ESCULIN MEDIA

PRODUCTS:

Plated Media:[a]

Bile Esculin Azide Agar	303427
Bile Esculin Media	P3715

Tubed Media:

Bile Esculin Azide Agar	T6194
Bile Esculin Media	T6193, T6190, T3080, T3090
Esculin Agar	T6395, T3150
Esculin Broth	T6392

[a]see catalog for ordering options

PURPOSE:

Bile Esculin media are selective media used primarily for the presumptive identification and the differential isolation of esculin-hydrolyzing Lancefield group D streptococci. Esculin media is used as a test media to identify microorganisms capable of hydrolyzing esculin.

PRINCIPLE:

Esculin-containing nutrient agar was originally developed for the detection of group D streptococci. Swan[7] introduced a media containing esculin and bile for the presumptive identification of group D streptococci; bile salts inhibit gram-positive organisms other than group D streptococci. Isenberg[5] further modified Bile Esculin Media (BEM) by adding sodium azide, which made the media more selective for group D streptococci and useful as primary plated media; sodium azide inhibits gram-negative organisms. The most common and clinically significant group D streptococci recovered from bile esculin media are:[3]

Enterococci	Non-enterococci
E. faecalis	*S. bovis*
E. faecium	*S. equinus*
E. durans	
E. avium	

Esculin Agar without bile or sodium azide is now used to detect a variety of microorganisms capable of hydrolyzing esculin. Dowell et al.[1] demonstrated the usefulness of Esculin Agar in the differentiation of *Bacteroides* species. It is also recommended in the identification scheme for a variety of microorganisms including microorganisms in the families *Vibrionaceae* and *Pseudomonadaceae*.[5]

FORMULAS:

Approximate, per liter of deionized filtered water.

(1) Bile Esculin Azide Agar (BEAA):

Pancreatic Digest of Casein	16.00 g
Yeast Enriched Meat Peptone	9.50
Oxgall (Bile)	10.00
Sodium Chloride	5.00
Sodium Citrate	1.00
Esculin	1.00
Ferric Ammonium Citrate	0.50
Sodium Azide	0.25
Agar	14.00

Final pH 7.1 ± 0.2 at 25°C

(2) Bile Esculin Media (BEM):

Beef Extract	3.00 g
Pancreatic Digest of Gelatin	5.00
Oxgall (Bile)	40.00
Esculin	1.00
Ferric Citrate	0.50
Agar	15.00

Final pH 6.6 ± 0.2 at 25°C

(3) Esculin Agar:

Beef Extract	3.00 g
Pancreatic Digest of Gelatin	5.00
Esculin	1.00
Ferric Chloride	0.50
Agar	15.00

Final pH 7.0 ± 0.2 at 25°C

(4) Esculin Broth:

Pancreatic Digest of Gelatin	5.0 g
Esculin	0.3
Ferric Citrate	0.5
Potassium Diphosphate	1.0

Final pH 7.2 ± 0.2 at 25°C

PRECAUTIONS: *

For *in vitro* diagnostic use. Observe approved biohazard precautions.

Storage: Upon receipt store at 2-8°C away from direct light. Media should not be used if there are signs of contamination, deterioration (shrinking, cracking, or discoloration), or if the expiration date has passed.

Limitations: Some strains of *S. intermedius*, *S. mutans*, *S. uberis*, *S. sanguis I*, *S. constellatus*, and *Aerococcus* species can grow in the presence of bile and can hydrolyze esculin.[3]

A heavy inoculum may make interpretation of the bile esculin test difficult; excess inoculum decreases the ability of bile to inhibit growth of other gram-positive microorganisms that can hydrolyze esculin. Some strains of *Staphylococcus* species, *Aerococcus* species, and *Listeria monocytogenes* are examples of such microorganisms that can hydrolyze esculin. In addition, *Listeria monocytogenes* and *Aerococcus* species grow in 6.5% sodium chloride, which could lead to misidentification. Colonial morphology, Gram staining the microorganism growing in thioglycollate broth, and the performance of other identification testing[3,5] may be necessary to assure a correct identification.

A few streptococci tolerate bile but do not hydrolyze esculin. Growth alone on the Bile Esculin Media does not constitute a positive test.

PROCEDURE:*

Specimen Collection: Information on specimen collection is found in standard reference material on the subject. In general, specimens should be protected from extreme heat and cold and delivered to the laboratory without delay.

Method of Use for Bile Esculin Media (BEM) and Esculin Agar/Broth: Specimen collection is not applicable since the media is not used for primary isolation from clinical specimens. Prior to inoculation, the media should be brought to room temperature. Lightly tap the top of one or two well-isolated and morphologically similar colonies with a sterile needle or loop and inoculate the broth or inoculate the slant in a fishtail motion. If using a multipoint inoculation system, lightly tap the top of one or two well-isolated and morphologically similar colonies and inoculate a broth culture. Deposit a broth spot 5-6 mm in diameter onto the surface of the agar plate using the replicator or any comparable device that could be readily adaptable. Incubate tubes or plates aerobically at 35°C for 18-72 hours.

Method of Use for Bile Esculin Azide Agar (BEAA): Prior to inoculation, the media should be brought to room temperature. BEAA is a primary isolation media and enhances the recovery and isolation of individual colonies from clinical specimens. Inoculate a quarter of the plate with the clinical specimen and streak the plate in a manner that allows for the growth of isolated colonies. Incubate aerobically at 35°C for 18-48 hours.

Interpretation:

For agar slants:	Positive:	One-half or more of the slant is blackened.
	Negative:	Less than one-half or no blackening of the slant after 72 hours of incubation.
For plated/broth media:	Positive:	Any blackening of the media.
	Negative:	No blackening of the media after 48 hours of incubation.

Materials Required but Not Provided: Standard microbiological supplies and equipment commonly found in a microbiological laboratory are not provided.

QUALITY CONTROL:*

Media Used	Microorganisms Used (ATCC #):	Expected Results:
Bile Esculin Azide Agar and Bile Esculin Media:	*Enterococcus faecalis* (29212)	(+)
	Streptococcus pyogenes (19615)	(-); Inhibition
	Escherichia coli (25922)	(-); Inhibition, BEAA only
Esculin Agar/Broth:	*Klebsiella pneumoniae* (13883)	(+)
	Escherichia coli (25922)	(-)
	Key: See "Interpretation"	

User Quality Control: Check for signs of contamination and deterioration. Esculin agars should appear firm, translucent and light olive in color. Esculin broths should appear clear and light olive in color.

BIBLIOGRAPHY:

1. Dowell, V. R., Jr., and G. L. Lombard, *Presumptive Identification of Anaerobic Nonsporeforming Gram-Negative Bacilli*, Centers for Disease Control, Atlanta, 1977.
2. Facklam, R. R., and M. D. Moody, *Appl. Microbiol.*, 20:245, 1970.
3. Finegold, S. M., and E. J. Baron, *Bailey and Scott's Diagnostic Microbiology*, 7th ed., C. V. Mosby, St. Louis, 1986.
4. Granato, P. A. and P. D. Ellner, *Hlth. Lab. Sci.*, 13:(4):258-261, 1976.
5. Isenberg, H. D., et al., *Appl. Microbiol.*, 20:433, 1970.
6. Lennette, E. H., et al., *Manual of Clinical Microbiology*, 4th ed., American Society for Microbiology, Washington, D. C., 1985.
7. Swan, A., *Am. J. Clin. Pathol.*, 7:160, 1954.

*For more detailed information, consult appropriate references.

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