

BRUCELLA AGAR w/ 5% SHEEP BLOOD, HEMIN, VITAMIN K

INTENDED USE

Remel Brucella Agar w/ 5% Sheep Blood, Hemin, Vitamin K is a solid medium recommended for use in qualitative procedures for the primary isolation of anaerobic bacteria.

SUMMARY AND EXPLANATION

Brucella agar is prepared according to the formulation of the American Public Health Association (APHA).¹ Finegold reported brucella agar w/ 5% sheep blood preferable to heart infusion blood agar for the cultivation of anaerobic bacteria.² Vitamin K was initially incorporated into the formula by Summanen et al.² Weinstein and Onderdonk further modified the medium with the addition of hemin.^{3,4} The incorporation of these additives, as well as sheep blood, has made this medium appropriate for the cultivation of many anaerobes.

PRINCIPLE

Casein and meat peptones supply nitrogenous compounds necessary to support the growth of fastidious anaerobic microorganisms. Dextrose provides a source of energy and yeast extract supplies B vitamins. Sheep blood provides growth factors required by some anaerobic bacteria and enables the demonstration of hemolytic reactions. Vitamin K and hemin are added to enhance the growth of *Bacteroides* species and gram-positive sporeformers.⁵ Agar is a solidifying agent.

REAGENTS (CLASSICAL FORMULA)*

Casein Peptone.....	15.0 g	Sodium Bisulfite	0.1 g
Meat Peptone	5.0 g	Vitamin K	10.0 mg
Sodium Chloride.....	5.0 g	Hemin	5.0 mg
Yeast Extract.....	2.0 g	Sheep Blood	5 %
Dextrose.....	1.0 g	Agar	15.0 g
		Deminerlized Water	1000.0 ml

pH 7.0 +/- 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE

1. Prior to use, reduce the plates for a minimum of 24 hours by placing them in an anaerobic jar at room temperature.
2. Specimens for anaerobic culture should be plated to both selective and non-selective media.
3. Incubate anaerobically for 48-72 hours at 35-37°C.
4. Confirm anaerobic growth by Gram stain and subculture to a blood agar plate incubated in ambient air.

QUALITY CONTROL

All lot numbers of Brucella Agar w/ 5% Sheep Blood, Hemin, Vitamin K have been tested using the following quality control organisms and have been found to be acceptable. This quality control testing meets or exceeds CLSI standards.⁶ Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL

- **Bacteroides fragilis* ATCC® 25285
- **Clostridium perfringens* ATCC® 13124
- **Fusobacterium nucleatum* ATCC® 25586
- **Peptostreptococcus anaerobius* ATCC® 27337
- **Prevotella melaninogenica* ATCC® 25845

*CLSI recommended organism

INCUBATION

- Anaerobic, up to 48 h @ 33-37°C
- Anaerobic, up to 48 h @ 33-37°C
- Anaerobic, up to 48 h @ 33-37°C
- Anaerobic, up to 48 h @ 33-37°C
- Anaerobic, up to 48 h @ 33-37°C

RESULTS

- Growth
- Growth, beta hemolysis
- Growth
- Growth
- Growth

BIBLIOGRAPHY

1. Marshall, R.T. 1993. Standard Methods for the Examination of Dairy Products. 16th ed. APHA, Washington, D.C.
2. Summanen, P., E.J. Baron, D.M. Citron, C. Strong, H.M. Wexler, and S.M. Finegold. 1993. Wadsworth Anaerobic Bacteriology Manual. 5th ed. Star Publishing Co., Belmont, CA.
3. Weinstein, W.M., A.B. Onderdonk, J.G. Bartlett, and S.L. Gorbach. 1974. Infect. Immun. 10:1250-1255.
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5. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD.
6. Clinical and Laboratory Standards Institute. 2004. Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard, 3rd ed. M22-A3. CLSI, Wayne, PA.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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