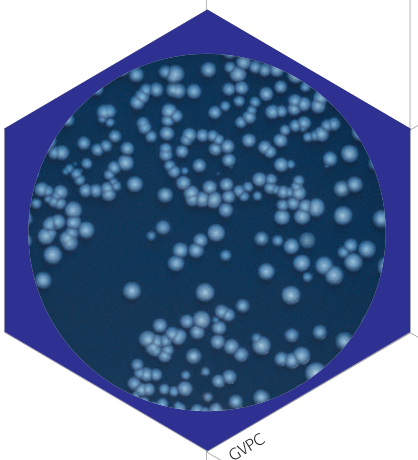
WATER

Detection and Enumeration of *Legionella*

Part 2 : Direct Membrane Filtration Method for waters with low bacteria counts

Standard NF EN ISO 11731-2 (July 2008)



PREPARATION

- Filter 10 ml to 1000 ml of water sample on a polycarbonate membrane
- **Acid treatment :**
  Add (30 ± 5) ml of acid buffer above the membrane
  Leave in contact 5 minutes
- Eliminate the buffer by filtration and wash the membrane with (20 ± 5) ml of Page's saline solution

INOCULATION

Apply the membrane on a
GVPC (ref. 43031 - 20 x 90 mm plates) or
BCYE (ref. 43013 - 10 x 90 mm plates)

INCUBATION

Incubate at (36 ± 2)°C for at least 10 days
Examine plates at least twice from the 3rd day

Colony features :

- White, grey, blue, purple, brown sometimes, pink, dark red, or lime
- Smooth surface, regular edge and sintered glass aspect
- Under UV lamp, different species of colonies give out a bright blue/white fluorescence, others appear in red. *L. pneumophila* colonies appear as an insipid green, usually tinted with yellow

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PIONEERING DIAGNOSTICS

Confirmation of presumed *Legionella* colonies

Prick out at least 5 typical colonies alternatively for each sample

If different types are present, prick out at least 2 colonies of each type

From one colony
Seed the 2 next media

Legionella agar
without L-cystein [1]

**(BCY - ref. 43023
10 x 90 mm plates)**

Legionella agar
with L-cystein

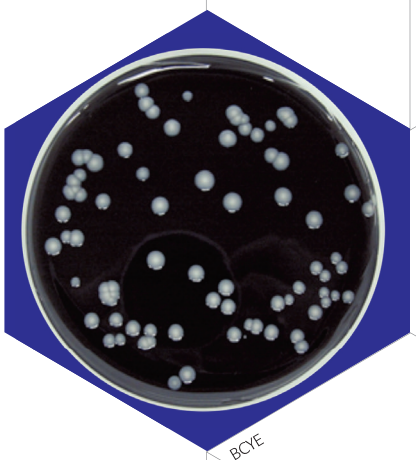
**(BCYE - ref. 43013
10 x 90mm plates)**

Incubate at (36 ± 1)°C for at least 2 days

Count as Legionella all colonies which grow on BCYE
but not on BCY (without cystein)

IDENTIFICATION

Perform a latex agglutination test
(SLIDEX® Legionella kit - ref. 73120 - 50 tests)
or an immunofluorescence test


BCYE

Slidex Legionella

(1) Or Blood Agar **(Trypcase Soy Agar + Sheep blood - ref. 43001 - 20 x 90mm plates)**

* Or Nutritive Agar **(2.8% Nutritive Agar - ref. AEB521960 - 20 x 90mm plates)**

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WATER

Detection and Enumeration of *Legionella*

Standard NF EN ISO 11731 (May 1998)

CLEAR WATER SAMPLES

DIRECT SEEDING

0.1 to 0.5 ml on GVPC * media

Or dilution

0.1 to 0.5 ml on GVPC * media

FILTRATION

Filter 1 liter on a sterile polycarbonate membrane

Place the membrane in a sterile flask, 5 to 25 ml add of:

- Page's saline solution **or**
- Ringer solution **or**
- Purified sterile water

Then

- Shake vigorously for at least 2 min. **or**
- Ultrasound for 2 to 10 min.

Obtaining a Concentrated solution

0.1 to 0.5 ml on GVPC * media

Heat treatment

(1 ± 0.5) ml in a sterile tube

Place in a water bath at (50 ± 1)°C for (30 ± 2) min.

0.1 to 0.5 ml on GVPC * media

Acid treatment

1 to10 ml in a sterile tube then centrifugation :

- 6000 g 10 min. or
- 3000 g 30 minutes

Remove the supernatant: half of initially added volume

Resuspend the base with the rest of the supernatant

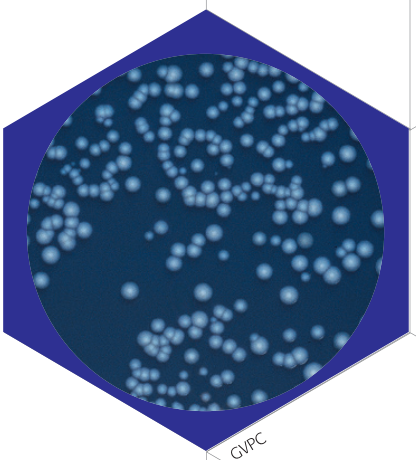
Add the acid buffer: same volume as that previously added

Contact time: (5 ± 0.5) min.

0.1 to 0.5 ml on GVPC * media

Incubate all plates at (36 ± 1)°C for 10 days

Check the plates at least 3 times during incubation every 2 - 4 days



* GVPC (ref. 43031 20x90 mm plates)

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PIONEERING DIAGNOSTICS

Colony features:

- White-grey-blue-purple, brown sometimes, pink, dark red, lime.
- Smooth surface, regular edge and sintered glass aspect.
- Under UV lamp, different species of colonies give out a bright blue/white fluorescence, others appear in red. *L. pneumophila* colonies appear as an insipid green, usually tinted with yellow.

Confirmation of presumed *Legionella* colonies

Select at least 3 typical *Legionella* colonies from each plate
From one colony
Seed the 2 next media

Legionella agar without L-cystein [1]	Legionella agar with L-cystein
BCY	**BCYE**
(ref. 43023 - 10 x 90 mm plates)	**(ref. 43013 - 10 x 90mm plates)**

Incubate at (36 ± 1)°C for at least 2 days

Count as *Legionella* all colonies which grow on BCYE but not on BCY (without cystein)

IDENTIFICATION

Perform a latex agglutination test
(SLIDEX® Legionella kit - ref. 73120 - 50 tests)
or an immunofluorescence test

(1) Or Blood Agar **(Trypcase Soy Agar + Sheep blood - ref. 43001 - 20 x 90mm plates)**
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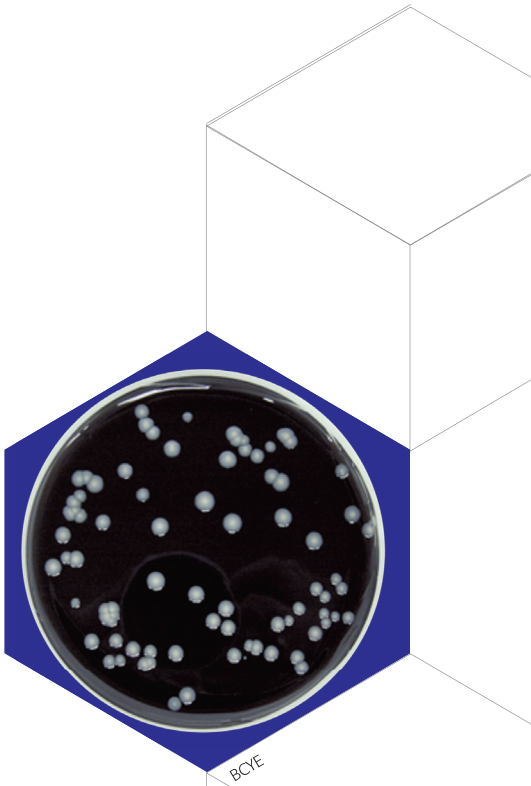

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