Folic Acid Casei Medium

Intended Use

Folic Acid Casei Medium is used for determining folic acid concentration by the microbiological assay technique.

Summary and Explanation

Vitamin assay media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Folic Acid Casei Medium is prepared for the microbiological assay of folic acid, particularly folic acid in serum. *Lactobacillus rhamnosus* ATCC™ 7469 is used as the test organism in this assay. Folic Acid Casei Medium is prepared according to the formulation described by Flynn et al.[1] and modified by Baker et al.[2] and Waters and Mollin.[3]

Total serum folic acid activity can vary depending on the disease state. It has been reported that normal subjects have a mean serum folic acid level of 9.9 ng per mL. Patients with uncomplicated pernicious anemia have a mean serum folic acid level of 16.6 ng per mL, while patients with megaloblastic anemia have levels less than 4.0 ng per mL.

User Quality Control

Identity Specifications

Difco™ Folic Acid Casei Medium

Dehydrated Appearance:	Off-white, homogeneous, with soft clumps.
Solution:	4.7% (single strength) soluble in purified water upon boiling 1-2 minutes. Single-strength solution is light amber, clear, may have a slight precipitate.
Prepared Appearance:	Single-strength solution is very light amber, clear, may have a very slight precipitate.
Reaction of 4.7% Solution at 25°C:	pH 6.7 ± 0.1

Cultural Response

Difco™ Folic Acid Casei Medium

Prepare the medium per label directions with the addition of 0.05% ascorbic acid. Dilute folic acid to produce a standard solution in folic acid buffer solution (consisting of per liter: monopotassium phosphate 10.65 g, dipotassium phosphate 3.744 g and ascorbic acid 1.0 g and having a final pH at 25°C of 6.1 ± 0.05). The medium supports the growth of *Lactobacillus rhamnosus* ATCC™ 7469 when prepared in single strength and supplemented with folic acid. The medium should produce a standard curve when tested using a folic acid reference standard at 0.0 to 1.0 ng per 10 mL. Incubate tubes with caps loosened at 35-37°C for 18-24 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Principles of the Procedure

Folic Acid Casei Medium is a folic acid-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *L. rhamnosus* ATCC 7469. The addition of folic acid in specified increasing concentrations gives a growth response that can be measured turbidimetrically.

Formula

Difco™ Folic Acid Casei Medium

Approximate Formula* Per Liter		
Charcoal Treated Pancreatic Digest of Casein	10.0	g
Dextrose	40.0	g
Sodium Acetate	40.0	g
Dipotassium Phosphate	1.0	g
Monopotassium Phosphate	1.0	g
DL-Tryptophan	0.2	g
L-Asparagine	0.6	g
L-Cysteine Hydrochloride	0.5	g
Adenine Sulfate	10.0	mg
Guanine Hydrochloride	10.0	mg
Uracil	10.0	mg
Xanthine	20.0	mg
Polysorbate 80	0.1	g
Glutathione (reduced)	5.0	mg
Magnesium Sulfate (anhydrous)	0.2	g
Sodium Chloride	20.0	mg
Ferrous Sulfate	20.0	mg
Manganese Sulfate	15.0	mg
Riboflavin	1.0	mg
p-Aminobenzoic Acid	2.0	mg
Pyridoxine Hydrochloride	4.0	mg
Thiamine Hydrochloride	400.0	µg
Calcium Pantothenate	800.0	µg
Nicotinic Acid	800.0	µg
Biotin	20.0	µg

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Directions for Preparation from Dehydrated Product

1. Suspend 9.4 g of the powder in 100 mL of purified water.
2. Add 50 mg ascorbic acid. Mix thoroughly.
3. Heat with frequent agitation and boil for 1-2 minutes to completely dissolve the powder.
4. Dispense 5 mL amounts into tubes, evenly dispersing the precipitate.
5. Add standard or test samples.
6. Adjust tube volume to 10 mL with purified water.
7. Autoclave at 121°C for 5 minutes.

Procedure

Preparation of Stock Cultures and Inoculum

Prepare stock cultures of the test organism, *L. rhamnosus* ATCC 7469, by stab inoculation into prepared tubes of Lactobacilli Agar AOAC. Incubate the cultures at 35-37°C for 18-24 hours. Store cultures in the refrigerator at 2-8°C. Stock transfers are made at monthly intervals.

Prepare the inoculum for assay by subculturing from a stock culture of *L. rhamnosus* into a tube containing 10 mL prepared Micro Inoculum Broth. Incubate at 35-37°C for 16-18 hours. Under aseptic conditions, centrifuge the tubes to sediment the cells and decant the supernatant. Wash the cells in 10 mL sterile single-strength Folic Acid Casei Medium. Resediment the cells by centrifuging aseptically and decant the supernatant. Repeat washing two more times. After the third washing, resuspend the cells in 10 mL sterile single-strength medium and dilute 1 mL with 99 mL of the same medium. One drop of this suspension is used to inoculate each of the assay tubes. Read the growth response of the assay tubes turbidimetrically after 18-24 hours incubation at 35-37°C. (Some laboratories use 0.85% saline instead of the single-strength basal medium to wash and dilute the inoculum.)

Preparation of the Standard

It is essential that a standard curve be constructed for each separate assay. Autoclave and incubation conditions can influence the standard curve readings and cannot always be duplicated. The standard curve may be 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).

The folic acid required for preparation of the standard curve may be prepared as follows:

Dissolve 50 mg dried folic acid in about 30 mL 0.01N NaOH and 300 mL purified water. Adjust to pH 7-8 with 0.05N HCl and dilute to 500 mL with purified water. Dilute 10 mL of this solution with 500 mL purified water. Further dilute 1 mL in 1 L purified water to make a stock solution containing 2 ng per mL folic acid. Prepare the standard solution containing 0.2 ng per mL folic acid by diluting 10 mL of stock solution with 90 mL of folic buffer solution (consisting of per liter: monopotassium phosphate 10.656 g, dipotassium phosphate 3.744 g and ascorbic acid 1.0 g and having a final pH at 25°C of 6.1 ± 0.05). Use 0.0, 0.5, 1, 2, 3, 4 and 5 mL per assay tube.

Prepare the stock solution fresh daily.

Preservation of Serum Specimens

1. Allow the blood specimen to clot and the serum to separate from the clot.
2. Aspirate the serum into a clean dry tube and centrifuge to remove any cells that may be present. Avoid hemolysis. Dispense 5 mL of each serum sample into clean dry test tubes and add 25 mg ascorbic acid to each tube.
3. If the test is not begun immediately, place tubes in a freezer and hold below -20°C.

Preparation of Serum Specimen

1. Thaw the serum containing ascorbic acid.
2. Add 5 mL of the uniform sample to 45 mL rehydrated folic buffer solution (see "Preparation of the Standard").
3. Incubate the serum-buffer solution at 37°C for 90 minutes. Autoclave the incubated mixture at 121°C for 2.5 minutes.
4. Remove the coagulated protein by centrifuging and transfer the clear supernatant to a clean dry tube. The clear solution is the sample to use in the folic acid assay.

Procedure for Total Folic Acid

1. Use 0.5, 1.0, 1.5 mL or other volumes of the prepared serum extracts as described above.
2. Fill each assay tube with 5 mL of rehydrated Folic Acid Casei Medium and sufficient purified water to give a total volume of 10 mL per tube.
3. Autoclave tubes at 121°C for 5 minutes.
4. Add 1 drop of inoculum described under "Preparation of Stock Cultures and Inoculum" to each assay.
5. Incubate at 35-37°C for 18-24 hours. Tubes are refrigerated for 15-30 minutes to stop growth before reading turbidimetrically.

Expected Results

The amount of folic acid in the test samples can be determined by interpolating the results with the values obtained on the standard curve, taking into consideration the dilutions of the samples.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.

References

1. Flynn, Williams, O'Dell and Hogan. 1951. Anal. Chem. *23*:180.
2. Baker, Herbert, Frank, Pasher, Hunter, Wasserman and Sobotka. 1959. Clin. Chem. *5*:275.
3. Waters and Molin. 1961. J. Clin. Pathol. *14*:335.

Availability

Difco™ Folic Acid Casei Medium

Cat. No. 282210 Dehydrated – 100 g*

**Store at 2-8°C.*