



## Asparagine Broth (Coccidioidin and Histoplasmin Broth)

M672

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

### Composition\*\*

Ingredients	Gms / Litre
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	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.

### Quality Control

#### Appearance

Off-white to yellow homogeneous free flowing powder

#### Colour and Clarity of prepared medium

Yellow coloured clear solution with brownish 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>	50-100	luxuriant
ATCC 10230		

## Storage and Shelf Life

Store dehydrated and prepared medium at 2-8°C in tightly closed container . Use before expiry date on label.

## 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.

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