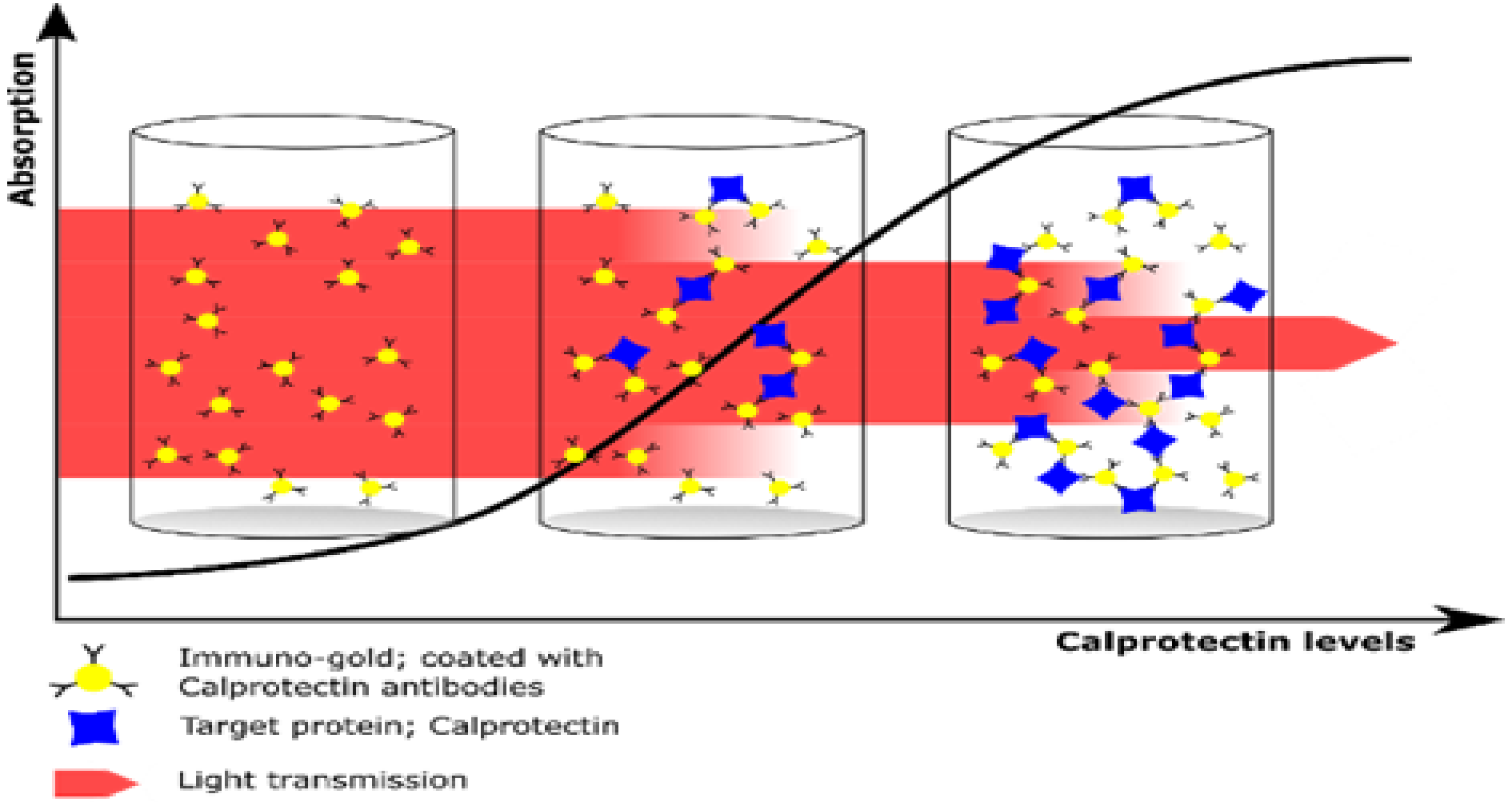
The turbidimetric (or tube assay) method.

THE Diffusion (Cylinder-Plate) method is based upon the diffusion of the antibiotic throughout solid culture media. A metal cylinder or paper disk containing the antibiotic is placed on a solid agar growth media containing a target microorganism.




Microbiological assay
Cup plate method
Disc diffusion method
Prepare Nutrient Agar plate inoculated with test organism, with a depth of 4-5mm and then allow it to solidify. Divide the NA plate into four equal portions. Then with the help of a sterile borer make four cavities one in each portion. Then in three the cavity fills antibiotic solution and in one fill the standard solution.
Prepare Nutrient agar plates and with the help of a cotton swab spread the broth solution containing the test microorganism uniformly. Then with the help of a sterile forceps put the required antibiotic disc slowly above the plate
Incubate the plates at 37⁰C for 24 hours
Measure the zone of inhibition

In turbidimetric assay, particle bound antibodies are mixed with the sample in appropriate buffer. This mixture is then analysed using a light source. Level of light transmitted reflects the amount of antigen in the solution. If no antigens are present, the light is hardly absorbed.



Assay performed with the aid of microorganisms.

MICROBIOLOGICAL ASSAY OF VITAMINS

DEFINITION:

Microbiological assay is a type of biological assay performed with the aid of microorganisms.

Many therapeutic agents, which either inhibit the growth of microorganisms **or** are essential for the growth of them are standardized by microbial assay. Principles of microbial assay were developed in1920s.

MICROBIOLOGICAL ASSAY OF VITAMINS:

PRINCIPLE: Vitamins and amino acids are essential for the growth of microorganisms. The basis of this assay is to measure the ability of test organism to utilize the substance being assayed under a proper nutritional condition. The organisms require these growth factors (vitamins & amino acids) in micro or nano grams.

The response (growth of test organism) is proportional to the dose (amount of factor) added to medium. Materials required for Microbial assay of vitamins & amino acids:

A stock solution. A inoculum media. Assay medium. A standard curve

Microbiological assay is applicable to the B vitamins for example. The rate of growth of a species of microorganism that requires a vitamin is measured in growth media that contain various known quantities of a foodstuff preparation containing unknown amounts of the vitamin.

MICROBIAL ASSAY OF VITAMIN B12 :

Also known as cyanocobalamin. Its a water soluble vitamin. Structure is similar to that of heme where the iron is replaced with cobalt as a centre of molecule. Its main sources are liver, eggs , milk meat & fish. VitB12 deficiency causes Macrolytic anemia, pernicious anemia. National Research Council, USA recommends a daily intake of about 5mg of vitB12. **The test organism selected must be capable of utilizing the vit.**

PRINCIPLE OF ASSAY:

The test organism selected must be capable of utilizing free cyanocobalamin.

Lactobacillus Liechmannii is found to satisfy the requirements. Gram negative bacilli, non-pathogenic, easy to culture & easily available. Isolated from milk, cheese, & other dairy products. Assay is performed by using either titrimetric or turbidimetric method.

INDUSTRIAL MICROBILOGY

Industrial microbiology is an important area of applied microbiology. It refers to the use of microorganisms in commercial enterprise and Cheap raw materials are converted to valuable products through the metabolism of microbes.

Microbes for this purpose could be exploited in different ways.

For instance this includes(i) synthesis of fermentation products as acids, alcohols or other organic compounds(ii) transformation of one compound into another desired type,(iii) the production of enzymes, antibiotics, or insecticides, or(iv) the use of microbes themselves as food.

Pharmaceutical products of microbial origin

There are a number of medicinal substances which derive from microorganisms, including **antibiotics, immunological products and products resulting from recombinant DNA technology.**

These are **dextran, used as a substitute for plasma, certain enzymes and a small group of organic chemicals**.

Chemicals which continue to be produced by fermentation include citric acid, ethanol, lactic acid, vitamins B2 and B12 and a considerable range of amino acids.

Pharmaceutical products of microbial origin

Various commercial products of economic value made by microbes are

(i) medicines i.e. pharmaceuticals, including antibiotics, steroids, human protein, vaccines, and vitamins,

(ii) organic acids,

(iii) amino acids,

(vi) organic solvents and

(vii) synthetic fuels.

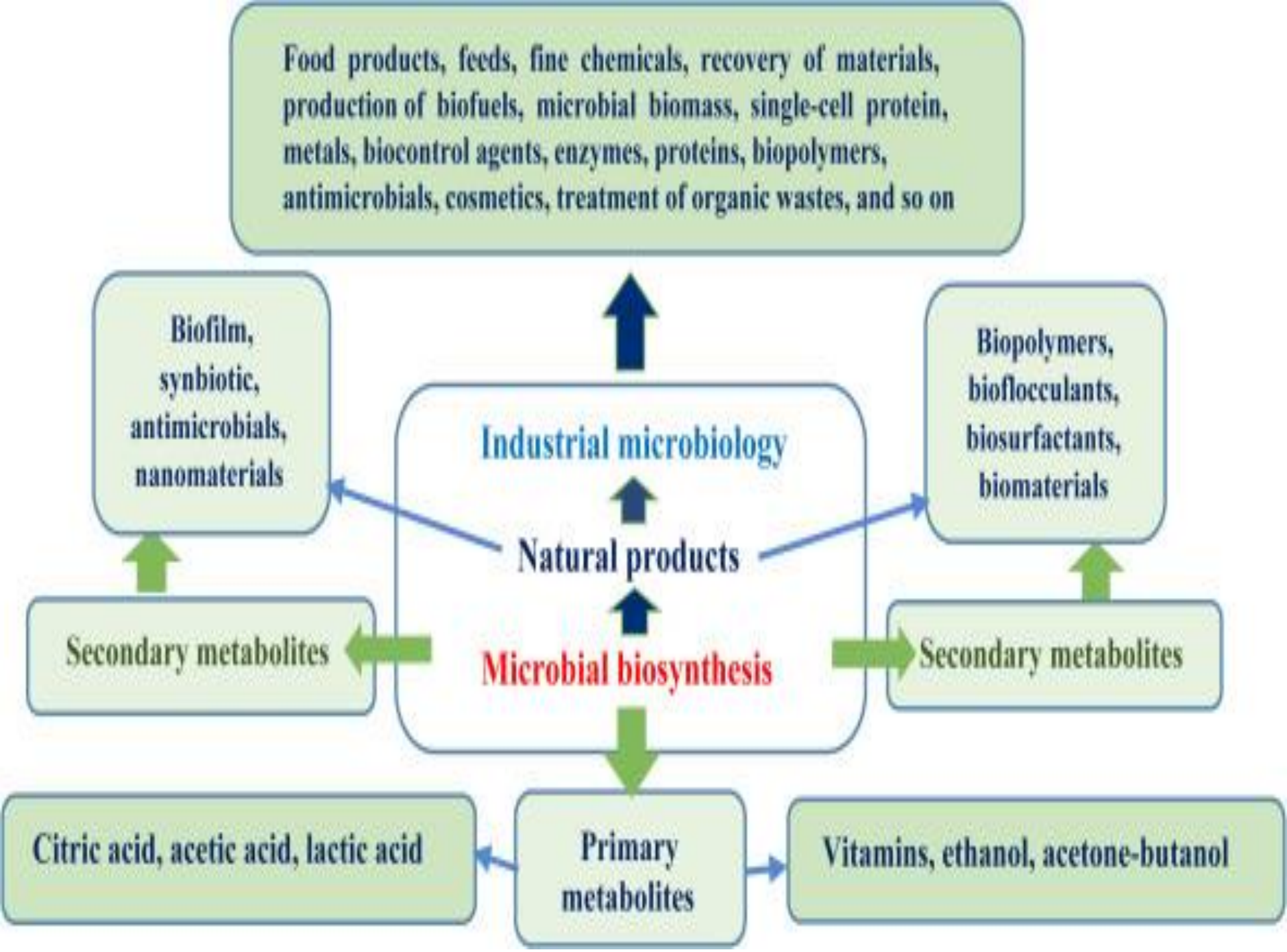
In addition to these, quite recently potential of microbes could also be realized in

(viii) recovery of metals from ores through bioleaching, (ix) recovery of petrol, and

(x) protein production.

(vi) enzymes,

(v) alcohols,


Food products, feeds, fine chemicals, recovery of materials, production of biofuels, microbial biomass, single-cell protein, metals, biocontrol agents, enzymes, proteins, biopolymers, antimicrobials, cosmetics, treatment of organic wastes, and so on
Biofilm, synbiotic, antimicrobials, nanomaterials
Biopolymers, bioflocculants, biosurfactants, biomaterials
Industrial microbiology
Natural products
Secondary metabolites
Microbial biosynthesis
Secondary metabolites
Citric acid, acetic acid, lactic acid
Primary metabolites
Vitamins, ethanol, acetone-butanol