

produce urease, upon which the specific color reaction depends.^{1,2}

References

1. Shepard and Lunceford. 1976. J. Clin. Microbiol. 3:613.
2. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
3. Shepard. 1983. In Razin and Tulley (ed.), Methods in mycoplasmaology, vol. 1. Academic Press, Inc., New York, N.Y.
4. Waites and Taylor-Robinson. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ A7 Agar, Modified

BS10 MCM7

Cat. No. 215048 Prepared Plates – Pkg. of 20*
292211 Prepared Plates (60 × 15 mm-style) – Pkg. of 10*

*Store at 2-8°C.

AC Broth

Intended Use

AC Broth is used for cultivating a wide variety of microorganisms and for the sterility testing of turbid or viscous solutions and other materials not containing mercurial preservatives.

Summary and Explanation

AC Broth possesses growth-promoting properties for a wide variety of microorganisms. Christensen¹ and Malin and Finn² reported that AC Medium does not exhibit the toxicity shown by media containing sodium thioglycollate. Several early studies reported on the wide variety of organisms able to grow on AC Medium.³⁻⁵ AC Broth is suitable for use in the detection of obligately aerobic contaminants in biologicals and other products. AC Broth is also useful in the isolation and cultivation of many common pathogenic and saprophytic aerobes.⁶ The medium can be used to test the sterility of biologicals and solutions that do not contain mercurial preservatives. Fluid Thioglycollate Medium should be employed for the sterility testing of solutions containing mercurial preservatives.

User Quality Control

Identity Specifications

Difco™ AC Broth

| | |
|------------------------------------|--|
| Dehydrated Appearance: | Light tan, free-flowing, homogeneous. |
| Solution: | 3.4% solution, soluble in purified water. Solution is medium to dark amber, clear to very slightly opalescent. |
| Prepared Appearance: | Light to medium amber, clear to very slightly opalescent. |
| Reaction of 3.4% Solution at 25°C: | pH 7.2 ± 0.2 |

Cultural Response

Difco™ AC Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

| ORGANISM | ATCC™ | INOCULUM CFU | RECOVERY |
|--|-------|----------------------------------|----------|
| <i>Corynebacterium diphtheriae</i> biotype mitis | 8024 | 10 ² -10 ³ | Good |
| <i>Streptococcus mitis</i> | 9895 | 10 ² -10 ³ | Good |
| <i>Streptococcus pneumoniae</i> | 6305 | 10 ² -10 ³ | Good |
| <i>Streptococcus pyogenes</i> | 19615 | 10 ² -10 ³ | Good |

Principles of the Procedure

Peptone, beef extract and malt extract provide the carbon and nitrogen sources required for good growth of a wide variety of organisms. Vitamins and cofactors required for growth as well as additional sources of nitrogen and carbon are provided by yeast extract. Dextrose is a carbon energy source. Ascorbic acid is added to clarify the solution.

Formula

Difco™ AC Broth

| Approximate Formula* Per Liter | | |
|--------------------------------|------|---|
| Proteose Peptone No. 3 | 20.0 | g |
| Beef Extract | 3.0 | g |
| Yeast Extract | 3.0 | g |
| Malt Extract | 3.0 | g |
| Dextrose | 5.0 | g |
| Ascorbic Acid | 0.2 | g |

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

Directions for Preparation from Dehydrated Product

1. Dissolve 34 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. Store prepared medium at 15-30°C.
4. After prolonged storage, reheat in flowing steam or a boiling water bath for a few minutes to drive off dissolved gases. Cool without agitation.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

See appropriate references for specific procedures.

Expected Results

Refer to appropriate references and procedures for results.

Limitation of the Procedure

When reheating prepared media to drive off dissolved gases, do not overheat because this may result in decreased growth.

References

- Christensen. 1944. Paper read at New York Meeting. American Public Health Association.
- Malin and Finn. 1951. *J. Bacteriol.* 62:349.
- Reed and Orr. 1943. *J. Bacteriol.* 45:309.
- Schneiter, Dunn and Caminita. 1945. *Public Health Rep.* 60:789.
- Kolb and Schneiter. 1950. *J. Bacteriol.* 59:401.
- MacFaddin. 1985. *Media for isolation-cultivation-identification-maintenance of medical bacteria*, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

Difco™ AC Broth

Cat. No. 231710 Dehydrated – 500 g

AK Agar #2 (Sporulating Agar)

Intended Use

AK Agar #2 (Sporulating Agar) is a culture medium for the preparation of spore suspensions for use in procedures for the detection of antibiotic residues in milk and dairy products.

Summary and Explanation

AK Agar #2 was devised by Arret and Kirshbaum for specific use in the production of spores of *Bacillus subtilis* ATCC™ 6633 for use in the Penicillin Milk Test procedure.¹ This medium was formerly specified in the spore preparation phase of the American Public Health Association disc assay procedure for the detection of sulfa drugs and antibiotics in milk.²

Principles of the Procedure

The peptones and beef extract are sources of nitrogen, sulfur, amino acids and essential trace ingredients. Yeast extract is a rich source of B vitamins. Dextrose is an energy source for bacterial replication. Manganous sulfate plays an important role in the sporulation process.

Formula

BBL™ AK Agar #2

| Approximate Formula* Per Liter | | |
|------------------------------------|------|---|
| Pancreatic Digest of Gelatin | 6.0 | g |
| Pancreatic Digest of Casein | 4.0 | g |
| Yeast Extract | 3.0 | g |
| Beef Extract | 1.5 | g |
| Dextrose | 1.0 | g |
| Agar | 15.0 | g |
| Manganous Sulfate | 0.3 | g |

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

Directions for Preparation from Dehydrated Product

- Suspend 30.8 g of the powder in 1 L of purified water. Mix thoroughly.
- Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- Dispense and autoclave at 121°C for 15 minutes.
- Test samples of the finished product for performance using stable, typical control cultures.

Procedure

- For preparation of spore suspensions for use in the FDA procedure for the Penicillin Milk Test.¹ Transfer cultures of *Bacillus subtilis* ATCC 6633 monthly to fresh Seed Agar slants. Wash the growth from a fresh slant culture with sterile physiological saline onto the surface of a Roux bottle containing 300 mL of AK Agar

#2. Incubate the bottles for 5 days at 35 ± 2°C and wash off the resulting growth into 50 mL of sterile physiological saline. Centrifuge the suspension and decant and discard the supernatant fluid. Resuspend the sediment in sterile saline and heat shock the suspension at 70°C for 30 minutes. The resultant spore suspension can be stored for several months. Consult the reference for the test procedure utilizing this *B. subtilis* spore suspension.¹

- For preparation of spore suspension for use in the APHA procedure for detection of sulfa drugs and antibiotics in milk.²

Transfer cells of *Bacillus megaterium* ATCC 9855 by streaking the entire surface of sterile AK Agar #2 contained in a prescription (180 mL capacity) or Roux bottle. Incubate inoculated bottles at 35 ± 2°C for 48 hours. After incubation, wash the spores and vegetative cells from the agar surface with buffered MS (microbiologically suitable) water. Sediment the spores and cells by centrifugation at 5,000 × g for 15 minutes at 3°C. Store the spore suspension in buffered MS water under refrigeration. Consult the reference for the test procedure utilizing this *B. megaterium* spore suspension.²

User Quality Control

Identity Specifications

BBL™ AK Agar #2

| | |
|-------------------------------------|---|
| Dehydrated Appearance: | Fine, homogeneous, free of extraneous material. |
| Solution: | 3.08% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to moderately hazy. |
| Prepared Appearance: | Light to medium, yellow to tan, clear to moderately hazy. |
| Reaction of 3.08% Solution at 25°C: | pH 6.6 ± 0.2 |

Cultural Response

BBL™ AK Agar #2

Prepare the medium per label directions. Inoculate plates and incubate at 35 ± 2°C for 18-24 hours. Reincubate plates at 35 ± 2°C and prepare slides after 2 days (and again after 5 days for *B. subtilis* only).

| ORGANISM | ATCC™ | INOCULUM CFU | RECOVERY | SPORE PRODUCTION |
|----------------------------|-------|----------------------------------|----------|------------------|
| <i>Bacillus megaterium</i> | 9855 | 10 ² -10 ³ | Good | + |
| <i>Bacillus subtilis</i> | 6633 | 10 ² -10 ³ | Good | + |