GTP FOR MICROBIOLOGICAL ANALYSIS FOR VITAMIN B12

REAGENTS AND CULTURE MEDIUM:

1.0 B12 culture agar (Hi media)
2.0 B12 Inoculum broth (Hi media)
3.0 B12 assay medium (Hi media)
4.0 Distilled water

Instruments:
1.0 37°C water bath or incubator
2.0 spectrophotometer

Inoculum:
Pure culture of *Lactobacillus leichmanii* ATCC 7844.

Standards:
Vitamin B12 working standard

Procedure:
Preparation of standard stock solution (1.0µg/ml):
weigh accurately about 112mg of vitamin B12 working standard (activity-88.75%) in a 100 ml volumetric flask. dissolve in 25 ml alcohol and make up the volume with purified water. this gives a solution of 1 mg/ml (solution A). Further dilute 1 ml of solution A to 100 ml with purified water (solution B)
take 10 ml of solution B and dilute it to 100 ml with purified water to obtain a stock solution of 1.0µg/ml. Store the stock solution in the refrigerator for 15 days.

Preparation of standard solution (0.02mµg/ml)
prepare the standard solution from the standard stock solution on the day of assay dilute 1ml of standard stock solution with purified water to 250 ml and mix well further dilute 1ml of this solution to 200 ml with purified water to obtain a final concentration of 0.02 µg/ml of vitamin B12.
preparation of sample solution:
make the dilution concentration eqveualent to 0.02 µg/ml

Preparation of stock culture of *Lactobacillus leichmanii*:
prepare B12 culture agar .Allow the medium to solidify in an upright position. Prepare a fresh stab every week using a pure culture of *Lactobacillus leichmanii* incubate at 37°C for 18-24 hours. Store under refrigerator for one week.
Preparation of *Lactobacillus leichmanii* Inoculum:
Prepare B12 Inoculum medium. Inoculate cells from the freshly prepared stab culture (not more than one week old) to the Inoculum medium. Incubate at 37°C for 18-24 hours under aseptic conditions, centrifuge the culture and decant the supernatant. Centrifuge the cells once again after suspending in 10 ml sterile normal saline. suspend the cells in 10 ml sterile normal saline. Just before use, dilute the culture by adding 0.1 ml of Inoculum in 10 ml normal saline.
Assay:
Prepare B12 assay medium:

To similar tubes in duplicate add 1.0, 2.0, and 3.0, 4.0and5.0 ml respectively of the standard preparation .to similar tubes in duplicate add 2.0, 3.0 and 4.0 ml respectively of the sample preparation. to each of the above tubes and to four similar tubes (blank) add5.0 ml of assay medium and sufficient purified water to make up the volume to 10 ml.

place all the tubes in one rack, cover suitably and autoclave at 121°C for 5 minutes. Cool the medium as rapidly as possible. Add 1 drop of diluted Inoculum to each tube except 2of the4 tubes kept as uninoculated blank. Incubate the tubes at 37°C for 16-24hours in a water bath.
Observation and calculation:
After incubation, determine the % transmission of each tube with the transmittance set at 100% using standard response curve by plotting % transmission against each level of standard solution.
determine the amount of vitamin B12 in the sample for each level of assay solution from the standard curve

Calculate the content of vitamin b12 using the following formula.

$$\text{Content of vitamin B12} = \frac{\text{Value per ml X wt. of std.}}{\text{0.02 x wt. of sample}} \text{ X dilution factor X potency of the working std.}$$

NOTE: PROCEDURE FOR RETESTING:

For the purpose of confirming a doubtful result by any of the procedures

Proceed as directed under Procedure, but make allowance for the larger specimen size.