TECHNICAL DATA SHEET

BCYE AGARS WITH AND WITHOUT CYSTEINE

CONFIRMATION OF *LEGIONELLA*

1 INTENDED USE

The two confirmation agars for *Legionella*, BCYE and BCYE without cysteine are destined for the confirmation of colonies obtained on GVPC agar, which itself is used for the enumeration and isolation of *Legionella* species in water and in other samples susceptible of harboring the bacteria.

The typical composition of the media responds to the composition described in the water control directives ISO 11731, NF T 90-431 and NF EN ISO 11731-2.

2 HISTORY

In 1977, MacDade *et al.* were the first to isolate the agent responsible for Legionnaire's Disease, a bacterium now known as *Legionella pneumophila*. After this discovery, numerous occurrences of *Legionella* isolation were reported in fresh water environments such as water distribution systems, air conditioning, cooling towers, and spas. 48 species of *Legionella* are currently known.

In 1978, Weaver succeeded in cultivating *Legionella* on Mueller-Hinton chocolate agar. Feeley *et al.*, deduced that cysteine and ferric pyrophosphate could replace the vitamin and hemoglobin supplements found in the Mueller Hinton chocolate agar. Their work led to the formulation of a medium dubbed F-G agar. They determined as well that an atmosphere enriched at 2.5% CO_2 was necessary for *Legionella* culture..

In 1979, Feeley *et al.* modified the F-G medium by replacing acid hydrolysate of casein by yeast extract, and adding activated charcoal while eliminating starch. The resulting CYE media allowed better growth of *Legionella*.

In 1980, Pasculle *et al.* supplemented the CYE medium with ACES buffer. They demonstrated that this new medium, designated BCYE, offered a better recovery of *Legionella* and could be incubated aerobically..

In 1981, Edelstein increased the sensitivity of the medium by adding α-ketoglutarate (BCYEα medium). Colonies are considered to be *Legionella* if they develop on BCYEα medium, but demonstrate no growth on BCYEα without cysteine (BCYEα - cys).

3 PRINCIPLES

Yeast extract constitutes a primary nutrient leading to *Legionella* growth.

Activated charcoal decomposes hydrogen peroxide (toxic metabolic by-product), captures the carbon dioxide and modifies the surface tension.

The ACES/KOH buffer maintains the pH and permits aerobic incubation.

Cysteine and ferric pyrophosphate represents indispensable nutritive elements for the growth of *Legionella.*

α-ketoglutarate is a growth activator for *Legionella*.

4 TYPICAL COMPOSITION

The compositions may be adjusted to obtain optimal performance..

BCYE agar with cysteine

For 1 liter of media :

- Yeast extract ..... 10,0 g
- Activated charcoal ..... 2,0 g
- α-ketoglutarate, monopotassium salt ..... 1,0 g
- ACES ..... 10,0 g
- Potassium hydroxide ..... 2,8 g
- Ferric pyrophosphate ..... 0,25 g
- Bacteriological agar ..... 12,0 g
- L-cysteine, hydrochloride..... 0,4 g

pH of the ready-to-use media at 25 °C : 6,9 ± 0,2

BCYE agar without cysteine

For 1 liter of media :

- Yeast extract ..... 10,0 g
- Activated charcoal ..... 2,0 g
- α-ketoglutarate, monopotassium salt ..... 1,0 g
- ACES ..... 10,0 g
- Potassium hydroxide ..... 2,8 g
- Ferric pyrophosphate..... 0,25 g
- Bacteriological agar ..... 12,0 g

pH of the ready-to-use media at 25 °C : 6,9 ± 0,2

5 INSTRUCTIONS FOR USE

- Re-streak each type of colony onto a plate of BCYE agar without cysteine (BM073) and to a plate of BCYE with cysteine (BM072).
- Incubate at 36 ± 2 °C for 3 to 4 days.

✓ **Inoculation :**
Surface inoculation

✓ **Incubation :**
3 to 4 days at 36 ± 2 °C

6 RESULTS

Consider as positive for *Legionella* all colonies that develop on BCYE (BM072) agar with cysteine but present no growth on BCYE – cys (BM073).

Colonies of *Legionella* spp. present a white to gray coloration on BCYEα with cysteine (BM072). They can also have blue, pink, purple, maroon, greenish-yellow or dark red pigmentation that fades, becoming whiter and filamentous with age. Their surface is smooth with precise edges. Some strains may give a ground glass or “fried egg” aspect when observed through a binocular scope, while others may present a brilliant white fluorescence under a UV light.

See ANNEX 1 : PHOTO SUPPORT.

7 QUALITY CONTROL

BCYE agar with cysteine
Prepared media in plates : black agar, with visible particles of activated charcoal.
Typical cultural response after 72 hours of incubation at 36 °C (NF EN ISO 11133) :

Microorganisms		Growth (Productivity Ratio : P_R)
Legionella pneumophila	WDCM 00107	$P_R \geq 70$ %

BCYEα without cysteine
Prepared media in plates : black agar, with visible particles of activated charcoal.
Typical cultural response after 72 hours of incubation at 36 °C :

Microorganisms		Growth
Legionella pneumophila	WDCM 00107	Negative
Escherichia coli	WDCM 00179	Positive

8 STORAGE / SHELF LIFE

Complete pre-poured media : 2-8 °C.
The expiry date is indicated on the label.

9 PACKAGING

BCYE agar with cysteine, pre-poured in Petri dishes (ø 90 mm)
20 plates BM07208

BCYEα without cysteine, pre-poured in Petri dishes (ø 90 mm)
20 boîtes BM07308

10 BIBLIOGRAPHY

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NF EN ISO 11731-2. Juillet 2008. Qualité de l'eau. Recherche et dénombrement des *Legionella*. Partie 2 : Méthode par filtration directe sur membrane pour les eaux à faible teneur en bactéries.

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11 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

Document code : BCYE AGARS_EN_v2
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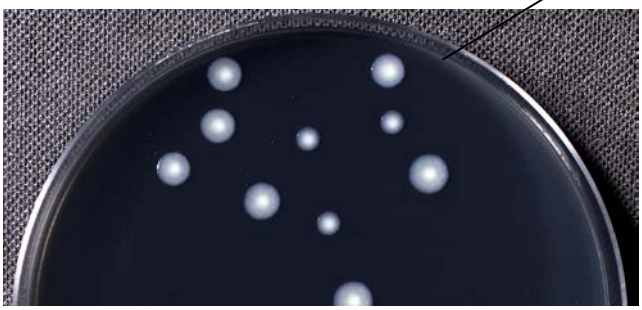
Agars BCYEα & BCYE without cysteine

Confirmation of *Legionella*.

Results :

Growth obtained after 4 days of incubation at 36 °C.



Legionella pneumophila

Characteristic colony :
White to gray color with a smooth surface, sometimes presenting a ground glass appearance when viewed through a stereoscope.

Microorganisms	Growth BCYEα media	Growth on BCYEα without cysteine
Legionella	White to gray colonies with a smooth surface.	Absence of colonies