MICROBIOLOGICAL ASSAY OF VITAMINS

- Vitamins are important growth factor needed for growth and multiplication of microorganism .

- They are very sensitive to small amount of growth factor .

DEPTH OF BIOLOGY

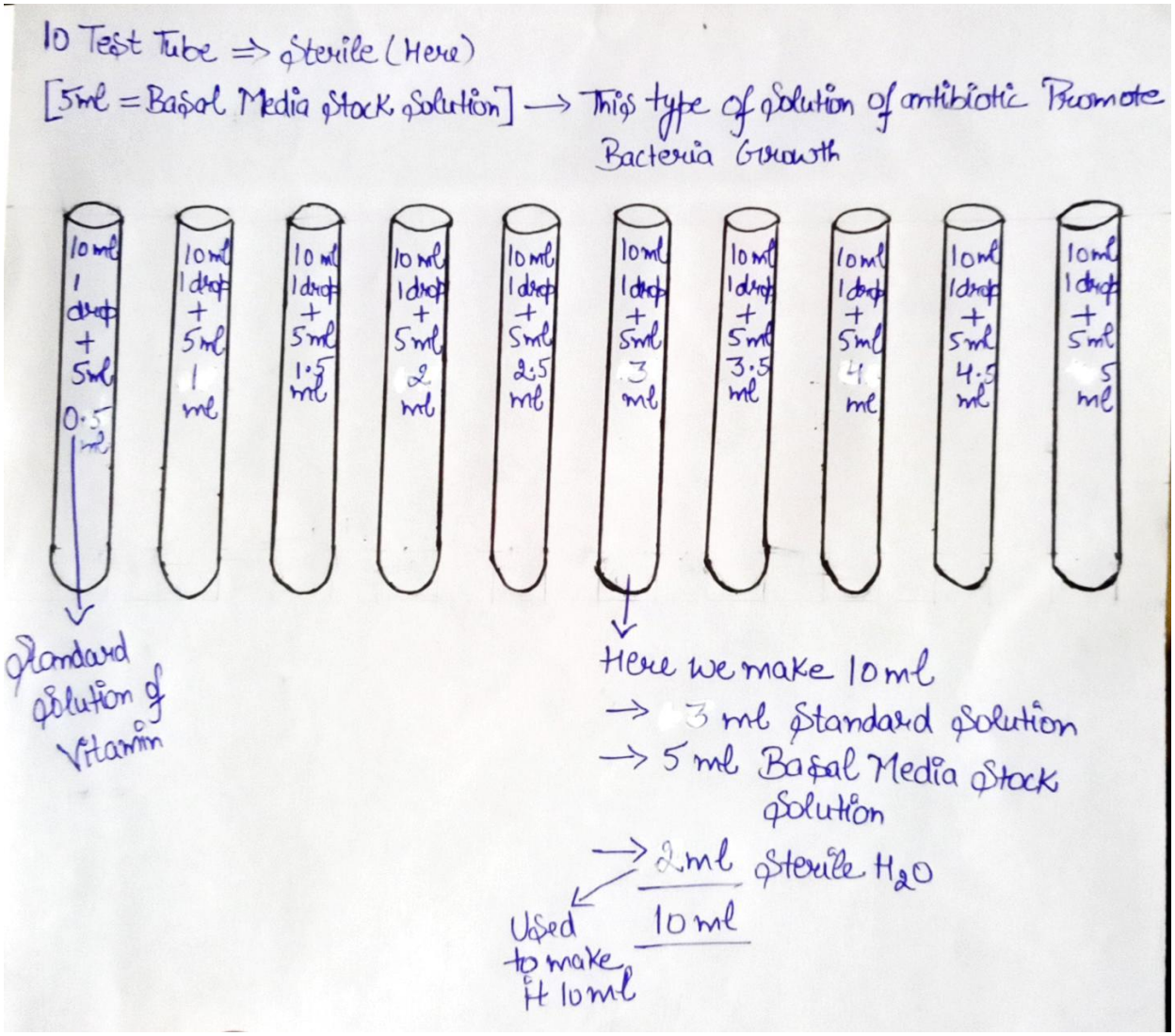
- Test microorganism used for assaying the water Soluble vitamins such as Vitamin B12 , Vitamin B6 or test microorganism used such as – Lactobacillus leichamannii etc

Assay of Cyanocobalanin (vit.B12) :

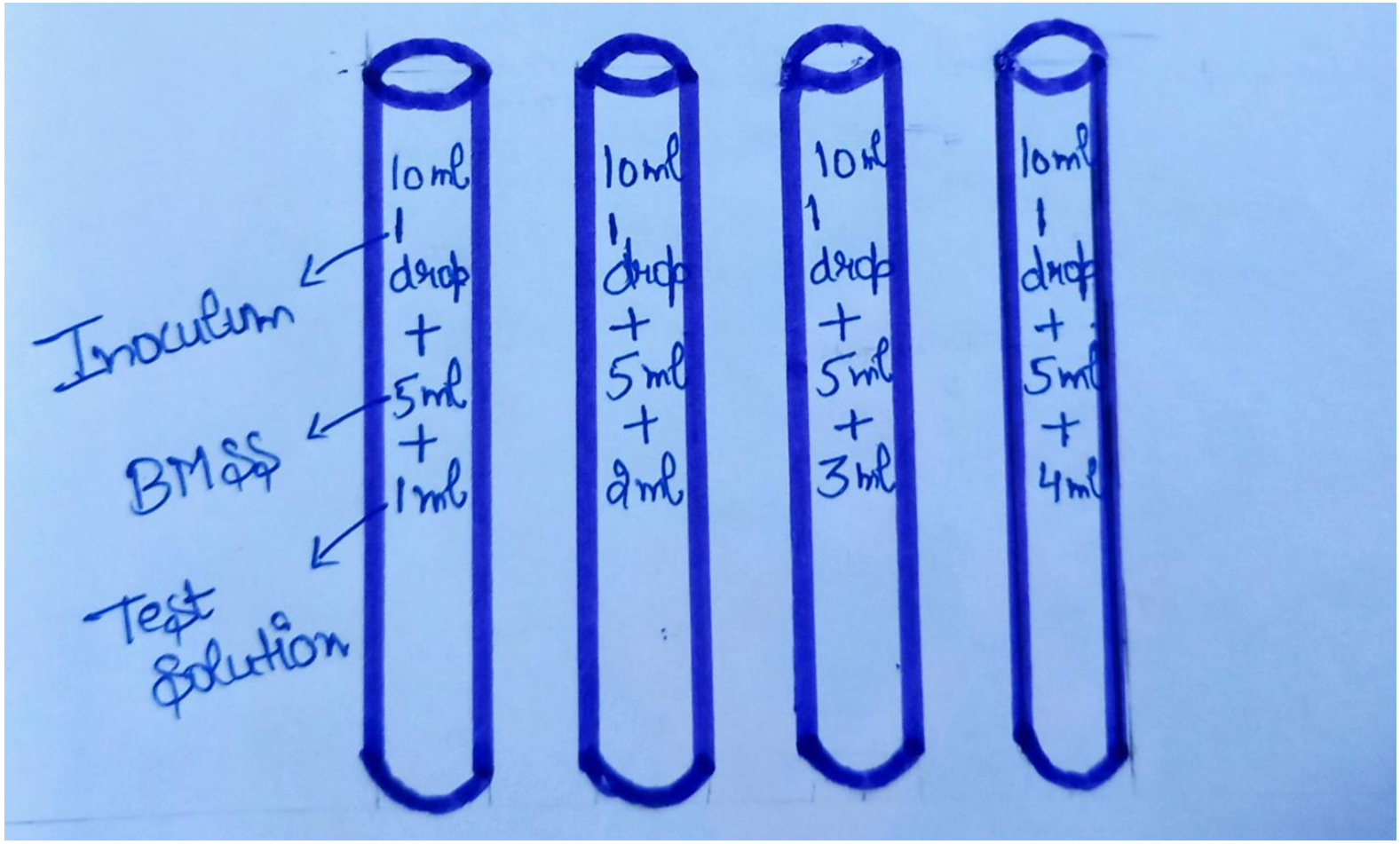
(A) Titrimetric Method

(B) Turbidimetric Method

DEPTH OF BIOLOGY



DEPTH OF BIOLOGY



Remaining we add sterile H_2O

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To make up the required volume (10ml)

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And now we placed it for sterlisation (Autoclave) 121°C for 5 minutes

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Now after this it free from Bacteria

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Place at room temperature for cool

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And now we add Inoculum

Inoculated = Here we grow Microorganism

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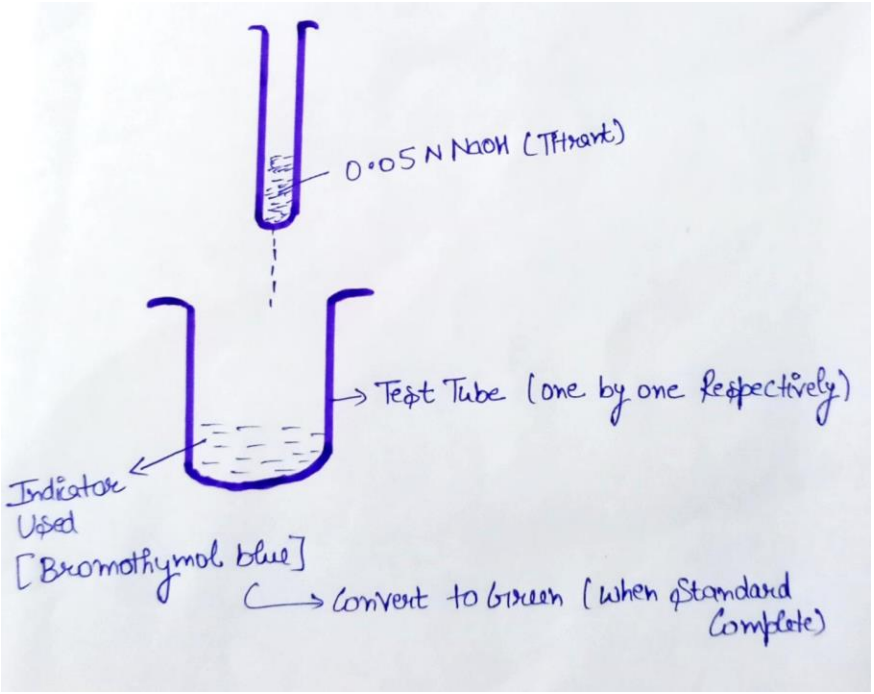
Now after add Inoculum

We placed it on Incubator for 64 – 72 hrs and 30 – 37 °C



- Now we each test tube (Note the reading then draw a graph for both)

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We do average and then plot a graph

This is standard graph Here titration value increases with growth of microorganism . If this graph and test graph is equal then we can say our test solution us good for bacterial growth .

2. Turbidimetric Method →

→ Use device photoelectric colorimeter .

→ Turbidity increases (microorganism)

DEPTH OF BIOLOGY

- Apparatus, Reagent and procedure are same as titrimetric method .

- But this include 2 more test tube which is uninoculated blank test (not standard + not test solution + no microorganism) .

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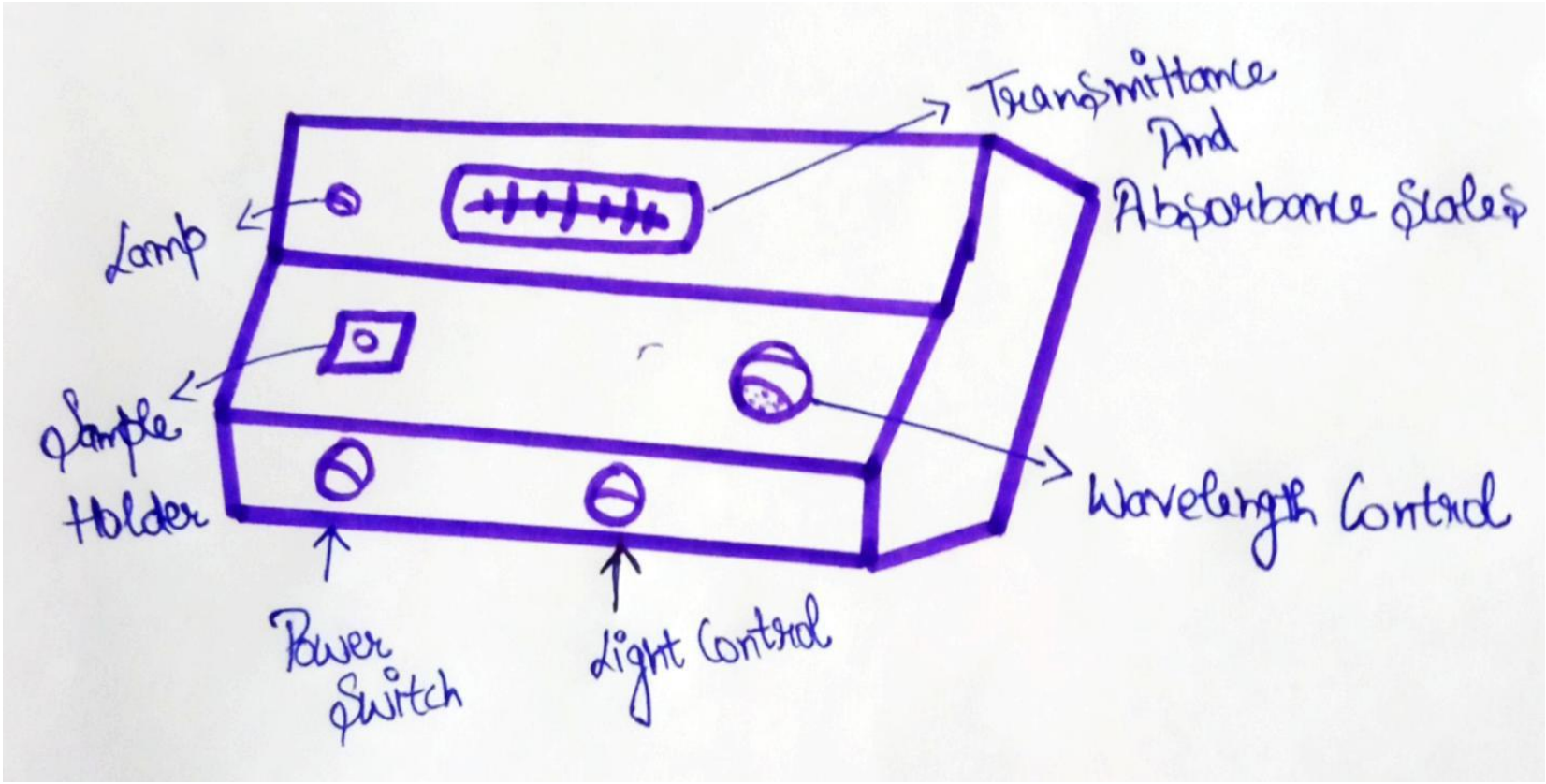
Incubate all test tube at 30 – 37 °C for 16 – 24 hrs .

Now by using unioculated blank tube adjust the transmittance at 640 mμ to 100% in the photoelectric container .

Now take reading and plot a graph and compared with test tube If equal then test is good (Not then test is not good) .

DEPTH OF BIOLOGY



★ Assesment of New antibodies (Minimum Inhibitory Concentration) MIC

- It is the lowest concentration of antimicrobial compound found to inhibit the growth of a particular test microorganism .

- It may applied to access new

DEPTH OF BIOLOGY

Disinfectants , antiseptics , preservatives and antibiotics .

Unit = µg/ml or units/ml

1. Liquid dilution method (Test tube method)

- Take 10 clean test tube and add (0.5 , 1 , 1.5 , 2.0 , 2.5 , 3.0 , 3.5 , 4.0 , 4.5 , 5.0) ml respectively of test chemical .

- Take another two test tube , one is in- inoculated (without chemical

And test microorganism) and other is controlled test tube medium for growth of microorganism .

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Now add 5 ml double – strength medium in all test tube .

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Make up required volume upto 10 ml by sterile H_2O .

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Incubate 37°C for 2 – 3 days .

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After incubation , all test tube are examined for the growth in the form of turbidity .

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Now , minimum Inhibitory

Concentration (MIC) is calculated .

2. Solid Dilution method :

Test chemical is first mixed into molten agar , then poured into petri plates .

After solidification , the inoculation is spread on the surface of agar

medium .

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Incubated at 37°C for 2 – 3 days .

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After incubation , all plates are observed for growth of inoculum and the MIC of test chemical is calculated .