



BD™ Legionella Agar with Vancomycin and Colistin

INTENDED USE

BD Legionella Agar with Vancomycin and Colistin is a selective media for the isolation of *Legionella* species from clinical specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

Since the first description of *Legionella pneumophila* as the agent of legionaire's disease, a large number of species has been described, some of which are also associated with human disease.^{1,2} Although *Legionella* is not extremely fastidious, it needs cysteine and ferric ions for growth.²⁻⁴ Since the organism is quite sensitive to oxygen radicals, media containing charcoal are superior to Chocolate Agar for the growth of *Legionella*. The medium described by Edelstein, BCYE α (Buffered Charcoal Yeast Extract α -Ketoglutarate) Agar, is the medium used most frequently as a base for *Legionella* media.⁴ In order to inhibit the accompanying flora, several supplements have been described.^{2,5,6}

In **BD Legionella Agar with Vancomycin and Colistin**, yeast extract supplies nutrients, and L-cysteine, alpha-ketoglutarate, and ferric pyrophosphate satisfy the specific nutritional requirements of *Legionella*. ACES [N-(2-acetamido)-2-aminoethanesulfonic acid] is a buffer. Vancomycin inhibits Gram positive organisms and colistin inhibits sensitive Gram negative bacteria, such as *Enterobacteriaceae*, except *Proteus*.

REAGENTS

BD Legionella Agar with Vancomycin and Colistin

Formula* Per Liter Purified Water

Yeast Extract	10.0 g
Ferric Pyrophosphate	0.25
ACES Buffer	10.0
Charcoal, activated	2.0
Alpha-Ketoglutarate	1.0
Agar	15.0
L-Cysteine HCl	0.4
Vancomycin	0.002 g
Colistin	0.015 g

pH 6.9 +/- 0.2

*Adjusted and/or supplemented as required to meet performance criteria.

PRECAUTIONS

IVD . For professional use only.

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate for 3 to 4 days aerobically at 35 to 37° C in a moist chamber.

Strains	Growth results
<i>Legionella pneumophila</i> ATCC™ 33152	Growth good to excellent; bluish-gray colonies
<i>Legionella bozemanii</i> ATCC 33217	Growth good to excellent; bluish-gray colonies
<i>Legionella micdadei</i> ATCC 33218	Growth good to excellent; bluish-gray colonies
<i>Legionella anisa</i> DSM 17627	Growth satisfactory to excellent; bluish-gray colonies
<i>Escherichia coli</i> ATCC 25922	Inhibition partial to complete
<i>Staphylococcus aureus</i> ATCC 25923	Inhibition partial to complete
Uninoculated	Shiny black

PROCEDURE

Materials Provided

BD Legionella Agar with Vancomycin and Colistin (90 mm **Stacker™** plates).
Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

This is a partially selective medium for the isolation of *Legionella* from lower respiratory tract specimens, including sputa (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**). Consult the references for further details on specimens and specimen collection.^{2,6} The medium may also be used for the detection of *Legionella* from water and water supply systems. Faucets and showers should be swabbed after removal of the aerator or showerhead. Specimens or samples on swabs may be transported in a small volume of sterile tap water to avoid desiccation.^{2,6}

Test Procedure

All clinical specimens with high counts of accompanying flora may first be treated by heat (waterbath: 30 min 50° C) or acid-treated (dilute specimen in 1/10 ratio with 0.2 M KCl/HCl buffer pH 2.2; allow to incubate for 5 to 15 min at room temperature, neutralize afterwards to pH 7.0 before inoculation) to enhance recovery. Clinical specimens may also be homogenized by shaking in Trypticase Soy Broth or other suitable liquids and should then be streaked on the medium.

Water samples must first be concentrated by centrifugation and filtration before it is plated. For details of the pretreatment and collection procedures, consult the reference.²

Streak the specimen onto the medium using an approved isolation technique. Avoid desiccation during specimen handling and cultivation.

Incubate for 3 to 4 days, eventually up to 2 weeks, in ambient air in a moist chamber at 35 to 37° C.

Results

Legionella species appear as small to large, shiny, often mucoid, colorless, bluish or reddish colonies on BCYE-based media. Several *Legionella* species autofluoresce, but not *L. pneumophila*. Confirm the diagnosis using appropriate staining methods and serological tests.²

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD Legionella Agar with Vancomycin and Colistin is one of the formulations used for the isolation of *Legionella* from lower respiratory tract specimens and from water.^{2,5}

This medium is only partially selective. Specimens or water containing contaminating flora must be pretreated to reduce an overgrowth of fungi or bacteria.

Confirmatory tests are necessary for the identification of the genus and species. Antigen and antibody tests used for confirmation of an infection with *Legionella* have been described.^{2,6}

REFERENCES

1. McDade, J.E., C.C. Shepard, D.W. Fraser, T.R. Tsai, M.A. Redus, W.R. Dowdle, and the Laboratory Investigation Team. 1977. Legionnaires' disease: isolation of a bacterium and demonstration of its role in other respiratory disease. N. Engl. J. Med. 297:1197-1203.
2. Winn, W.C. 1995. *Legionella*, p. 533-544. In: P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. Feeley, J.C., G.W. Gorman, R.E. Weaver, D.C. Mackel, and H.W. Smith. 1978. Primary isolation media for Legionnaires' disease bacterium. J. Clin. Microbiol. 8:320-325.
4. Edelstein, P.H. 1981. Improved semiselective medium for isolation of *Legionella pneumophila* from potable water. J. Clin. Microbiol. 14: 298-303.
5. MacFaddin, J. D. 1985. Media for isolation-cultivation-identification- maintenance of medical bacteria, vol. 1, p. 275-284. Williams & Wilkins, Baltimore, MD.
6. Stout, J.E., J.D. Rihs, and V.L. Yu. 2003. *Legionella*. In: Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Tenover (ed.). Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.

PACKAGING/AVAILABILITY

BD Legionella Agar with Vancomycin and Colistin

Cat. No. 254414	Ready-to-use Plated Media, cpu 20
Cat. No. 254543	Ready-to-use Plated Media, cpu 120

FURTHER INFORMATION

For further information please contact your local BD representative.



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