

**Legionella BCYE agar with L-cysteine (BCYE)***For microbiological control only*Medium for confirmation of suspected *Legionella***SUMMARY AND EXPLANATION**

Legionella BCYE medium with L-cysteine is used to confirm or exclude the presence of *Legionella* in water by subculture of typical colonies obtained previously using Legionella GVPC medium.

This medium should be inoculated at the same time as the BCYE medium without L-cysteine. If the strain does not grow on BCYE medium without L-cysteine, it can be confirmed as belonging to the genus *Legionella*.

Legionella BCYE medium with L-cysteine complies with the standards NF T90-431 (1) and ISO 11731 (2).

**PRINCIPLE**

Legionella BCYE medium with L-cysteine is a non-selective medium.

L-cysteine is an essential amino-acid for the growth of *Legionella*.

The activated charcoal absorbs any toxic substances in the yeast extract and thus stimulates the growth of *Legionella*.

The ACES buffer stabilizes the pH of the medium at 6.9, which is the optimal pH for growth.

**CONTENT OF THE KIT**

	<b>Ready-to-use medium</b>
<b>REF 43 013</b>	Pack of 10 plates (90 mm)
	<b>BCYE *</b>

\* printed on each plate.

**COMPOSITION****Theoretical formula**

This medium can be adjusted and/or supplemented according to the performance criteria required:

Yeast extract.....	10 g
Active charcoal .....	2 g
ACES / KOH buffer (pH adjustment).....	12.8 g
$\alpha$ Ketoglutarate.....	1 g
Agar.....	12 g
L.cysteine hydrochloride.....	0.4 g
Ferric pyrophosphate.....	0.25 g
Purified water.....	1 l

pH 6.9

**REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED****Reagents:**

- Legionella GVPC agar (Ref. 43 031)
- Legionella BCYE agar without L-cysteine (Ref. 43 023)

**Material:**

- Bacteriology incubator.

**WARNINGS AND PRECAUTIONS**

- **For microbiological control only.**
- **For professional use only.**
- All specimens, microbial cultures and inoculated products should be considered infectious and handled appropriately. Aseptic technique and usual precautions for handling the bacterial group studied should be observed throughout this procedure. Refer to "CLSI M29-A, Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline" – Current Revision. For additional handling precautions, refer to "Biosafety in Microbiological and Biomedical Laboratories, CDC/NIH – Latest edition, or to the regulations currently in use in each country.
- Culture media should not be used as manufacturing material or components.
- Do not use reagents after the expiry date.
- Do not use reagents if the packaging is damaged.
- Do not use contaminated plates or plates that exude moisture.
- The medium must be used according to the procedure indicated in this package insert. Any change or modification in the procedure may affect the results.

**STORAGE CONDITIONS**

- **Store the plates at 2-8°C in their box until the expiry date.**
- If not in the box, plates can be stored for 2 weeks at 2-8°C in the cellophane sachet.
- Legionella BCYE medium with L-cysteine can be stored in its box for 1 week at 18-25°C.

**SPECIMENS**

This medium is used to subculture typical colonies obtained with Legionella GVPC medium.

**INSTRUCTIONS FOR USE**

1. Allow plates to come to room temperature.
2. For inoculation, follow the reference standard: in general, suspect colonies previously obtained with Legionella GVPC medium, are subcultured on BCYE medium with L-cysteine and BCYE medium without L-cysteine. BCYE medium without L-cysteine can be associated with a blood agar.
3. After inoculation, plates must be incubated with the cover bottom side at 37 °C  $\pm$  1 °C for 2 or 3 days, according to the reference standard. Three days of incubation is preferable for certain *Legionella* species.

## READING AND INTERPRETATION

To confirm that a strain belongs to the genus *Legionella*, growth must be obtained on Legionella BCYE medium with L-cysteine, whereas no significant growth should be observed on Legionella BCYE medium without L-cysteine. It is also recommended to check that the colonies obtained are Gram-negative bacteria, before going on to identify the species.

## QUALITY CONTROL

BCYE medium with L-cysteine is designed and developed to meet the strictest quality requirements.

The quality control is performed in conformity with the recommendations of the standard NF T90-461 and includes other complementary controls (3, 4).

The results of the strains tested in the batch by batch quality control are given on the quality control certificate available on request.

## LIMITATIONS OF THE METHOD

- Very weak growth of *Legionella* (traces) may be observed after 3 days of incubation on BCYE medium without L-cysteine. This growth is however, considerably weaker than that obtained on the medium with L-cysteine.

## WASTE DISPOSAL

Unused reagents may be considered as non-hazardous waste and disposed of accordingly.

Dispose of all used reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.








It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

## LITERATURE REFERENCES

1. Norme NF T90-431 (sept. 2003) – Recherche et dénombrement de *Legionella* spp et *Legionella pneumophila* - Méthode par ensemencement direct après concentration par filtration sur membrane ou centrifugation.
2. ISO 11731 (1998/05/01) – Water quality – Detection and enumeration of Legionella.
3. NF T90-461 (July 2001) – Quality control of culture media.
4. NF T90-461 / A2 (May 2007) – Quality control of culture media.
5. PASCULLE W.- Update on *Legionella* - Clinical microbiology Newsletter, July 1, 2000, Vol. 22, 13, p 97-101
6. SQUINAZI F., NAHAPETIAN K. - *Legionella pneumophila* méthodes de recherche chez l'homme et dans l'environnement - Revue française des laboratoires, avril 1988, Vol.172, p 33-41

7. Circulaire DGS N° 97/311 du 24 avril 1997, relative à la surveillance et à la prévention des Légionellose.
8. Circulaire DGS n° 98/771 du 31 décembre 1998, relative à la mise en œuvre de bonnes pratiques d'entretien des réseaux d'eau dans les établissements de santé et aux moyens de prévention du risque lié aux Legionelles dans les installations à risque et dans celles des bâtiments recevant du public.

## INDEX OF SYMBOLS

Symbol	Meaning
 or REF	GB : Catalogue number US : Catalog number
	Manufacturer
	Temperature limitation
	Use by
	Batch code
	Consult instructions for use
	Contains sufficient for <n> tests

## WARRANTY

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