TECHNICAL DATA SHEET

CONFIRMATION AGARS FOR *LEGIONELLA* (BCYEα AND BCYEα WITHOUT CYSTEINE)

CONFIRMATION FOR ENUMERATION AND ISOLATION OF *LEGIONELLA*

BM07208
BM07308

1 INTENDED USE

The two confirmation agars for *Legionella*, BCYEα and BCYEα without cysteine, are destined for the confirmation of colonies previously isolated on GVPC agar. Collectively, these media are used for the enumeration and isolation of *Legionella* species in water and other samples susceptible of harboring the bacteria.

2 HISTORY

- In 1977, MacDade *et al.* Were the first to isolate the agent responsible for Legionnaire's Disease, a bacteria now known as *Legionella pneumophila*. After this discovery, numerous occurrences of *Legionella* isolation of *Legionella* 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% CO2 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.
- End in 1981, Edelstein increased the sensitivity of the medium by adding α-cetoglutarate (BCYEα medium). Colonies are considered to be *Legionella* if they develop on BCYEα medium, but demonstrate no growth on BCYEα without cysteine.

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-cetoglutarate is a growth activator for *Legionella*.

4 INSTRUCTIONS FOR USE

- Select at least three characteristic *Legionella* colonies from each plate of GVPC agar (**BM071**).
- Re-streak each colony onto a plate of BCYEα agar without cysteine (**BM073**) and a plate of BCYEα agar (**BM072**).
- Incubate at 36 ± 1°C for at least 2 days.
- Consider as positive for *Legionella* all colonies that develop on BCYEα agar but present no growth on BCYEα agar without cysteine.
- Proceed with serological identification of the *Legionella* species.

Biokar Diagnostics
Rue des Quarante Mines – ZAC de Ther – Allonne – B.P. 10245 – 60002 Beauvais Cedex – France
Tél : + 33 (0)3 44 14 33 33 – Fax : + 33 (0)3 44 14 33 34 – www.biokar-diagnostics.fr

5 RESULTS

The present of colonies on BCYEα Agar with cysteine (**BM072**) and the absence of colonies on BCYEα Agar without cysteine (**BM072**) proves the present of *Legionella*.

Colonies of *Legionella* spp. present a white to gray coloration on BCYEα Agar 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 APPENDIX 1 : PHOTO SUPPORT)

6 TYPICAL COMPOSITIONS

Typical compositions can be adjusted to obtain optimal performance.

- **BCYEα Agar with cysteine**

For 1 liter of media :

- Yeast extract ..... 10,0 g
- Activated charcoal ..... 2,0 g
- α-cetoglutarate, monopotassium salt ..... 1,0 g
- ACES (2-[2-amino-2-oxoethyl)-amino] ethanesulfonic acid) ..... 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 ± 0.1

- **BCYEα agar without cysteine**

For 1 liter of media :

- Yeast extract ..... 10,0 g
- Activated charcoal ..... 2,0 g
- α-cetoglutarate, monopotassium salt ..... 1,0 g
- ACES (2-[2-amino-2-oxoethyl)-amino] ethanesulfonic acid) ..... 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

7 QUALITY CONTROL

BCYEα agar (BM072) / BCYEα agar without cysteine (BM073)

- Prepared media in plates : black agar, with visible particles of activated charcoal.
- Typical cultural response after 72 hours incubation at 36°C :

Microorganisms		Growth	
		BCYEα agar	BCYEα agar without cysteine
Legionella pneumophila	WDCM 00107	positive	negative
Escherichia coli	WDCM 00179	positive	positive

8 STORAGE

Pre-poured media (complete) in Petri dishes BM072 and BM073:

Store between 2 - 8°C, shielded from light.
The expiration date is indicated on the label

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9 PRESENTATION

Pre-poured media in Petri plates (Ø 90 mm):

BCYEα agar – 20 plates BM07208
BCYEα agar without cysteine – 20 plates BM07308

10 BIBLIOGRAPHY

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11 OTHER INFORMATIONS

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

Code document : BM072/A/2012-05 : 1.
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APPENDIX 1 : PHOTO SUPPORT

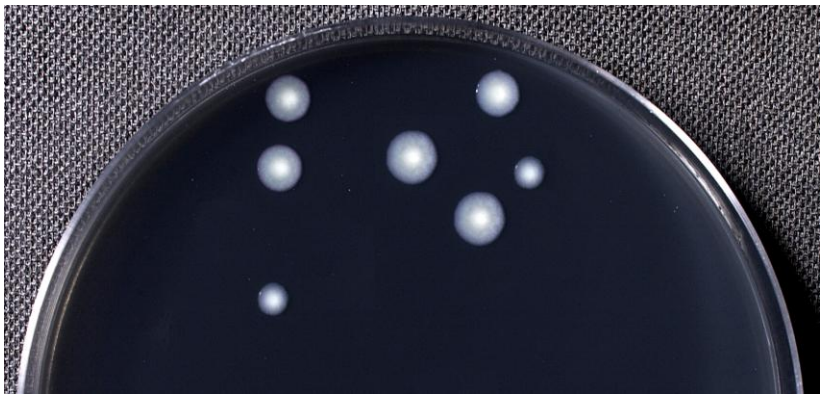
CONFIRMATION AGARS FOR *LEGIONELLA* BCYEα AND BCYEα WITHOUT CYSTEINE

Methodology:

- Re-streak 3 characteristic *Legionella* colonies from plate of GVPC agar onto BCYEα (**BM072**) agar and BCYEα agar without cysteine (**BM073**).
- Incubate at 36 ± 1°C for 2 days.

Results:

Growth on BCYEα agar and BCYEα agar without cysteine after incubate at 36°C for 2 days



Legionella pneumophila on BCYEα agar.

Microorganisms	Characteristic colonies on BCYEα agar	Characteristic colonies on BCYEα without cysteine agar
Legionella	White to gray colonies with a smooth surface ; may present a ground glass appearance under a binocular scope	Absence of colony

Product codes:

BM07208 : BCYEα agar - 20 plates
BM07308 : BCYEα agar without cysteine - 20 plates

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