BD BBL™ Prepared Plated Media for Isolation of Gram-Positive and Gram-Negative Enteric Organisms

Columbia CNA Agar with Sheep Blood//Levine EMB Agar

8801591JAA(02)
2013-07

INTENDED USE

This combination of media is used in qualitative procedures with clinical specimens. One side of the plate contains Columbia CNA Agar with Sheep Blood, which is used for isolating gram-positive cocci. The other side of the plate contains Levine EMB Agar, which is used for isolating gram-negative enteric bacilli.

SUMMARY AND EXPLANATION

Columbia CNA Agar with Sheep Blood is used for selectively isolating and cultivating gram-positive cocci. It is based on a medium developed by Ellner et al.[1] They found that adding 10 mg/L colistin and 15 mg/L nalidixic acid to Columbia Agar enriched with 5% sheep blood would support the growth of staphylococci, hemolytic streptococci and enterococci while inhibiting the growth of *Proteus*, *Klebsiella* and *Pseudomonas* species. In this medium, the concentration of nalidixic acid has been reduced to 10 mg/L to increase the recovery of gram-positive cocci from clinical specimens.

Levine EMB is used for isolating gram-negative enteric organisms and is especially useful for isolating and differentiating coliform organisms.[2,3] Levine developed the formula as a variation of the eosin methylene blue medium that Holt-Harris and Teague introduced in 1916.[2,4] Levine simplified the orginal formula by using a single peptone as a base and supplementing it with dipotassium phophate as a buffer, and by deleting the sucrose and increasing the concentration of lactose. The concentration of methylene blue and eosin were later reduced because of the purity of the dyes used in these products.[3]

PRINCIPLES OF THE PROCEDURE

Columbia CNA Agar with Sheep Blood consists of two antimicrobial agents, colistin and nalidixic acid, in Columbia Agar that has been enriched with 5% sheep blood. The colistin and nalidixic acid inhibit the growth of gram-negative organisms that could interfere with recovery of staphylococci and other pathogenic cocci. Defibrinated sheep blood provides the nutrients necessary to support the growth of fastidious organisms and indicates hemolytic reactions.[5] Some beta-hemolytic streptococci may produce greenish reactions that could be mistaken for alpha hemolysis because of the presence of carbohydrates from the yeast extract contained in the medium.

Levine EMB Agar contains pancreatic digest of gelatin, a peptone, to supply nutrients. Eosin Y and methylene blue dyes inhibit gram-positive organisms and aid in the differentiation of the enteric organisms by pigmentation of lactose fermenters. A decrease in pH of the medium surrounding colonies of lactose fermenters may reduce the solubility of the methylene blue-eosin complex. The colonies then absorb the dye precipitate and become pigmented. Colonies that do not ferment lactose remain colorless because ammonia produced during utilization of peptones increases the pH of the surrounding medium, causing the methylene blue-eosinate complex to become soluble.[6]

REAGENTS

Columbia CNA Agar with Sheep Blood

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	12.0 g
Peptic Digest of Animal Tissue	5.0 g
Yeast Extract	3.0 g
Beef Extract	3.0 g
Corn Starch	1.0 g
Sodium Chloride	5.0 g
Agar	13.5 g
Colistin	10.0 mg
Nalidixic Acid	10.0 mg
Sheep Blood, defibrinated	5.0%

Levine EMB Agar

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Gelatin	10.0 g
Lactose	10.0 g
Dipotassium Phosphate	2.0 g
Agar	15.0 g
Eosin Y	0.4 g
Methylene Blue	65.0 mg

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

Warnings and Precautions: For *in vitro* Diagnostic Use

If excessive moisture is observed, invert the 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.

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, cracking or other signs of deterioration.

SPECIMEN COLLECTION AND HANDLING

Refer to appropriate texts for details of specimen collection and handling procedures.[7-9]

Observe established precautions against microbiological hazards throughout all procedures. All specimens should be handled according to CDC-NIH recommedations for any potentially infectious human serum, blood or other body fluids. Prior to discarding, sterilize specimen containers and other contaminated materials by autoclaving.

PROCEDURE

Material Provided: Columbia CNA Agar with Sheep Blood//Levine EMB 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 and moist, but without excessive moisture.

Inoculate the medium as soon as possible after the specimen arrives at the laboratory. To culture specimens from swabs, inoculate the medium by rolling the swab over a small area of the agar on one side of the plate, and streak the remainder of the agar on that side with a sterilized inoculating loop to obtain isolated colonies. Using the same specimen, repeat the procedure to inoculate the other side of the plate. Material not being cultured from swabs should 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 °C for 18 – 48 h in an aerobic or CO_2-enriched atmosphere, depending on the source of the specimen or the microorganisms suspected of being present.

User Quality Control:

1. Examine plates for signs of deterioration as decribed under “Product Deterioration.”
2. Check performance by inoculating a representative sample of plates with pure cultures of stable control organisms that give known, desired reactions. The following test strains are recommended:

MEDIUM	TEST STRAIN	EXPECTED RESULTS
Columbia CNA Agar w/5% Sheep Blood (CO_2)	*Staphylococcus aureus* ATCC™ 25923	Growth
	Streptococcus pneumoniae ATCC 29212	Growth, alpha hemolysis.
	Streptococcus pyogenes ATCC 19615	Growth, beta hemolysis.
	Proteus mirabilis ATCC 12453	Inhibition, (partial to complete)
Levine EMB Agar (O_2)	*Escherichia coli* ATCC 25922	Growth, blue-black colonies with green, metalic sheen.
	Salmonella enterica subsp. *enterica* serotype Typhimurium ATCC 14028	Growth, colorless to amber colonies.
	Enterococcus faecalis ATCC 29212	Inhibition, (partial to complete)

RESULTS

After 18 – 48 h of incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

On the Columbia CNA Agar with Sheep Blood, most species of gram-negative organisms are inhibited. Small, grayish-white colonies surrounded by zones of hemolysis may be presumptively indentified as streptococci. Enterococci produce slightly larger, blue-gray colonies surrounded by zones of alpha hemolysis. Staphylococci produce larger, cream or yellow to gray, opaque colonies, some of which may be surrounded by zones of beta hemolysis.

Levine EMB Agar inhibits most species of gram-positive bacteria. Lactose fermenters produce blue-black colonies while lactose nonfermenters produce colorless colonies. *Escherichia coli* colonies have a characteristic green, metallic sheen, which may also be evident in the surrounding medium.

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

LIMITATIONS OF THE PROCEDURE

These prepared plated media are intended for primary isolation. Some diagnostic tests may be performed with the primary plate. However, a pure culture is recommended for biochemical tests and serological procedures. Consult appropriate references for further information.[5,8,10-12]

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. The agents in selective media may inhibit some strains of the desired species or permit growth of a species they were designed to inhibit, especially if the species is present in large numbers in the specimen. Specimens cultured on selective media should, therefore, also be cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

AVAILABILITY

Cat. No.	Description
295618	**BBL™** Columbia CNA Agar with Sheep Blood//Levine EMB Agar, Ctn. of 100 plates

REFERENCES

1. Ellner, P.D., C.J. Stoessel, E. Drakeford, and F. Vasi. 1966. A new culture for medical bacteriology. Am. J. Clin. Pathol. *45*:502-504.
2. Levine, M. 1918. Differentiation of *E. coli* and *E. aerogenes* on a simplified eosin-methylene blue agar. J. Bacteriol. *23*:43-47.
3. Levine, M. 1943. The effect of concentration of dyes on differentiation of enteric bacteria on eosin-methylene blue agar. J. Bacteriol. *45*:471-475.
4. Holt-Harris, J.E., and O. Teague. 1916. A new culture medium for the isolation of *Bacillus typhosus* from stools. J. Inf. Dis. *18*:596-600.
5. Facklam, R.R., and J.A. Washington II. 1991. *Streptococcus* and related catalase-negative gram-positve cocci, p. 238-257. *In* A. Balows, W.J. Hausler, Jr., K.L. Herrmann, H.D. Isenberg, and H.J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
6. Estevez, E.G. 1984. Bacteriologic plate media: review of mechanisms of action. Lab. Med. *4*:258-262.
7. Isenberg, H.D., F.D. Schoenknecht, and A. von Graevenitz. 1979. Cumitech 9, Collection and processing of bacteriological specimens. Coordinating ed., S.J. Rubin. American Society for Microbiology, Washington, D.C.
8. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 1998. Baileys & Scott's diagnostic microbiology, 10th ed. Mosby, Inc., St. Louis.
9. Miller, J.M., and H.T. Holmes. 1995. Specimen collection, transport, and storage, p. 19-32. *In* P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
10. Kloos, W.E., and T.L. Bannerman. 1995. *Staphylococcus* and *Micrococcus*, p. 282-298. *In* P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
11. Farmer, J.J., III. 1995. *Enterobacteriaceae*: introduction and identification, p. 483-449. *In* P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
12. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual® of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.

Technical Information: In the United States, contact BD Technical Service and Support at 800-638-8663 or www.bd.com/ds.

Becton, Dickinson and Company
7 Loveton Circle
Sparks, MD 21152 USA