

# **Technical Data**

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\*\*

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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)	$7.9\pm0.2$

<sup>\*\*</sup>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

#### pН

7.70-8.10

## **Cultural Response**

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

Organism	Growth
<b>Cultural Response</b>	
Mycoplasma bovis ATCC	good-luxuriant
25523	
Mycoplasma gallinarium	good-luxuriant
ATCC 19708	
Mycoplasma pneumoniae	good-luxuriant
ATCC 15531	
Streptococcus pneumoniae	good-luxuriant
ATCC 6303	

# **Storage and Shelf Life**

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

### Reference

- 1. Murray P.R., Baron E. J., Pfaller M.A., Tenover F.C., Yolken R.H.(Eds.),1995, Manual of Clinical Microbiology, 6th Ed., ASM Press.
- 2. Collee J.G, Fraser A.G., Marmion B.P., Simmons. A (Eds.), 1996, Mackie and McCartney Practical Medical Microbiology, 14th Ed, Churchill Livingstone.
- 3. Barile, Yaguchi, Eveland, 1958, Am. J. Clin. Path. 30:171.
- 4. Barile, 1962, National Cancer Institute Monograph, No.7: 5.5. Barile, 1962, J. Bacteriol., 83:430.

Revision: 1 / 2011

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