Legionella BCYE Agar Base

Basal medium for detection and enumeration of *Legionella* spp, according to ISO 11731.

TYPICAL FORMULA	(g/l)
Yeast Extract	10.0
Activated Charcoal	2.0
Alpha-Ketoglutarate	1.0
ACES Buffer	10.0
Potassium Hydroxide	2.8
Agar	13.0[a]
Final pH 6.9 ± 0.2 at 25°C	

[a] Adjusted to provide adequate gelling strength.

DESCRIPTION

Legionella BCYE Agar Base is a medium used with supplements for isolation and confirmation of *Legionella* colonies from clinical specimens and environmental samples.

PRINCIPLE

Yeast extract provides amino acids, nitrogen, carbon, vitamins and minerals. Activated charcoal decomposes hydrogen peroxide, a metabolic product toxic to *Legionella* spp, and may also collect carbon dioxide and modify surface tension. Alpha-ketoglutarate stimulates organism growth. ACES buffer and potassium hydroxide are included to maintain the proper pH for optimal growth. Agar is the solidifying agent.

This medium is supplemented with Legionella Growth Supplement (ref. 80056) to give Buffered Charcoal Yeast Extract (BCYE) Agar.

To raise selectivity, one of the following supplements can be further added:

- Legionella AB Supplement (ref. 81099) containing polymyxin B, cefazolin and pimaricin
- Legionella GVPC Supplement (ref. 81008) containing vancomycin, polymyxin B and cycloheximide
- Legionella MWY Supplement (ref. 81019) containing vancomycin, polymyxin B and anisomycin

PREPARATION

Suspend 3.9 g of powder in 90 ml of deionized or distilled sterile water. Heat to boiling until completely dissolved. Sterlize in autoclave at 121°C for 15 minutes. Cool to 45-50°C and aseptically add rehydrated contents of 1 vial (10 ml) of Legionella Growth Supplement.

To prepare BCYE+AB agar, add the entire contents of 1 vial (5 ml) of Legionella AB Supplement reconstituted with sterile distilled water.

To prepare GVPC agar, add the contents of 1 vial (1 ml) of Legionella GVPC Supplement reconstituted with sterile distilled water.

To prepare MWY agar, add the entire contents of 1 vial (2 ml) of Legionella MWY Supplement reconstituted with sterile distilled water.

Mix well and pour in Petri dishes.

TECHNIQUE

Inoculate clinical specimens from swab by rolling it over the agar surface in order to obtain isolated colonies.

According to ISO 11731, the method and culture media to be used will depend on the characteristics of the sample. See the scheme on page 2. For more details refer to the ISO standard.

To ensure detection, water samples may be concentrated by membrane filtration or, alternatively, by centrifugation (when the number of legionellae in any given sample is not known, concentration technique are usually performed). Dilution is necessary when high concentrations of *Legionella* and/or other bacteria are expected. Heat treatment, acid treatment, or a combination of both may be also required before culturing on selective media.

Incubate inoculated plates at 36 ± 2°C for 7 to 10 days in humidified atmosphere (air with 2.5% CO_2 can be beneficial for the growth of some Legionella but is not essential).

INTERPRETATION OF RESULTS

Inspect plates for the first time either on day 2, 3, 4 or 5 followed by a final inspection at the end of the incubation period. Examine for growth and fluorescence under long-wave UV light.

Colonies of *Legionella* are white-grey in general but can also appear in other colours. They are smooth with an entire edge and exhibit a characteristic ground-glass appearance. Under UV light, colonies usually exhibit brilliant white fluorescence.

For confirmation, regard as *Legionella* those colonies which grow on Legionella BCYE Agar but fail to grow on the medium without cysteine (Legionella BCYE Agar w/o Cysteine, ref. 10412).

STORAGE

10-30°C away from light, until the expiry date on the label or until signs of deterioration or contamination are evident.

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. it is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for *in vitro* diagnostic use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

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Samples with an expected	Detection methods
high level of *Legionella* spp (>10^4 cfu/l) and low level of contaminant organisms	Direct plating • Spread 0.1 to 0.5 ml of the sample on one plate of BCYE agar and one plate of BCYE+AB agar
low level of *Legionella* spp and low level of contaminant organisms	Membrane filter on plate • Filter the sample • Place the untreated membrane filter on one plate of BCYE agar • Place the membrane filters treated with acid on one or more of the selective plates of BCYE+AB agar or GVPC agar or MWY agar
	Filtration with washing procedure • Concentrate the sample • Spread 0.1 to 0.5 ml of each concentrated portion of the sample (untreated, heat treated and acid treated) on one plate of BCYE agar and on one or more of the selective plates of BCYE+AB agar or GVPC agar or MWY agar
high level of contaminant organisms	Plating after dilution • Use samples unconcentrated (direct), concentrated or diluted (1:10) • Spread 0.1 to 0.5 ml of each portion of the sample (untreated, heat treated and acid treated) on one plate of GVPC agar or MWY agar
extremely high concentration of contaminant organisms	Plating after dilution • Use samples unconcentrated (direct) and diluted (1:10 and 1:100) • Spread 0.1 to 0.5 ml of each portion of the sample (untreated, heat treated, acid treated and heat/acid treated) on one plate of GVPC agar or MWY agar

REFERENCES

1. ISO 11731:2017. Water quality – Enumeration of *Legionella*.
2. EN ISO 11133:2014. Microbiology of food, animal feed and water − Preparation, production, storage and performance testing of culture media.
3. Edelstein P.H. (1981) Improved semiselective medium for the isolation of *Legionella pneumoniae* from contaminated clinical and environmental specimens. J. Clin. Microbiol. 14(3):298.

PRODUCT SPECIFICATIONS

NAME
Legionella BCYE Agar Base

PRESENTATION
Dehydrated medium

STORAGE
10-30°C

PACKAGE

Ref.	Content	Packaging
610049	500 g	500 g of powder in plastic bottle
620049	100 g	100 g of powder in plastic bottle

pH OF THE MEDIUM
6.9 ± 0.2

USE
Legionella BCYE Agar Base is a medium used with supplements for isolation and confirmation of *Legionella* colonies from clinical specimens and environmental samples

TECHNIQUE
Refer to technical sheet of the product

APPEARANCE OF THE MEDIUM
Dehydrated medium
Appearance: free-flowing, homogeneous
Colour: grey-black
Prepared medium
Appearance: opaque
Colour: black

SHELFLIFE
4 years

QUALITY CONTROL
1. Control of general characteristics, label and print
2. Microbiological control
   Inoculum for productivity: 50-100 CFU
   Inoculum for selectivity: 10^4-10^6 CFU
   Incubation conditions: 2-5 days at 36 ± 2°C

Microorganism		Growth on GVPC agar, BCYE+AB agar, MWY agar
Legionella pneumophila	WDCM 00107	Good
Enterococcus faecalis	WDCM 00087	Inhibited
Escherichia coli	WDCM 00013	Partially to totally inhibited

TABLE OF SYMBOLS

LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Consult instruction for use	Keep away from heat sources

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