

Asparagine Broth, Granulated (Coccidioidin and Histoplasmin Broth, Granulated

GM672

Asparagine Broth, Granulated is used for the preparation of Coccidioidin and Histoplasmin antigens for immunodiagnostic work.

Composition**

Ingredients	g/ L
L-Asparagine	7.000
Ammonium chloride	7.000
Dipotassium phosphate	1.310
Sodium citrate	0.900
Magnesium sulphate	1.500
Ferric citrate	0.300
Dextrose (Glucose)	10.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 28.01 grams in 1000 ml distilled water containing 25 ml glycerol. Mix thoroughly and then dispense in a wide bottom flask, to give a depth of 1 to 1.5 inches. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Histoplasma capsulatum, a dimorphic fungus causes histoplasmosis, a systemic fungal disease. *H.capsulatum* is an obligate intracellular organism residing in macrophages of the reticuloendothelial system. Of current concern is the increased incidence of histoplasmosis in patients with AIDS (1). *Coccidioides immitis*, the causative agent of coccidioidomycosis (Valley fever) is endemic in hot regions with dry climate and alkaline soil. Patients with AIDS are at a risk of developing coccidioidomycosis.

Asparagine Broth is a chemically defined medium used for the preparation of Coccidioidin and Histoplasmin antigens.

Histoplasma capsulatum or *Coccidioides immitis* are cultured in this medium for 1-3 months at 37°C till the static phase is obtained. At this stage, cells are autolyzed and a mixture of antigen haptens is prepared. Cell free filtrate from this medium is sterilized by filtration and used as the antigen (2,3). Preparation, standardization and administration of histoplasmin and the interpretation of delayed cutaneous hypersensitivity tests are identical to those for Coccidioidin (4).

The amino acid asparagine, favours the synthesis of antigens from *Histoplasma* and *Coccidioides*. Salts included in the medium buffer the medium well. Dextrose and slightly acidic pH of the medium helps for the luxuriant growth of the fungi.

Type of specimen

Clinical samples: skin scrapings

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In vitro diagnostic use only. For professional 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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

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

Off-white to yellow granulated free flowing powder

Colour and Clarity of prepared medium

Light yellow to yellow coloured clear solution with precipitate

Reaction

Reaction of 2.8% w/v aqueous solution containing 2.5 ml glycerol at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

M672: Cultural characteristics observed with added glycerol, after an incubation at 35-37°C for 1 week.

Organism	Inoculum CFU	Growth
<i>Coccidioides immitis</i>	50-100	luxuriant
<i>Histoplasma capsulatum</i> ATCC 10230	50-100	luxuriant

Storage and Shelf Life

Store dehydrated and prepared medium at 2-8°C in tightly closed container . Use before expiry date on 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. 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

1. Jone P. G., Cohen R. L., Bates D. H., et al., 1983, Six Transm. Dis, 10: 202-204.
2. Smith C. E., Pappagianis D., Levine H. B., and Saito M., 1961, Bact. Rev., 25:310.
3. Emmons C. W., Olson B. J., and Eldridge W. W., 1945, Pub. Hlth. Rept., 60:1383.
4. Emmons W. W., Binford C. H., Utz J. P., and Kwon-Chung K. J., (Eds.), 1977, Medical Mycology, 3rd Ed., Lea and Febiger, Philadelphia.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. 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.

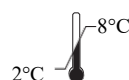
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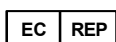
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Plot No.C-40, Road No.21Y,
MIDC, Wagle Industrial Area,
Thane (W) -400604, MS, India



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