TECHNICAL DATA SHEET

GVPC AGAR FOR *LEGIONELLA*

DETECTION AND ENUMERATION OF *LEGIONELLA* BM07108

1 INTENDED USE

GVPC agar for *Legionella* is used for the enumeration, isolation and culture 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 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-cetoglutarate (BCYEα medium), and Wadowsky & Yee suggested incorporating glycine, vancomycin and polymyxin B (GVP medium) to obtain a selective culture media. In 1984, Dennis *et al.* formulated the current GVPC medium by adding cycloheximide into GVP medium. They demonstrated that this selective medium allowed a greater level of *Legionella* isolation.

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 represent indispensable nutritive elements for the growth of *Legionella*.

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

Secondary microflora are inhibited by the association of glycine, vancomycin , polymyxin B and cycloheximide.

4 INSTRUCTIONS FOR USE

Surface inoculation :

- To the surface of pre-poured plates (BM071), or to plates prepared as above, transfer 0.1 mL of the sample to be tested and its serial dilutions.
- Spread the inoculum with a sterile triangle or "hockey stick".
- Incubate at 36 ± 2 °C for 10 days.
- During the incubation period, examine the plates starting from the third day and three successive moments at intervals of 2 to 4 days.

✓ **Inoculation :**
On surface
After membrane filtration

✓ **Incubation :**
10 days at 36 ± 2°C

After membrane filtration :

- Aseptically filter through a membrane a known volume of the sample to test (10 mL to 1000 mL of water sample).

- Deposit the membrane on the surface of pre-poured plates (BM071), filtered side up and making sure that the membrane and agar are in close contact. The plates should be brought to room temperature before use.
- Incubate at 36 ± 2 °C for 10 days.
- During the incubation period, examine the plates starting from the third day and three successive moments at intervals of 2 to 4 days.

5 RESULTS

Colonies of *Legionella* spp. present a white to gray coloration. 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.
Colonies of Legionella that develop on white membrane filters may have a different aspect to those that develop against a black background.

Enumerate each colony type separately. Select at least three characteristic colonies of *Legionella* on each of the agar plates. Re-streak each colony onto a plate of BCYEα without cysteine (BM073) and a plate of BCYEα (BM072) from the BT007 kit.

See ANNEX 1 : PHOTO SUPPORT.

6 TYPICAL COMPOSITION

The typical composition can be adjusted to obtain optimal performance.

For 1 liter of medium :

- 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
- L-cysteine, hydrochloride ..... 0.4 g
- Ferric pyrophosphate ..... 0.25 g
- Glycine ..... 3.0 g
- Vancomycin ..... 1.0 mg
- Polymyxin B ..... 80000 IU
- Cycloheximide ..... 80.0 mg
- Bacteriological agar ..... 12.0 g

pH of ready-to-use medium at 25°C : 6.9 ± 0.1.

7 QUALITY CONTROL

Prepared media in plates : black agar, with visible particles of activated charcoal.

Typical cultural response after 5 days incubation at 36 °C (NF T 90-461) :

Microorganisms		Growth
Legionella pneumophila	WDCM 00107	66% ≤ R_2 ≤150%
Enterococcus faecalis	WDCM 00176	Inhibited
Escherichia coli	WDCM 00179	Inhibited

8 STORAGE

Pre-poured media in Petri dishes : 2-8°C, shielded from light.

Expiration dates are indicated on the labels.

9 PRESENTATION

Pre-poured Petri dishes (Ø 90 mm)
20 plates BM07108

10 BIBLIOGRAPHY

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FD T 90-522. July 2006. Water quality. Sampling technical guide for the detection of *Legionella* in water.

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11 ADDITIONAL INFORMATIONS

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

Document code	:	BM071/EN/2004-06 : 5.
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Update	:	2013-06
Grounds for revision	:	General revision (§ 7 : Quality control).

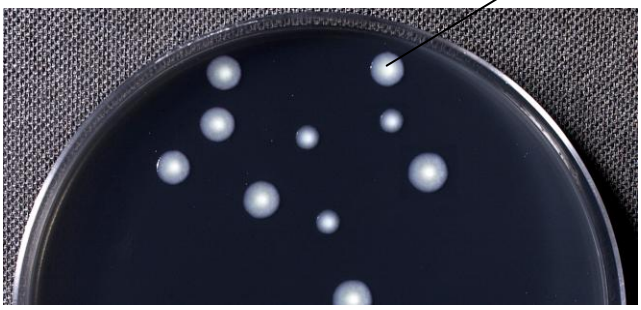
ANNEX 1 : PHOTO SUPPORT

GVPC Agar for *Legionella*

Detection and enumeration of *Legionella*.

Reading :

Growth obtained after 10 days of incubation at 36 °C.



Legionella pneumophila

Characteristic colony
White to gray color with a smooth surface ; some colonies may present a ground glass appearance under a binocular scope