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Legionella Buffered Charcoal Yeast Extract (BCYE) Agar Base

DM258

Intended Use

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

Contents

See pack label.

Formulation*

Material:	Concentration in medium:
Activated charcoal	1.5 g/litre
Yeast extract	10.0 g/litre
ACES buffer	6.0 g/litre
Ferric pyrophosphate	0.25 g/litre
α -ketoglutarate	1.0 g/litre
Agar	12.0 g/litre
Final pH: 6.9 \pm 0.2	

Storage and shelf life

All dehydrated culture media containers should be kept tightly closed and stored in a dry place at 10 to 25°C until the expiry date shown on the pack label.

Precautions

For *in vitro* diagnostic use only. Observe approved hazard precautions and aseptic techniques. To be used only by adequately trained and qualified laboratory personnel. Sterilise all biohazard waste before disposal. Refer to Product Safety Data sheet (available on request or via MAST® website).

Materials required but not provided

Standard microbiological supplies and equipment such as loops, MAST® selective supplements, swabs, applicator sticks, incinerators and incubators, etc., as well as serological and biochemical reagents and additives such as blood.

Procedure

1. Refer to pack label for quantities and volumes required. Prepare MAST® Legionella BCYE Agar Base (DM258D) by suspending the powder in distilled or deionised water. For sachet packs, dissolve the entire contents of the sachet in the volume shown on the label.
2. Autoclave at 121°C (15 p.s.i.) for 15 minutes.
3. Cool to 50 to 55°C and hold at this temperature in a water bath. Add MAST® L-cysteine growth supplement (SV35).
4. If required the medium can be made selective by the addition of Legionella MAST® SELECTATAB (MWY) (MS36) or Legionella MAST® SELECTAVIAL (PNV) (SV37).

5. After supplementation, swirl 3 to 4 times and invert to ensure that the charcoal is evenly suspended.
6. Pour culture plates (20ml per plate) and allow to set. Single vent Petris may be preferred as they help retain the humidity favoured by *Legionella*.
7. Prepared culture plates may be used immediately or stored in plastic bags at 2 to 8°C for up to one week before use.
8. Poured plates should be dried before use and the specimen cultured directly onto the surface of the supplemented medium. If selective plates have been prepared it is advisable, as with all selective culture techniques, to include a non-selective plate in parallel.
9. Incubate at 35 to 37°C in a humidified atmosphere, preferably under 2.5% CO₂ for 3 to 7 days.

Interpretation of results

After incubation record growth of organisms. Typical characteristics to note include colony size and morphology and pigmentation. *L. pneumophila* colonies are bluish-white and translucent in appearance.

Based on the absolute requirement of L-cysteine for growth of *Legionella* spp., a test organism that grows on BCYE Agar supplemented with L-cysteine-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

Quality control

Check for signs of deterioration. Quality control must be performed with at least one organism to demonstrate expected performance. Do not use the product if the result with the control organism is incorrect. The list below illustrates a range of performance control strains which the end user can easily obtain.

Test Organisms	Result*
<i>Legionella pneumophila</i> ATCC® 33152	Growth
<i>Escherichia coli</i> ATCC® 25922	No Growth

*Results shown are representative of those obtained on MAST® Selective Legionella Agar

References

Bibliography available on request.