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CE

TECHNICAL SHEET

LEGIONELLA BCYE-AGAR (with L-cysteine)/LEGIONELLA BCYE-AGAR (w/o L-cysteine)

IVD in Class A, EU Reg. 2017/746
For in vitro diagnostic use **IVD**

Medium for detection and enumeration of Legionella spp, according to ISO 11731.

DESCRIPTION

Legionella Buffered Charcoal Yeast Extract (BCYE) Agar (with L-cysteine) is used for primary isolation and cultivation of Legionella pneumophila and other Legionella species from environmental samples and clinical specimens.
Legionella BCYE Agar (with L-cysteine) is based on Edelstein's modification of previously described media. In 1979, Feely et al. described Charcoal Yeast Extract (CYE) Agar as a modification of an existing medium, F-G Agar. They replaced the starch in the F-G Agar with activated charcoal and substituted yeast extract for casein hydrolysate, resulting in better recovery of L. pneumophila. In 1980, Pasculle reported that CYE Agar could be improved by buffering the medium with ACES Buffer. A year later, Edelstein further increased the sensitivity of the medium by adding alpha-ketoglutarate (BCYE Agar).

PRINCIPLE

Legionella BCYE Agar (with L-cysteine) is an enriched medium for isolation and cultivation of Legionella species. Yeast extract supplies the protein and other nutrients necessary to support growth. L-Cysteine, an essential amino acid, and soluble ferric pyrophosphate, an iron supplement, are incorporated to satisfy specific nutritional requirements of Legionella species. Alpha-ketoglutarate is added to stimulate growth. Activated charcoal decomposes hydrogen peroxide, a metabolic product toxic to Legionella species, and may also collect carbon dioxide and modify surface tension. ACES buffer is added to maintain the proper pH for optimal growth.

Legionella BCYE AGAR (with L-cysteine):

COMPOSITION	g/L
Yeast Extract	10.0 g
Activated Charcoal	2.0 g
α-Ketoglutarate	1.0 g
ACES Buffer	10.0 g
Potassium Hydroxide	2.8 g
L-Cysteine HCl	0.4 g
Iron(III) Pyrophosphate	0.25 g
Agar	12.0 g

Final pH 6,9 ± 0,2 at 25°C

Legionella BCYE AGAR (without L-cysteine):

COMPOSITION	g/L
Yeast Extract	10.0 g
Activated Charcoal	2.0 g
α-Ketoglutarate	1.0 g
ACES Buffer	10.0 g
Potassium Hydroxide	2.8 g
Iron(III) Pyrophosphate	0.25 g
Agar	12.0 g

Final pH 6,9 ± 0,2 at 25°C

WARNING AND PRECAUTIONS

For in vitro diagnostic use.
Observe the precautions normally taken when handling laboratory reagents.
Prepared Medium: The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous.
Safety Data Sheet is available on request for professional users.
All wast must be disposed of according to local directives.

STORAGE AND STABILITY	
Prepared medium:	10-25°C

The product is stable until the expiration date indicated on the label under the recommended storage conditions.

PREPARATION

Prepared medium (plates): ready to use.

PROCEDURE

Inoculate clinical specimens from swab by rolling it over the agar surface (90 mm plate) in order to obtain isolated colonies.
For water testing according to ISO 11731, the choice of the method is down to the individual laboratory, as it depends on the type of the sample (e.g., whether the water has low, high, or extremely high bacterial background flora) and the reason of the investigation. 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 (REF. 85000), 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% CO2 can be beneficial for the growth of some Legionella but is not essential).

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, 90 mm Plate, REF. 1592308/20).

QUALITY CONTROL
Prepared medium: opaque, black.

Typical response after incubation at 36±2°C for 2-5 days on Legionella BCYE Agar (with L-cysteine) plates:

MICROORGANISM	GROWTH
Legionella pneumophila WDCM 00107	Good (white-gray colonies)

Typical response after incubation at 36±2°C for 2-5 days on Legionella BCYE Agar (w/o L-cysteine) plates:

MICROORGANISM	GROWTH
Legionella pneumophila WDCM 00107	Inhibited

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.

PRESENTATION	Packaging	REF.
Prepared medium:		
LEGIONELLA BCYE AGAR (with L-cysteine)		
	20 pcs (90 mm ready-to-use plates)	**1554183/20**
LEGIONELLA BCYE AGAR (w/o L-cysteine)		
	20 pcs (90 mm ready-to-use plates)	**1592308/20**

SYMBOLS

Read the instructions	**Biological hazard**
CE Mark (product complies with the requirements of Regulation (EU) 746/2017)	
Temperature limitation	**Use by**
For in vitro diagnostic use	**Manufacturer**