

QUALITY CONTROL PROCEDURES (Optional)

I INTRODUCTION

BCYE (Buffered Charcoal Yeast Extract) Agar is used for the isolation and cultivation of *Legionella* species.

II PERFORMANCE TEST PROCEDURE

- Inoculate representative samples with the cultures listed below.
 - Add 0.1 mL of a culture containing 30–300 CFU/0.1 mL to each plate and spread-inoculate using a sterile glass spreader.
 - Incubate plates at 35 ± 2 °C in an aerobic atmosphere.
 - Include plates of a previously tested lot of BCYE Agar and **Trypticase™** Soy Agar with 5% Sheep Blood plates as positive and negative growth controls, respectively.
- Examine plates after 18–24 and 48–72 h for growth, pigmentation and fluorescence.
- Expected Results

CLSI Organisms	ATCC®	Recovery	Colony Color/Fluorescence
* <i>Legionella pneumophila</i>	33152	Growth by 72 h	White-gray, gray to blue-gray**
* <i>Fluoribacter (Legionella) bozemanii</i>	33217	Growth by 72 h	White-gray, gray to blue-gray; blue-white fluorescence under long-wave UV light
<i>Tatlockia (Legionella) micdadei</i>	33204	Growth	
Additional Organism			
<i>Fluoribacter (Legionella) dumoffii</i>	33279	Growth	White-gray, gray to blue-gray; blue-white fluorescence under long-wave UV light

*Recommended organism strain for User Quality Control.

**CLSI standard M22-A3 includes "yellow-green fluorescence under long-wave UV light." In fact, this species does not fluoresce.^{1,2} The CLSI subcommittee has been advised of this discrepancy.

NOTE: User QC testing of exempt media used for fastidious organisms such as *Legionella* sp. is strongly recommended by CLSI M22-A3.

III ADDITIONAL QUALITY CONTROL

- Examine plates as described under "Product Deterioration."
- Visually examine representative plates to assure that any existing physical defects will not interfere with use.
- Determine the pH potentiometrically at room temperature for adherence to the specification of 6.9 ± 0.2 .
- Note the firmness of plates during the inoculation procedure.
- Incubate uninoculated representative plates at 35 ± 2 °C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Buffered Charcoal Yeast Extract (BCYE) Agar is used for primary isolation and cultivation of *Legionella pneumophila* and other *Legionella* species from environmental samples and clinical specimens.

V SUMMARY AND EXPLANATION

BCYE Agar 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.^{1,2} 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.³ A year later, Edelstein further increased the sensitivity of the medium by adding alpha-ketoglutarate (BCYE Agar).⁴

VI PRINCIPLES OF THE PROCEDURE

BCYE Agar 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.

VII REAGENTS

BCYE Agar

Approximate Formula* Per Liter Purified Water

Yeast Extract	10.0 g	Charcoal, Activated	2.0 g
L-Cysteine HCl	0.4 g	Alpha-Ketoglutarate	1.0 g
Ferric Pyrophosphate	0.25 g	Agar	15.0 g
ACES Buffer	10.0 g		

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"⁵⁻⁸ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2 – 8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2 – 8 °C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

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

VIII SPECIMEN COLLECTION AND HANDLING

A variety of swabs and containers have been devised for collecting specimens. Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory. Several holding media or transport systems, such as **BBL** specimen collection and transport products, have been devised to prolong the survival of microorganisms when a significant delay is expected between collection and definitive culturing.

Refer to appropriate texts for details of specimen collection and handling procedures.^{9,10}

IX PROCEDURE

Material Provided: BCYE Agar

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

The agar surface should be smooth, firm and moist, but without excessive moisture.

Culture the specimen as soon as possible after it is received in the laboratory. To culture a specimen from a swab, inoculate the medium by rolling the swab over a third of the agar surface and streaking the remainder of the plate to obtain isolated colonies. Material not being cultured from swabs may be streaked onto the medium with a sterilized inoculating loop. The streak plate technique is used primarily to obtain isolated colonies from specimens containing mixed flora.

Incubate the plates in an inverted position (agar side up) at 35 ± 2 °C for a minimum of 3 days. Growth is usually visible within 3–4 days, but may take up to 2 weeks to appear.

User Quality Control: See “Quality Control Procedures.”

Each lot of media has been tested using appropriate quality control organisms and this testing meets product specifications and CLSI standards, where relevant. As always, QC testing should be performed in accordance with applicable local, state, federal or country regulations, accreditation requirements, and/or your laboratory's standard quality control procedures.

X RESULTS

After sufficient incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. *Legionella pneumophila* produces small to large, smooth, colorless to pale, blue-gray, slightly mucoid colonies. Consult references for morphology, presence and color of fluorescence, etc., of other species.^{11,12}

A Gram stain, biochemical tests and serological procedures should be performed to confirm findings.

XI LIMITATIONS OF THE PROCEDURE

Some diagnostic tests may be performed with the primary plate. However, a pure culture is recommended for biochemical tests and other identification procedures. Consult appropriate texts for detailed information and recommended procedures.^{11,13,14}

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of BCYE Agar are tested for performance characteristics. Representative samples of the lot are tested with cell suspensions of *Legionella*, inoculated by spreading the cell suspension, diluted in normal saline to yield 30–300 CFU/plate, over the agar surface. Plates are incubated at 35–37 °C for three days in an aerobic atmosphere. Moderate to heavy growth and correct color of colonies and fluorescence under long-wave UV light are observed.

XIII AVAILABILITY

Cat. No.	Description
221808	BD BBL™ BCYE Agar
215102	BD BBL™ BCYE Agar

XIV REFERENCES

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