Folic Acid Medium, AOAC

M126

Intended Use:

Recommended for microbiological assay of folic acid using *Enterococcus hirae ATCC 8043*.

Composition**

Ingredients	Gms / Litre
Vitamin free acicase#	10.000
L-Asparagine	0.600
L-Tryptophan	0.200
L-Cystine hydrochloride	0.760
Dextrose (Glucose)	40.000
Adenine sulphate	0.010
Guanine hydrochloride	0.010
Uracil	0.010
Xanthine	0.020
p-Amino benzoic acid (PABA)	0.001
Pyridoxine hydrochloride	0.004
Thiamine hydrochloride	0.0004
Calcium pantothenate	0.0008
Nicotinic acid	0.0008
Biotin	0.00002
Riboflavin (Vitamin B2)	0.001
Glutathione	0.0052
Polysorbate 80	0.100
Sodium citrate	52.000
Dipotassium hydrogen phosphate	6.400
Magnesium sulphate	0.400
Manganese sulphate	0.020
Sodium chloride	0.020
Ferrous sulphate	0.020
Final pH (at 25°C)	6.7±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Casein acid hydrolysate, vitammin free

Directions

Suspend 11.1 grams in 100 ml purified / distilled water. Heat to boiling for 2-3 minutes. Agitate to dispense the slight precipitate evenly. For assay, dispense 5 ml medium into each assay tube (containing increasing amounts of standard or unknown) and make up the total volume to 10 ml per tube with distilled water. Sterilize by autoclaving at 15 lbs pressure (121°C) for 5 minutes. Cool to 45-50°C. Satisfactory results are obtained with Folic acid at levels of 0, 2, 4, 6, 8, 10 nanograms per assay tube (10 ml)

Principle And Interpretation

Folic Acid Assay Medium contains all the necessary nutrients for the growth of the test organism except folic acid. The medium contains nutrients like amino acids, carbohydrates, purine, pyrimidines, salts, and vitamins. Folic acid is required for the growth of *Enterococcus hirae*. Hence growth of this organism will occur only if the sample being assayed contains folic acid. The exact folic acid concentration in the test sample can be determined by comparing the growth obtained to that of known standard concentrations of folic acid (standard curve).

Folic Acid Assay Medium is prepared according to the formula described by Capps et al (1) and is recommended by AOAC (4) for the determination of folic acid content of the pharmaceutical products and other materials using *Enterococcus hirae ATCC 8043* as the test organism.

Standard reference for assay of folic acid should be referred (4).

Procedure : Stock cultures of *Enterococcus hirae* ATCC 8043 are prepared by stab inoculation of Lactobacilli Agar AOAC (M366). Following incubation at 35-37°C for 24 hours, the tubes are stored in the refrigerator. Transplants are made at monthly intervals. Inoculum for assay is prepared by subculturing from a stock culture of *Enterococcus hirae* ATCC 8043 into a tube containing 10 ml of Lactobacilli Broth AOAC (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 resuspended in 10 ml of sterile 0.85% NaCl. The cell suspension is then diluted 1:100 with sterile 0.85% NaCl. One drop of this later suspension is used to inoculate each of the assay tubes. It is essential that a standard curve be set up for each separate assay since conditions of autoclaving, 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, 2, 4, 6, 8 and 10 ng per assay tube (10 ml). Tubes are refrigerated for 15-30 minutes to stop growth before reading. Turbidimetric readings should be read after 16-18 hours incubation at 35-37°C and Acidimetric after 72 hours at 35-37°C. To prepare stock solution of folic acid, 20 mg folic acid is used.

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 0.1 N NaOH to dissolve the acid and then adjust pH to 7.0 with 0.05 N HCl. This solution contains 200 mcg folic acid per ml. Dilute 1 ml of this solution with 999 ml of distilled water to get 200 ng 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 5.0 ml per assay tube.

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 erroneous results.

Type of specimen

Pure isolates

Specimen Collection and Handling:

Stock cultures of Enterococcus hirae ATCC 8043 are prepared by stab inoculation of Lactobacilli Agar AOAC (M366). Following incubation at 35-37°C for 24 hours, the tubes are stored in the refrigerator. Transplants are made at monthly intervals. Inoculum for assay is prepared by subculturing from a stock culture of *Enterococcus hirae* ATCC 8043 into a tube containing 10 ml of Lactobacilli Broth AOAC (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 resuspended in 10 ml of sterile 0.85% NaCl. The cell suspension is then diluted 1:100 with sterile 0.85% NaCl. One drop of this later suspension is used to inoculate each of the assay tubes. It is essential that a standard curve be set up for each separate assay since conditions of autoclaving, 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, 2, 4, 6, 8 and 10 ng per assay tube (10 ml). Tubes are refrigerated for 15-30 minutes to stop growth before reading. Turbidimetric readings should be read after 16-18 hours incubation at 35-37°C and Acidimetric after 72 hours at 35-37°C. To prepare stock solution of folic acid, 20 mg folic acid is used.

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

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

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

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

Reaction
Reaction of 11.1% w/v aqueous solution at 25°C. pH : 6.7±0.2

pH
6.50-6.90

Cultural Response
Gradual increase in growth with increasing standard Folic acid concentration 0,2,4,6,8,10 ng per assay tube is recorded as equivalent increase in absorbance at 620nm

Cultural Response
Microbiological assay of Folic acid is carried out using *Enterococcus hirae ATCC 8043* as per AOAC.After an incubation at 35-37°C for 18-24 hours ,good growth is obtained.

Storage and Shelf Life

Store dehydrated and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. Capps, Hobbs and Fox, 1948, J. Bacteriol., 55:869

2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

4. Official Methods of Analysis of AOAC International, 2005, 19th Ed., Vol. II, Association of Analytical Chemists, Washington, D.C.

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Disclaimer :

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