

the equivalent of 500 g of fresh heart tissue. Beef Heart for Infusion supplies the nutritional requirements for growth of microorganisms in Heart Infusion media.

One of the first media used for the cultivation of bacteria was a liquid medium containing an infusion of meat. Huntoon¹ used fresh beef heart and Bacto Peptone to prepare a “hormone” broth to retain growth-promoting substances. Highly pathogenic organisms, such as meningococci and pneumococci, could be grown on infusion medium without enrichments.¹

Beef Heart for Infusion is a component of Heart Infusion media. Heart Infusion media are used in the mass production of microorganisms for vaccine production and are specified in standard methods for other multiple applications.²⁻⁷

User Quality Control

Identity Specifications

Difco™ Beef Heart for Infusion

Dehydrated Appearance:	Tan to medium brown, fine, homogeneous.
Solution:	5.0% solution, not completely soluble in purified water. Solution, after filtration, is light to medium amber, clear to slightly opalescent, may have a precipitate.
Reaction of 5.0% Solution at 25°C:	pH 7.5-7.8

Cultural Response

Difco™ Beef Heart for Infusion

Prepare a 5% solution of Beef Heart for Infusion. Infuse for one hour at 50 ± 2°C. Heat to boiling for 3-5 minutes and filter. Add 2% Proteose Peptone No. 3, 0.5% sodium chloride and 0.005% dextrose to the filtrate. Adjust pH to 7.5-7.8. Boil and filter before autoclaving. Inoculate and incubate tubes at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Escherichia coli</i>	25922	10 ² -10 ³	Good
<i>Klebsiella pneumoniae</i>	13883	10 ² -10 ³	Good
<i>Staphylococcus aureus</i>	25923	10 ² -10 ³	Good
<i>Streptococcus pyogenes</i>	19615	10 ² -10 ³	Good

Principles of the Procedure

Beef Heart for Infusion provides nitrogen, amino acids and vitamins in microbiological culture media.

Typical Analysis

Refer to Product Tables in the Reference Guide section of this manual.

Directions for Preparation from Dehydrated Product

Infusions can be prepared using 50 g of Beef Heart for Infusion per liter of purified water. For best results, infuse at 50° C for 1 hour. Heat the infusion to boiling for a few minutes to coagulate some of the proteins and filter. Add peptone and remaining ingredients of the medium to the filtrate. Adjust the pH to 7.5-7.8. Boil the medium and filter before autoclaving. Consult appropriate references for further directions on preparation of specific products.

Procedure

See appropriate references for specific procedures using Beef Heart for Infusion.²⁻⁴

Expected Results

Refer to appropriate references and procedures for results.

References

- Huntoon. 1918. J. Infect. Dis. 23:168.
- Ruoff. 1995. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
- U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
- Horwitz (ed.). 2000. Official methods of analysis of AOAC International, 17th ed. AOAC International, Gaithersburg, Md.
- U.S. Environmental Protection Agency. 2000. Improved enumeration methods for the recreational water quality indicators: Enterococci and *Escherichia coli*. EPA-821/R-97/004. Office of Water, USEPA, Washington, D.C.
- Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
- U.S. Department of Agriculture. 1998. Microbiology laboratory guidebook, 3rd ed. Food Safety and Inspection Service, USDA, Washington, D.C.

Availability

Difco™ Beef Heart for Infusion

AOAC BAM EPA SMWW USDA

Cat. No. 213210 Dehydrated – 500 g

Bile Esculin Agar

Intended Use

Bile Esculin Agar is used to differentiate enterococci and the *Streptococcus bovis* group from other streptococci.^{1,2}

Summary and Explanation

Rochaix noted the value of esculin hydrolysis in the identification of enterococci.³ The enterococci were able to split esculin, but other streptococci could not. Meyer and Schonfeld incorporated bile into the esculin medium and showed that 61 of 62 enterococci were able to grow and split esculin, whereas the other streptococci could not.⁴ Swan used an esculin medium containing 40% bile salts and reported that a positive

reaction on the bile esculin medium correlated with a serological group D precipitin reaction.⁵

Principles of the Procedure

Enterococci and certain streptococci hydrolyze the glycoside esculin to esculetin and dextrose. Esculetin reacts with an iron salt to form a dark brown or black complex.⁶ Ferric citrate is incorporated into the medium as an indicator of esculin hydrolysis and resulting esculetin formation. Oxgall is used to inhibit gram-positive bacteria other than enterococci.

Formula

BBL™ Bile Esculin Agar

Approximate Formula* Per Liter

Pancreatic Digest of Gelatin	5.0	g
Beef Extract	3.0	g
Oxgall	20.0	g
Ferric Citrate	0.5	g
Esculin	1.0	g
Agar	14.0	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 43.5 g of the powder in 1 liter of purified water. Mix thoroughly.

User Quality Control

Identity Specifications

BBL™ Bile Esculin Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material, may contain a moderate amount of very small dark particles.
Solution:	4.35% solution, soluble in purified water upon boiling. Solution is dark, tan olive to olive green with a blue tint, trace hazy to hazy.
Prepared Appearance:	Dark, tan olive to olive green with a blue tint, trace hazy to hazy.
Reaction of 4.35% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

BBL™ Bile Esculin Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 42-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	REACTION
<i>Enterococcus faecalis</i>	29212	10 ³ -10 ⁴	Good	Blackening
<i>Streptococcus pyogenes</i>	19615	10 ⁴ -10 ⁵	Partial to complete inhibition	No blackening

2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate the medium with two or three colonies and incubate overnight at 35 ± 2°C in an aerobic atmosphere.

Expected Results

Any blackening of the plated medium indicates a positive result; if no blackening occurs, the test is negative.

For slants, if more than half of the slant is blackened within 24-48 hours, the test is positive; if less than half is blackened or no blackening occurs within 24-48 hours, the test is negative.

Limitations of the Procedure

1. Strains of *Lactococcus*, *Leuconostoc* and *Pediococcus* that give a positive bile-esculin reaction have been isolated from human infections.^{1,2}
2. Occasional strains of viridans streptococci blacken the medium or display weakly positive reactions.²

References

1. Ruoff, Whiley and Beighton. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
2. Facklam, Sahn and Teixeira. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
3. Rocharix. 1924. Compt. Rend. Soc. Biol. 90:771.
4. Meyer and Schonfeld. 1926. Zentralbl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. Orig. 99:402.
5. Swan. 1954. J. Clin. Pathol. 7:160.
6. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed., Lippincott Williams & Wilkins, Baltimore, Md.

Availability

BBL™ Bile Esculin Agar

ISO		
Cat. No.	299068	Dehydrated –500 g
	221838	Prepared Plates – Pkg. of 10*
	221409	Prepared Slants – Pkg. of 10*
	221410	Prepared Slants – Ctn. of 100*

*Store at 2-8°C.

Biosate™ Peptone

Intended Use

Biosate Peptone is used as a component in microbiological culture media or in fermentation applications.

Summary and Explanation

Biosate Peptone is a mixed hydrolysate comprised of casein and yeast extract at a ratio of 65:35. The synergistic effect of two or more types of hydrolysates is well documented and has been utilized for decades in culture media formulation. The combination of pancreatic digest of casein and yeast extract provides nutritional benefits that are not provided by the components alone. It has been reported that the combined use of these two peptones has shown improved toxin production in clostridia.^{1,2} Additionally, the combination of pancreatic

digest of casein and yeast extract has been used successfully as components in media which supported the hatching and culture of *Giardia* spp. from cysts and the first-time culturing of a nematode without the need of its symbiotic bacteria.^{3,4}

Principles of the Procedure

Biosate Peptone provides nitrogen, amino acids and vitamins in microbiological culture media. In addition, the yeast extract component of the product provides proteins, carbohydrates and some micronutrients.

Typical Analysis

Refer to Product Tables in the Reference Guide section of this manual.

