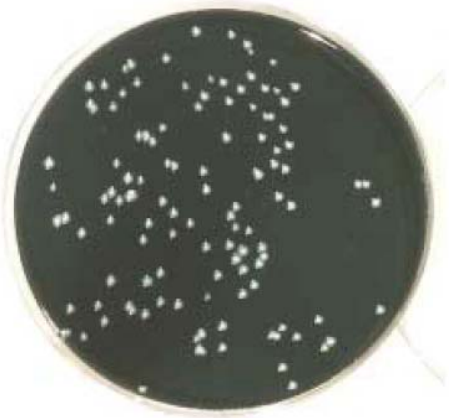
LAB195

BCYE Legionella Isolation Medium



DESCRIPTION

BCYE (Buffered Charcoal Yeast Extract) Legionella Isolation Medium (LAB195) is a base medium used for the isolation of Legionella from clinical and environmental samples. This medium is based on the charcoal yeast extract formulation of Feeley *et al*.[1&2]. The performance of this medium is further enhanced by the additions of ACES (N-2-acetamido-2-aminoethane - sulphonic acid) buffer and α-ketoglutarate as defined by Edelstein[3]. This medium is also detailed in internationally recognized methodology[4] for the isolation of *Legionella* spp. from water.

Specimens or samples are often heavily contaminated with other bacteria and consequentially a range of selective supplements have been developed to aid isolation. Lab M provide the GVPC supplement (X195) which is most effective for the isolation of *L. pneumophila*. It is recommended that this supplement is used in conjunction with heat and acid sample treatments, to further reduce the growth of non-*Legionella* bacteria.

This product contains the ACES buffer and ferric pyrophosphate in the base medium. This negates the need for complex freeze dried supplements. A complementary growth supplement is provided (X196) which contains the L-cysteine and α-ketoglutarate. In addition, an α-ketoglutarate supplement (X197) is also available for the preparation of confirmatory media for suspected *Legionella* colonies.

BCYE Legionella Isolation Medium (LAB195)

Formula	**Amount / Litre**
Yeast Extract	10.00g
Charcoal	2.00g
Ferric Pyrophosphate	0.25g
ACES Buffer	10.00g
Potassium Carbonate	2.28g
Agar	14.00g
Supplements	
GVPC Selective (X195)	
Glycine	3000mg
Vancomycin	1mg
Polymyxin B	79200IU
Cycloheximide	80mg
Growth Supplement (X196)	
L-Cysteine	400mg
α-ketoglutarate	1000mg
Presumptive ID (X197)	
α-ketoglutarate	1000mg

Add one vial per 500mL of sterilised medium as appropriate

pH 6.9 ± 0.1

PRINCIPLE OF ISOLATION

Water samples are concentrated either by membrane filtration or centrifugation (turbid samples may also be centrifuged). To reduce the growth of unwanted bacteria, separate portions of the concentrated sample may be subjected to heat and acid treatments. Treated and untreated portions are then inoculated onto *Legionella* selective media.

METHOD FOR RECONSTITUTION

Selective Isolation. Weigh 38.5 grams of powder and disperse in 1 litre of deionised water. Soak for 10 minutes, swirl to mix and sterilise by autoclaving at 110ºC for 10 minutes. Cool to 47ºC and aseptically add 2 vials of reconstituted growth supplement X196 and 2 vials of reconstituted

selective supplement X195. Mix well and pour into sterile Petri dishes.
Maintenance. Weigh 38.5 grams of powder and disperse in 1 litre of deionised water. Soak for 10 minutes, swirl to mix and sterilize by autoclaving at 110°C for 10 minutes. Cool to 47°C and aseptically add 2 vials of reconstituted growth supplement X196. Mix well and pour into Petri dishes.
Presumptive Identification. Weigh 38.5 grams of powder and disperse in 1 litre of deionised water. Soak for 10 minutes, swirl to mix and sterilize by autoclaving at 110°C for 10 minutes. Cool to 47°C and aseptically add 2 vials of reconstituted growth supplement X197. Mix well and pour into Petri dishes.

INOCULATION

The concentrated sample should be split into 3 portions. One portion is used without any further treatment, the other 2 portions should be treated, one with heat and the other with acid.
Heat Treatment. Take 1ml of the concentrated sample and place in a water bath at 50°C for 30 minutes.
Acid Treatment. Take 1-10ml of the concentrated sample and centrifuge at 6000g for 10 minutes. Decant the supernatant to leave half the original volume. Vortex to re-suspend the pellet and make up to the original volume using an HCl-KCl buffer. Leave to stand for 5 minutes.
Inoculate the first plate of GVPC supplemented media with 0.1mL of the untreated portion and spread over the entire surface of the plate. Inoculate the second plate of GVPC supplemented media in the same way with 0.1ml of the heat treated portion as soon as possible after removal from the water bath. Inoculate the third plate of GVPC supplemented media in the same way with 0.1mL of the acid treated portion immediately after acid treatment.

INCUBATION

Incubate at 36 ± 1°C in a humid atmosphere under aerobic conditions for up to 10 days.

INTERPRETATION

The plates should be examined for growth on days 3, 5, 7 and 10. Suspect colonies should be sub-cultured on to "maintenance" supplemented BCYE medium and "presumptive ID" supplemented BCYE medium, incubate as before. Isolates that fail to grow on the "presumptive ID" medium but grow on the maintenance medium and have typical morphology should be regarded as presumptive *Legionella*.

Presumptive isolates should be confirmed using a serological method, e.g. Microgen M45 Latex.

MINIMUM Q.C. ORGANISMS

Legionella spp. - Growth

Staphylococcus epidermidis - Growth

Escherichia coli - Inhibited

PACKAGING

BCYE Legionella Isolation Medium:

1 litre sample	LAB195-E38.5
500g	LAB195-A
2.5kg	LAB195-B2.5
5kg	LAB195-B5
10kg	LAB195-B10

X195 GVPC Selective Supplement - 10 vials, each sufficient for 500ml of media.
X196 BCYE Growth Supplement - 10 vials, each sufficient for 500ml of media.
X197 BCYE Growth Supplement (omitting L-Cysteine) - 10 vials, each sufficient for 500ml of media.

LATEX KITS: Product M45 . Lab M also distributes the Microscreen® Legionella Latex Kit. This provides reagents for the confirmation of *Legionella pneumophila* serogroups 1 and 2-15.

REFERENCES

1. Feeley, J.C., Gibson, R.J. *et al.* (1979). *Journal of Clinical Microbiology* **10**: 437-441

2. Pesculle, A.H., Feeley, J.C. *et al.* (1980). *Journal of Infectious Disease* **141**: 727-732

3. Edelstein, P.H. (1982). *Journal of Clinical Microbiology* **14**: 298-303

4. International Standard. ISO 11731:1998(E). Water Quality – Detection & Enumeration of *Legionella*.