MAST® Culture Media and Supplements

Technical Information Sheet

Product Code DM 258

Legionella Buffered Charcoal Yeast Extract (BCYE) Agar Base

A basal medium used in the isolation of *Legionella* spp. from environmental and clinical specimens.

1. Description

The original solid medium for isolation of *Legionella* spp. F-G agar, was described in 1978 by Feeley et al[1]. The main components included to support growth, were ferric salts and L-cysteine hydrochloride. Growth was best at pH 6.9, a temperature of 35°C and under 2.5% CO2. In 1979, the same workers added yeast extract and activated charcoal to F-G agar which greatly enhanced its ability to support the growth of *Legionella* spp.[2] The new medium, called Charcoal Yeast Extract (CYE) Agar was further modified to include ACES buffer[3] to minimise fluctuations in pH and aketoglutarate[4] to improve growth. The final medium is a great improvement on the original and is known as Buffered Charcoal Yeast Extract (BCYE) Agar.

2. Technical Formula*

Formula	grams per litre
Activated charcoal	1.5
Yeast extract	10.0
ACES buffer	6.0
Ferric pyrophosphate	0.25
Â-ketoglutarate	1.0
Agar	12.0
pH approx. 6.9	

*Formulation may be modified to meet performance criteria

3. Directions

1. Suspend 30.75g of powder in 1 litre or the contents of a sachet in the stated volume of distilled or deionised water and mix thoroughly.

2. Sterilise by autoclaving at 121°C for 15 minutes and cool to 50-55°C. Hold in a waterbath at this temperature and add one vial of L-cysteine growth supplement Selectavial (SV35) per 500ml medium.

3. Reconstitute the contents of one vial using 5ml sterile water. The best method is to aseptically add the diluent using a sterile needle and syringe. Draw the diluent into the syringe and after removing the plastic cap of the vial inject through the rubber stopper of the vial. The lyophilised supplement will rapidly dissolve and may be withdrawn into the syringe.

4. Add the antibiotic solution to 500ml of medium and discard the needle into an approved container. DO NOT TRY TO RESHEATH AN EXPOSED NEEDLE. Unused, reconstituted supplement should be discarded and not frozen.

5. Selective supplement Selectatabs (MS36 or SV37) if desired are added at this stage.

6. After Selectatab(s) have broken up swirl 3-4 times and invert to complete dissolution and to ensure that the charcoal is evenly suspended.

7. Pour plates.

8. Prepared plates can be used immediately or stored in plastic bags at 2-8°C for up to 1 week before use.

4. In Use

Poured plates are dried before use and the specimen cultured directly onto the surface of the supplemented medium. Plates are then incubated at 35-37°C in a humidified atmosphere, preferably under 2.5% CO2. Growth of *Legionella* spp. should occur after 3-7 days incubation. *L.pneumophila* colonies are bluish-white and translucent in appearance. If selective plates have been prepared it is advisable, as with all selective culture techniques, to include a non-selective plate in parallel.

Based on the absolute requirement of L-cysteine for growth of *Legionella* spp., a test organism that grows on BCYE Agar supplemented with Lcysteine-HCl, and fails to grow on unsupplemented BCYE Agar, can be presumptively identified as *Legionella* spp. This test is easy to perform using the MAST format as L-cysteine is the sole component of the growth supplement

5. References

1. Feely JC, Gorman GW, Weaver RE, Mackel DC, Smith HW. *J Clin Microbiol* 1978; **8:** No. 3; 320-325.

2. Feeley JC, Gibson RJ, Gorman GW, Langford NC, Rasheed JK, Mackel DC, Baine WB. *J Clin Microbiol* 1979; **10:** No. 4; 437-441.

3. Pascule AN, Feeley JC, Gibson RJ, Cordes LG, Myerowitz RL, Patton CM, Gorman GW, Carmach CL, Ezzel JW, Dowling JN. *J Inf Dis* 1980; **141:** 727- 732.

4. Edelstein PH. *J Clin Microbiol* 1981; **14:** 298-303.

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