PRODUCT SPECIFICATION SHEET

Blood Agar Base /Infusion Agar (DM040)

Intended Use
Blood Agar Base / Infusion Agar (DM040) is used with blood for the isolation and cultivation of a wide variety of microorganisms.

Product Summary and Explanation
Blood Agar Bases are typically supplemented with 5-10% sheep, rabbit, or horse blood, and used for isolating, cultivating, and determining hemolytic reactions of fastidious pathogenic microorganisms. Without enrichment, this blood agar base can be used as a general purpose medium. Blood Agar Base is a nutritious base, and a variety of supplements, including blood, are often added to enhance this medium. Blood Agar Base is formulated to achieve good growth and improve hemolytic reactions of important pathogenic bacteria.

Principles of the Procedure
Blood Agar Base is prepared using specially selected raw materials to support good growth of a wide variety of fastidious microorganisms. Blood Agar Base contains Brain heart infusion and Tryptose, which provide nitrogen, carbon, and essential vitamins to stimulate organism growth. Along with essential nutritive properties, the peptones improve and enhance hemolysin production. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent. In general, blood agar bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of a-hemolytic streptococci.[3] Supplementation with blood (5 - 10%) provides additional growth factors for fastidious microorganisms, and aids in determining hemolytic reactions. Hemolytic patterns may vary with the source of animal blood and the type of basal medium used.[4]

Formula / Liter

Ingredients	Gms / Litre
Beef heart infusion	10.00
Tryptose	10.00
Sodium chloride	5.00
Agar	15.00
Final pH: 7.3 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 40 g of the medium in one liter of distilled water.
2. Heat to boiling to completely dissolve the medium.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes.

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4. Prepare 5% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile molten agar base, cooled to 45 - 50°C.

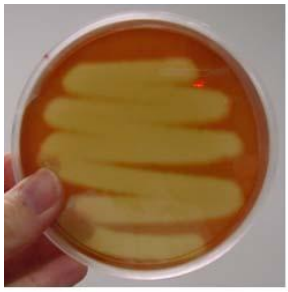
Quality Control Specifications

Dehydrated Appearance	Cream to yellow colored, homogeneous, free flowing powder
Solution	4% Solution in Distilled or deionized water is soluble on boiling, Light to Amber colored, and very slightly to slightly opalescent.
Prepared Medium	Without Blood – light to medium amber – slightly opalescent. With 5% sheep blood the medium is cherry red and opaque.
Reaction of 4.0% Solution	pH 7.3 ± 0.2 at 25°C
Gel Strength	Firm, compared to 1.5% Agar Gel.

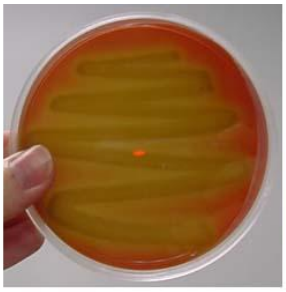
Expected Cultural Response: Cultural response on Blood Agar Base supplemented with 5-10% defibrinated sheep blood at 35 - 37°C after 18 - 48 hours incubation.

Sr. No.	Organisms	Results to be achieved		
		Growth w/o blood	Growth w/ blood	Haemolysis
1.	*Escherichia coli ATCC 25922*	good-luxuriant	good-luxuriant	slight beta hemolysis
2.	*Neisseria meningitidis ATCC 13090*	fair	good-luxuriant	none
3.	*Staphylococcus aureus ATCC 25923*	good-luxuriant	good-luxuriant	Beta haemolysis
4.	*Staphylococcus epidermidis ATCC 12228*	good-luxuriant	good-luxuriant	none
5.	*Streptococcus pneumonia ATCC 6303*	fair to good	good-luxuriant	Alpha haemolysis
6.	*Streptococcus pyogenes ATCC 19615*	fair to good	good-luxuriant	Beta haemolysis

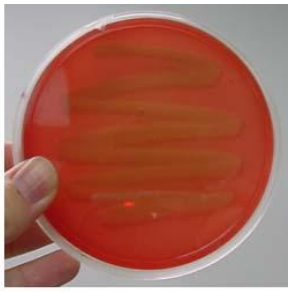
The organisms listed are the minimum that should be used for quality control testing.



Beta Hemolysis



Alpha Hemolysis



Gamma Hemolysis

Test Procedure

1. Inoculate directly onto the surface of the medium. Streak for isolation with an inoculating loop, and stab the agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions.[4]
2. Incubate plates aerobically, anaerobically, or under conditions of increased CO_2 (5 - 10%).

Results

Examine the medium for growth and hemolytic reactions after 18 - 24 and 48 hours incubation. There are four types of hemolysis on blood agar media described as: [4]

1. Alpha hemolysis (α) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This produces a green discoloration of the medium.
2. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony.
3. Gamma hemolysis (γ) indicates no hemolysis.
4. Alpha-prime-hemolysis (Ü) is a small zone of complete hemolysis, surrounded by an area of partial lysis.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. The incubation environment can influence hemolytic reactions of beta-hemolytic streptococci.[4]
2. Hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood. [4]

Packaging

Product Name : Blood Agar Base
Product Code : DM040
Available Pack sizes : 100gm / 500gm

References

1. United States Pharmacopeial Convention. 1995. The US pharmacopeia, 23rd ed. The US Pharmacopeial Convention, Rockville, MD.
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3. Casman, E. P. 1947. A noninfusion blood agar for neisseriae, pneumococci and streptococci. Am. J. Clin. 17:281-289.
4. Ruoff, K. L. 1995. *Streptococcus*, p. 299-305. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
5. Isenberg, H. D. (ed.). 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol. 1 p. 1.61-1.67. American Society for Microbiology, Washington, D.C.

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Further Information

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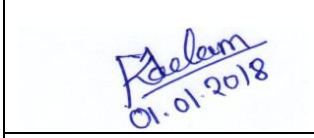	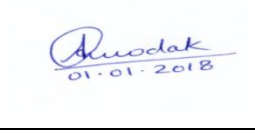	
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