Technique

Stock cultures of *Lactobacillus casei* ATCC 7469 are prepared by stab inoculation of Lactobacilli Agar AOAC (M366). Following incubation at 35-37°C for 18-24 hours the tubes are stored in a refrigerator. Transfers are made at monthly intervals.

Inoculum for assay is prepared by subculturing from stock culture of *Lactobacillus casei* ATCC 7469 into a tube containing 10 ml of Micro Vitamin Test Inoculum Broth (M133) or Lactobacilli Broth (M367). After 24 hours incubation at 35-37°C, the cells are centrifuged, under aseptic conditions, and the supernatant liquid is decanted. The cells are then resuspended in 10 ml of sterile single strength Folic Acid Casei Medium, resedimented as before and washed one more time. Finally, the washed cells are resuspended in 10 ml of sterile single strength Folic Acid Casei Medium (M543) and diluted 1:100 with the same medium. One drop of this suspension is used to inoculate each of the assay tubes. 0.85% NaCl can be used instead of the single strength basal medium to wash and dilute the inoculum.

A standard curve for each assay should be prepared, since the conditions of sterilization, temperature of incubation, etc, which influence the standard curve readings, cannot be duplicated exactly from time to time. The standard curve is obtained by using folic acid at levels of 0.0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ng per assay tube (10 ml).

Preparation of Folic Acid Concentrations

Dissolve 20 mg dried folic acid in 100 ml distilled water containing 20 ml ethanol. Adjust the pH of the solution to 10.0 with 0.1N NaOH to dissolve the acid and then adjust pH to 7.0 with 0.05N HCl. This solution contains 200 mcg folic acid per ml.

Dilute 1 ml of this solution with 999 ml of distilled water to get 200ng per ml and finally, dilute 1 ml of this solution with 999 ml of Folic Acid Buffer A (M544) to get a standard solution containing 0.2 ng folic acid per ml. Use 0.0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5 ml per assay tube.

Preservation of Serum Specimen

Allow the blood specimen to clot so as to separate the serum. Separate the serum into a clean dry tube and centrifuge to remove any blood cells present. Take care to avoid haemolysis of erythrocytes. Dispense 5 ml of serum sample into clean dry test tubes and add 25 mg ascorbic acid to each tube. Keep the tubes frozen below -20°C till assay.

Preparation of Serum Specimens

Thaw the serum containing ascorbic acid. Add 5 ml of this sample to 45 ml of rehydrated Folic Acid Buffer A (M544). Incubate this serum - buffer solution at 37°C for 90 minutes and then autoclave at 15 lbs pressure (121°C) for 2.5 minutes. Remove the coagulated protein by centrifuging and transfer the supernatant to a clean, dry tube. This clear solution obtained is used as a sample in the folic acid assay.

Procedure for determination of Total Folic Acid concentration in specimens

Use 0.5, 1.0, 1.5 ml or other volumes of the prepared serum extracts as described earlier. Fill each tube with 5 ml Folic Acid Casei Medium and sufficient distilled water to give total volume of 10 ml per tube. Sterilize tubes at 15 lbs pressure (121°C) for 5 minutes. Cool the tubes and add one drop of inoculum to each assay tube. Turbidimetric reading should be made after 18-24 hours incubation at 35-37°C. Tubes are refrigerated for 15-30 minutes to stop growth before reading. The turbidometric readings are recorded at 620 nm. The amount of folic acid in the test samples can be determined by interpreting the results with the values obtained on the standard curve taking into consideration the dilution of sample.

Extreme care should be taken to avoid contamination of media or glassware used for the assay. Detergent free clean glassware should be used. Even small amount of contamination by foreign material can be lead to erroroneus results.

Type of specimen

Clinical samples - Serum

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Further biochemical tests should be carried out for confirmation.

Quality Control

Appearance
Off-white to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light amber coloured, clear solution which may have a slight precipitate.

Reaction
Reaction of 9.4% w/v aqueous solution at 25°C. pH : 6.7±0.1

pH
6.60-6.80

Cultural Response
Microbiological assay of folic acid is carried out using *L.casei* ATCC 7469. After an incubation at 35-37°C for 16-18 hours.

Growth
Good growth is obtained. Gradual increase in growth with increasing concentration of standard folic acid 0,0.1,0.2, 0.4, 0.6, 0.8 and 1ng per assay tube was recorded as equivalent increase in absorbance at 620 nm.

Reference

1. Flynn, Williams, ODell and Hogan, 1951, Anal. Chem., 23, 180.
2. Baker, Herbert, Frank, Pasher, Hunter, Wasserman and Sobotka, 1959,Clin. Chem., 5, 275.
3. Waters and Mollin, 1961, J. Clin. Path., 14, 335.

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