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 Edelstein3.

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 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.

Typical Formulation

<table>
<thead>
<tr>
<th>Per litre</th>
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<tbody>
<tr>
<td>Yeast extract</td>
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<tr>
<td>Charcoal</td>
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<tr>
<td>Ferric pyrophosphate</td>
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<tr>
<td>ACES buffer</td>
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<tr>
<td>Potassium carbonate</td>
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<tr>
<td>Agar</td>
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Appearance
Finished medium: black gel

pH: 6.9 ± 0.1 at 25°C

Hazard Classification
Refer to appropriate SDS

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 110°C for 10 minutes. Cool to 47°C 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 3.85g of powder and disperse in 1 litre of deionized water. Soak for 10 minutes, swirl to mix and sterilise 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 3.85g of powder and disperse in 1 litre of deionized water. Soak for 10 minutes, swirl to mix and sterilise 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 2 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 6,000g 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°C ± 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.

Storage
Dehydrated culture media: 10-25°C
Final medium: 7 days at 2-8°C in the dark

Minimum QC Organisms
Legionella pneumophila WDCM 00107
Legionella pneumophila WDCM 00180

LAB195_01
References