

BYE Agar**M470**

BYE Agar is a simplified medium developed for the cultivation of *Mycoplasma*, or Pleuropneumonia like organisms and L-forms of bacteria.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
Calf brain, infusion from	200.000
Beef heart, infusion from	250.000
Dextrose	2.000
Sodium chloride	5.000
Disodium phosphate	2.500
Yeast extract	2.000
Agar	13.000
Final pH (at 25°C)	7.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 52 grams in 850 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add 150 ml of sterile human or animal blood or serum. Mix gently and pour into sterile Petri plates.

Principle And Interpretation

Mycoplasmas (mollicutes) are the smallest free-living microorganisms (1). Earlier *Mycoplasmataceae* were given the general title of pleuropneumonia like organism (PPLO), because of similarities to *Mycoplasma mycoides* (subsp. mycoides), the causative agent of bovine pleuropneumonia (2). BYE media are simple media developed for cultivation and routine studies of distribution, habitat and possible pathogenesis of *Mycoplasma* - Pleuropneumonia like organisms and L-forms of bacteria by Barile, Yaguchi and Eveland (3). These media can be used for isolation of PPLOs from urethritis, penile ulcerations and cervical specimens and L-forms of *Corynebacterium*, *Neisseria*, and *Streptococcus*. These are also used for detecting PPLO contamination of tissue culture and cell-lines (4) and for membrane filter work (5).

BYE Agar contains brain and heart infusion along with yeast extract, which provide carbon, nitrogen, vitamins and other growth factors required for the metabolism of *Mycoplasma* - Pleuropneumonia like organisms. Inoculations are made in duplicates. One set is incubated aerobically while the other anaerobically for 48 hours or more. Usually growth occurs within 3-5 days; however, negative results are reported after 10 days. Anaerobic conditions are most important for the first 3 days while secondary transfers can be incubated aerobically.

Quality Control**Appearance**

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.3% Agar gel.

Colour and Clarity of prepared medium

Yellow coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.2% w/v aqueous solution at 25°C. pH : 7.9±0.2

Cultural Response

M470: Cultural characteristics observed with added serum under humidified anaerobic conditions, after an incubation at 35-37°C for 5-10 days.

Organism	Growth					
<i>Mycoplasma bovis</i> ATCC 25523	good-luxuriant					
<i>Mycoplasma gallinarium</i> ATCC 19708	good-luxuriant					
<i>Mycoplasma pneumoniae</i> ATCC 15531	good-luxuriant					

Reference

- ### Storage and Shelf Life

Store below 30°C and the prepared medium at 2 - 8°C. Use before expiry date on the label.