TECHNICAL DATA SHEET

GVPC AGAR FOR *LEGIONELLA*

ENUMERATION OF *LEGIONELLA* IN WATER

1 INTENDED USE

GVPC for *Legionella* is used for the enumeration, isolation and culture of *Legionella* species in clean water (hot water production, swimming pools…) and in dirty water (industrial water, cooling towers…).

The typical composition corresponds to that defined in the standards NF T90-431 and NF EN ISO 11731.

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 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-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 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

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-oxoethyl)-amino] ethanesulfonic acid) ..... 10,0 g
- Potassium hydroxyde ..... 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 the ready-to-use media at 25 °C : 6,7-7,0.

5 INSTRUCTIONS FOR USE

Detection and enumeration of Legionella according to NF EN ISO 11731 :

Samples with a low concentration of *Legionella* species and a low concentration of interfering microorganisms :

- Filter a specific volume of water sample on membranes.

✓ **Inoculation : following the appropriate protocol**

✓ **Incubation : 7 to 10 days at 36 ± 2 °C**

Untreated sample :
- Place one membrane on a plate of BCYE agar..

Sample treated with acid solution:
- After treatment with acid solution place the membranes on one or more plates of selective medium such as GVPC agar or BCYE+AB agar.

Concentrated sample :
- Perform the wash procedure to obtain the concentrated sample.
- Divide each subsample into 3 portion : untreated, treatment with heat and treatment with acid solution.
- Spread 0,1 to 0,5 mL of each portion on one plate of BCYE agar (BM072) and on one or more plates of selective medium such as GVPC agar or BCYE+AB agar.
- Incubate at 36 ± 2 °C for 7 to 10 days.

Samples with a high concentration of interfering microorganisms :

Untreated sample :
- Divide each subsample into 3 portion : untreated, treatment with heat and treatment with acid solution.
- Spread 0,1 to 0,5 mL of each portion on one plate of GVPC agar (BM071).

Concentrated or diluate dample :
- Concentrate or dilute the sample to 1:10.
- Divide each subsample into 3 portion : untreated, treatment with heat and treatment with acid solution.
- Spread 0,1 to 0,5 mL of each portion on one plate of GVPC agar (BM071).
- Incubate at 36 ± 2 °C for 7 to 10 days.

Samples with an extremely high concentration of interfering microorganisms :

Untreated sample :
- Spread 0,1 to 0,5 mL of each portion on one plate of GVPC agar (BM071).

Heat and acid treated sample :
- Performed the heat treatment followed by the acid treatment.
- Dilute the sample to 1:10 and to 1 :100.
- Spread 0,1 to 0,5 mL of each portion on one plate of GVPC agar (BM071).
- Incubate at 36 ± 2 °C for 7 to 10 days.

NOTE :
Refer to NF EN ISO 11731 standard for the washing procedure and the treatment protocol.

Detection and enumeration of Legionella according to NF T 90-431 :

Clean water

Direct inoculation

- Dry the pre-poured plates (BM071) in an incubator, covers partially removed.
- Inoculate 0,2 mL of the water sample with a sterile triangle or « hockey stick ».
- Incubate at 36 ± 2 °C for 8 to 11 days.

Concentration by filtration

- Filter 10 and 100 mL of a water sample through a nitrocellulose membrane.
- Cover the membrane with a pH 2.0 buffer solution for 5 minutes.
- Rinse with sterile distilled water.
- Place the membrane on the surface of the agar, making sure to have good contact with the membrane and the agar surface.
- Incubate at 36 ± 2 °C for 8 to 11 days.

✓ **Inoculation :**
Direct : 0,2 mL
Filtration : 10 and 100 mL (acid treatment)

✓ **Incubation :**
8 to 11 days at 36 °C

Dirty water

Direct inoculation

- Dry the pre-poured plates (BM071) in an incubator, covers partially removed.
- Inoculate 0,2 mL of the water sample with a sterile triangle or « hockey stick ».
- Incubate at 36 ± 2 °C for 8 to 11 days.

Concentration by filtration or by concentration
(Refer to the standard NF T90-431).

- Inoculate 0,1 mL of the concentrate with a sterile triangle.
- Add the same volume of pH 2.0 acid buffer to a volume of sample concentrate and leave in contact for 5 minutes.
- Inoculate rapidly by spreading 0,2 mL on a plate.
- Place a volume of concentrated sample in a water batch at 50 ± 1 °C for 30 minutes.
- Spread 0,1 mL onto the surface of another plate of GVPC agar (BM071).
- With another sample of the concentrate, perform an associated treatment, starting with heat treatment then acid treatment.
- Spread 0,2 mL onto the surface of another plate of GVPC agar (BM071).
- Incubate all the plates at 36 ± 2 °C for 8 to 11 days.

✓ **Inoculation :**
Direct : 0,2 mL
After concentration :
Direct : 0,1 mL
Acid treatment : 0,2 mL
Heat treatment : 0,1mL
Associated treatment : 0,2 mL

✓ **Incubation :**
8 to 11 days at 36 °C

6 RESULTS

During the incubation period, examine the plates after the fourth day and then at regular intervals.

Legionella spp. colonies are white to grey. 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.

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 BCYE without cysteine (BM073) and a plate of BCYEα (BM072).

Consider as *Legionella* all the colonies that develop on BCYE with cysteine but do not grow on BCYE without cysteine.

Identify the species of *Legionella* by serology.

See ANNEX 1 : PHOTO SUPPORT.

7 QUALITY CONTROL

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

Typical culture response at 36 °C (NF EN ISO 11133) :

Microorganisms		Growth (Productivity Ratio : P_R)
(1) *Legionella pneumophila*	WDCM 00107	$P_R \geq 70$ %
(1) *Legionella pneumophila*	WDCM 00180	$P_R \geq 70$ %
(1) *Legionella anisa*	WDCM 00106	$P_R \geq 70$ %
(2) *Escherichia coli*	WDCM 00012	Partially to totally inhibited, score 0-1
(2) *Pseudomonas aeruginosa*	WDCM 00025	Partially to totally inhibited, score 0-1
(2) *Enterococcus faecalis*	WDCM 00087	Totally inhibited, score 0

(1) after 5 days of incubation
(2) after 3 days of incubation

8 STORAGE / SHELF LIFE

Pre-poured media in Petri plates : 2-8 °C.
The expiration date is indicated on the label.

9 PACKAGING

Pre-poured media in Petri plates (Ø 90 mm) :
20 plates BM07108

10 BIBLIOGRAPHY

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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.

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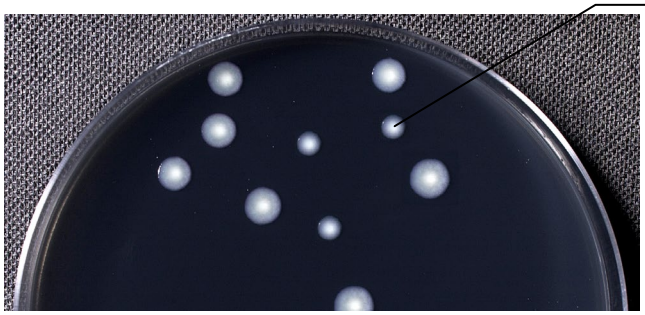
ANNEX 1 : PHOTO SUPPORT

GVPC Agar for *Legionella*

Detection and enumeration of *Legionella*.

Results :

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



Legionella pneumophila

Characteristic colony :
White to grey color with a smooth surface ; may present a ground glass appearance under stereo microscope.

Notes : Colonies of *Legionella* that develop on white membrane filters have a different aspect than those that develop on a black background.