Coagulase Mannitol Agar

Intended Use

Coagulase Mannitol Agar is used for the differentiation of *Staphylococcus aureus* from other species based on coagulase production and mannitol utilization.

Summary and Explanation

Staphylococci, both coagulase-positive and coagulase-negative *Staphylococcus* species (CoNS), have major medical significance.[1] Coagulase-producing staphylococci (*S. aureus*) may be differentiated and presumptively identified with this medium based on production of coagulase and mannitol utilization.

Chapman introduced the first selective medium for isolating and differentiating staphylococcal species.[2] Several years later, Zebovitz et al. and Marwin introduced tellurite-glycine media designed to selectively isolate coagulase-positive staphylococcal species.[3,4]

Esber and Faulconer developed the formula used in this medium.[5] In contrast to the earlier media, this formulation was developed as a general-purpose medium for fastidious organisms that also permitted differentiation of pathogenic staphylococci from other bacteria.

Principles of the Procedure

Coagulase Mannitol Agar aids in the differentiation of staphylococci by indicating the presence of coagulase and the utilization of mannitol. Coagulase production is dependant on the presence of mannitol, a protein factor in the brain heart infusion and blood serum (plasma).[5] During utilization of the mannitol, the pH of the medium drops, causing the bromcresol purple indicator to change from purple to yellow and producing yellow zones around these colonies. An opaque area of coagulated plasma forms around the colonies of organisms that also produce coagulase.

User Quality Control

Identity Specifications

BBL™ Coagulase Mannitol Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material, may contain some minute to very small tan flecks.
Solution:	4.7% solution, soluble in purified water upon boiling. Solution is medium to dark, purple, clear to slightly hazy.
Prepared Appearance:	Medium to dark, purple, clear to slightly hazy.
Reaction of 4.7% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

BBL™ Coagulase Mannitol Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM*	ATCC™	INOCULUM CFU	MANNITOL UTILIZATION	COAGULASE REACTION
Enterobacter aerogenes	13048	10^3-10^4	Yellow colonies with or without weak yellow zone	No zone
Proteus vulgaris	8427	10^3-10^4	Negative	No zone
Staphylococcus aureus	13150	10^3-10^4	Yellow zone	Opaque zone
Staphylococcus epidermidis	12228	10^3-10^4	Negative	No zone

**Recovery of all cultures should be good.*

In contrast, a coagulase-negative species that does not utilize mannitol, such as *Staphylococcus epidermidis*, does not change the color of the medium and it remains clear. Other coagulase-negative species may utilize mannitol and produce a yellow zone around the colonies, but an opaque zone will not be produced.

Formula

BBL™ Coagulase Mannitol Agar

Approximate Formula* Per Liter		
Brain Heart Infusion	5.0	g
Pancreatic Digest of Casein	10.5	g
Papaic Digest of Soybean Meal	3.5	g
Sodium Chloride	3.5	g
D-Mannitol	10.0	g
Agar	14.5	g
Bromcresol Purple	0.02	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 47 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 50°C and add 7-15% pretested, undiluted rabbit coagulase plasma with EDTA. Mix gently and pour into plates, approximately 18 mL per plate.
5. Test samples of the finished product for performance using stable, typical control cultures.

NOTE: The use of **BBL** Coagulase Plasma, Rabbit with EDTA, in place of citrated plasma, prevents false-positive coagulase reactions by citrate-utilizing microorganisms.

Procedure

Inoculate and incubate the plates in an inverted position (agar side up) at 35 ± 2°C, and examine for growth after 18-24 hours. Avoid prolonged incubation because it may cause the opaque zones surrounding coagulase-positive organisms to become clear.

Expected Results

After 18-24 hours of incubation, coagulase-positive organisms will produce opaque zones; coagulase-negative organisms will produce no opacity. Organisms that utilize mannitol produce yellow zones. *S. aureus* may be presumptively identified as those colonies with opaque, yellow zones around them.

Limitations of the Procedure

Some old or mutant strains of *S. aureus* may be weak coagulase producers or exhibit negative coagulase reactions and should be subcultured and retested if in doubt. *Escherichia coli* also uses mannitol and may be weakly coagulase-positive. Colonial morphology and a Gram stain should readily allow for differentiation from *S. aureus*.

References

1. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
2. Chapman. 1946. J. Bacteriol. *51*:409.
3. Zebovitz, Evans and Nivens. 1955. J. Bacteriol. *70*:686.
4. Marwin. 1958. Am. J. Clin. Pathol. *30*:470.
5. Esber and Faulconer. 1959. Am. J. Clin. Pathol. *32*:192.

Availability

BBL™ Coagulase Mannitol Agar

Cat. No. 211116 Dehydrated – 500 g