Legionella CYE-Agar (Base)

Selective culture medium for the growth and isolation of Legionella spp. from biological sample material. In combination with sample preparation by heat or acid, this culture medium is acknowledged as the best method of isolating Legionella pneumophila from natural water (DENNIS et al., 1984).

General Information

In 1977 McDADE et al. isolated a bacterium, which was first described in connection with an epidemic, which occured after a meeting of the "American Legion" in Philadelphia. For this reason the disease was called legionellosis ("Legionnaires' Disease"). The most important pathogen of legionellosis, among a total of 33 species, is Legionella pneumophila.

Principle

Microbioloical method

Mode of Action

The growth of Legionella is improved by the following components: the activated charcoal binds CO2, changes surfache tension and neutralises growth-inhibiting substances. L-cysteine HCl and a-ketoglutarate are directly used to form amino acid and chelate respectively. Ferric pyrophosphate serves as a source of iron and the optimal pH value for growth is adjusted by the ACES buffer. The accompanying flora is largely inhibited by the addition of glycine and the use of the antibiotic mixture of vancomycin, Polymyxin B and cycloheximide.

Typical Composition

1. Legionella CYE-Agar Base (g/liter): activated charcoal 2.0; yeast extract 10.0; agar-agar 16.0
2. Legionella BCYE a-growth supplement (composition of one vial; for 500 ml of culture medium) ACES buffer 5.0 g; ferric pyrophosphate 0.125 g; cysteine HCl 0.2 g; a-ketoglutarate 0.5; potassium hydroxide 1.4 g
3. Legionella GVPC selective supplement (composition of one vial; for 500 ml of culture medium) glycine 1.5 g, vancomycin-HCl 0.5 mg; Polymyxin B-sulfate 40,000 I.E.; cycloheximide 40 mg

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25 °C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25 °C

Preparation of BCYE Agar

1. Suspend 14,0 g CYE-Agar (Base) (Art. No. 110242) in 450 ml demin. water; sterilize by autoclaving at 121 °C for 15 minutes.
2. Reconstitute the contents of one bottle BCYE α-Growth-Supplement aseptically in 50 ml of sterile demin. water
3. Heat the BCYE α-Growth-supplement up 40 °C
4. Allow CYE-Agar (Base) (450 ml) to cool to 45-50 °C and aseptically add one bottle of the warm, reconstituted BCYE α-Growth-Supplement; mix gently and pour into sterile plates.

Final pH of Legionella-BCYE medium: 6.9 ± 0.2 (at +25 °C)

Preparation of GVPC-Agar

1. Suspend 14.0 g CYE-Agar (Base) (Art.No. 110242) in 440 ml demin. water; sterilize by autoclaving at 121 °C for 15 minutes.
2. Reconstitute the contents of one bottle BCYE-α-Growth-Supplement aseptically in 50 ml of sterile dermin. water.
3. Heat the BCYE-α-Growth-Supplement up to 40 °C
4. Reconstitute the contents of one bottle GVPC-Selective Supplement aseptically in 10 ml of sterile demin. water.
5. Allow CYE-Agar (Base) (440 ml) to cool to 45-50 °C and aseptically add one bottle of the warm reconstituted BCYE-α-Growth-Supplement and one bottle of GVPC-Selective-Supplement; mix gently and pour into sterile plates.

Final pH of Legionella-GVPC medium: 6.9 ± 0.2 (at +25 °C)

Experimental Procedure and Evaluation

Sample Preparation

It is recommended that three plates should be prepared for every sample: one after heat treatment, one after acid treatment and one without pre-treatment.

Heat treatment:

10 ml of concentrated examination material are incubated in a water bath at 50 °C for 30 minutes.

Acid treatment:

1. Centrifuge 10 ml of concentrated examination material at 2,500 R/min for 20 minutes in screw capped centrifuge vessels.
2. Pour off the supernatant to about 1 ml.
3. Add 9 ml HCl-KCl buffer*, shake gently and leave to stand for about 5 minutes.

* HCl-KCl buffer:
3.9 ml of 0.2 M HCl
25.0 ml of 0.2 M KCl
adjust pH to 2.2 ± 0.2 by adding 1 M KOH

Application

1. Spread 0.1 ml of pre-treated sample onto GVPC-Selective Agar.
2. Incubate for up to 7 days at 35 °C under microaerophilic conditions (see Anaerocult® C).

Evaluation

Legionella grows as a 2-3 mm, hour-glass shaped, grey-white colony. A few strains have a slightly blue colouring. Suspicious colonies are subcultured on to CASO Agar (Cat. No. 1.05458) with 5 % sheep-blood and B.C.Y.E.-Agar. Isolates that fail to grow on Blood Agar and poorly staining Gram-negative rods are presumptively identified as Legionella. These presumptive colonies should be serologically typified for further identification.

Legionella CYE-Agar (Base)

Literature

BOPP, C.A., SUMMER, J.W., MORRIS, G.K., a. WELLS, J.G.: Isolation of Legionella spp. from environmental water samples by low-pH treatment and use of a selective medium. - J. Clin. Microbiol., 13; 714-719 (1981).

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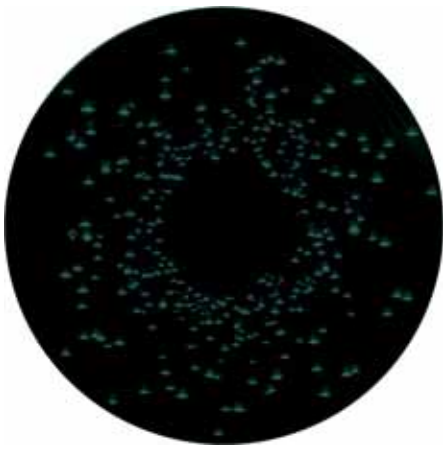
Ordering information

Product	Ordering No.	Pack size
CYE Agar Base	1.10242.0500	500 g
Legionella BCYE a-Growth-Supplement	1.10240.0010	1 x 10 vials
Legionella GVPC Selective Supplement	1.10241.0010	1 x 10 vials
Anaerobic jar	1.16387.0001	1 ey
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® C	1.16275.0001	1 x 10
Anaerocult® C mini	1.13682.0001	1 x 25
CASO Agar (Casein Peptone Soymeal Peptone Agar)	1.05458.0500	500 g 5 kg
Plate basket	1.07040.0001	1 ea
Legionella BCYE Agar	1.10097.0020	20 plates
Legionella GVPC Selective Agar	1.10098.0020	20 plates



Legionella pneumophila spp. fraseri ATCC 33216

Quality

Test strains	Inoculum (cfu)	recovery rate
Legionella auisa ATCC 35292	10 - 100	≥ 50 %
Legionella pneumophila ATCC 35096	10 - 100	≥ 50 %
Legionella pneumophila subsp. pneumophila ATCC 33152	10 - 100	≥ 50 %
Escherichia coli ATCC 25922	5000	no
Pseudomonas aeruginosa ATCC 27853	50 - 500	no
Enterococcus faecalis ATCC 19433	5000	no