

Precautions⁷

1. Biosafety Level 2 practices, containment equipment and facilities are recommended for activities with clinical specimens of human or animal origin containing or potentially containing pathogenic *Brucella* spp.
2. Biosafety Level 3 practices, containment equipment and facilities are recommended for all manipulations of cultures of the pathogenic *Brucella* spp. and for experimental animal studies.

Directions for Preparation from Dehydrated Product

1. Suspend 45.4 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. When an enrichment is being prepared, cool to 50-55°C prior to adding the desired enrichment.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion on bacteria and fungi from clinical specimens, refer to the appropriate procedures outlined in the references.^{8,9} For the examination of bacteria and fungi in food refer to standard methods.^{6,10}

Expected Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Eugon Agar is not recommended as a blood agar base for hemolytic reactions because of its high sugar content.
2. It is suggested that Eugon Agar be prepared as required. Do not melt and resolidify media containing enrichments.

References

1. Pelczar and Vera. 1949. Milk Plant Monthly 38:30.
2. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
3. Niven. 1949. J. Bacteriol. 58:633.
4. Harrison and Hansen. 1950. J. Bacteriol. 59:197.
5. Frank. 1955. J. Bacteriol. 70:269.
6. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
7. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 1999. Biosafety in microbiological and biomedical laboratories, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.
8. Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
9. Murray, Baron, Pfaller, Tenover and Tenover (ed.). 1999. Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
10. U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.

Availability

Difco™ Eugon Agar

COMPF

Cat. No. 258910 Dehydrated – 500 g

Difco™ Supplement B

Cat. No. 227610 Lyophilized – 6 × 10 mL with Reconstituting Fluid*
227620 Lyophilized – 100 mL with Reconstituting Fluid*

*Store at 2-8°C.

Bacto™ Eugon Broth • Eugonbroth™ Medium

Intended Use

Eugon Broth (Eugonbroth™) is a general-purpose medium used for the cultivation of fastidious and nonfastidious bacteria from a variety of clinical and nonclinical specimens.

Summary and Explanation

Eugon Broth (Eugonbroth) is the fluid form of Eugon Agar, a clear medium developed for use in the enumeration of bacteria in milk and other products.¹ The formulation was developed from a study conducted by Vera of various peptones, carbohydrates, salts and other constituents in various concentrations and combinations to yield eugonic (luxuriant) growth of bacteria.²

Principles of the Procedure

Peptones supply amino acids and other nitrogenous substances to support bacterial growth. L-cystine is an essential amino acid that improves growth. Dextrose is incorporated as a source of energy and sodium chloride provides osmotic equilibrium. Sodium sulfite along with the cystine content improves growth with chromogenicity.

Formula

Bacto™ Eugon Broth

Approximate Formula* Per Liter

Proteose Peptone No. 3	7.5	g
Pancreatic Digest of Casein	7.5	g
Soy Peptone	5.0	g
Dextrose	5.5	g
L-Cystine	0.7	g
Sodium Chloride	4.0	g
Sodium Sulfite	0.2	g

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

Precautions³

1. Biosafety Level 2 practices, containment equipment and facilities are recommended for activities with clinical specimens of human or animal origin containing or potentially containing pathogenic *Brucella* spp.
2. Biosafety Level 3 practices, containment equipment and facilities are recommended for all manipulations of cultures of the pathogenic *Brucella* spp. and for experimental animal studies.

Directions for Preparation from Dehydrated Product

1. Suspend 30.4 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. When an enriched medium is being prepared, cool to 50-55°C prior to adding the desired enrichment.
5. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Bacto™ Eugon Broth

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.04% solution, soluble in purified water upon boiling. Solution is light amber, clear, may have a slight precipitate.
Prepared Appearance:	Light amber, clear, may have a slight precipitate.
Reaction of 3.04% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Bacto™ Eugon Broth

Prepare the medium per label directions. Inoculate and incubate the medium unsupplemented at 35 ± 2°C (*Aspergillus niger* and *Candida albicans* at 30 ± 2°C) for up to 72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Aspergillus niger</i>	16404	10 ² -10 ³	Fair to good
<i>Brucella abortus</i>	11192*	10 ² -10 ³	Good
<i>Candida albicans</i>	26790	10 ² -10 ³	Good
<i>Lactobacillus fermentum</i>	9338	10 ² -10 ³	Good
<i>Shigella flexneri</i>	12022	10 ² -10 ³	Good
<i>Streptococcus pyogenes</i>	19615	10 ² -10 ³	Good

*If this strain is not available, verify performance with a known isolate.

Procedure

Organisms to be cultivated must first be isolated in pure culture on an appropriate solid medium.

Using a sterile inoculating loop or needle, transfer fresh growth from the subculture medium to the tubed medium.

Incubate under conditions appropriate for the organism being cultivated. Broth cultures should be held at least 1 week before discarding as negative.

Expected Results

Growth in tubes is indicated by the presence of turbidity compared to an uninoculated control.

If growth appears, cultures should be examined by Gram staining, subculturing onto appropriate media and incubating inoculated media aerobically with increased CO₂ and/or anaerobically.

References

1. Pelczar and Vera. 1949. Milk Plant Monthly. 38:30.
2. Vera. 1947. J. Bacteriol. 54:14.
3. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 1999. Biosafety in microbiological and biomedical laboratories, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.

Availability

Bacto™ Eugon Broth

Cat. No. 259010 Dehydrated – 500 g

BBL™ Eugonbroth™ Medium

Cat. No. 297424 Prepared Tubes – Ctn. of 100*

*Store at 2-8°C.