PRODUCT SPECIFICATION SHEET

Blood Agar Base No. 2 (DM041)

Intended Use

Blood Agar Base No. 2 (DM041) is a medium base which on addition of blood, medium permits maximum recovery of *Streptococci*, *Pneumococci* and other fastidious pathogenic microorganisms without interfering with their haemolytic reactions.

Product Summary and Explanation

A fastidious organism is one with complete nutritional requirements, needing additional cellular building-block molecules in order to survive.[1] In 1919, Brown experimented with blood agar formulations for the effects of colony formation and hemolysis.[2] Blood Agar Base No. 2 is a nutritionally rich medium for maximum recovery of fastidious microorganisms. Blood Agar Base media are specified in standard method procedures for food testing.[3-5] It also serves as a differential medium for *Brucella* and *Campylobacter* species by adding different antibiotic supplements for the respective bacteria.[6, 7] *Brucella* cultures are highly infective and must be handled with care. Incubate preferably in 5-10% carbon dioxide atmosphere. Blood agar bases are typically supplemented with 5 - 10% sheep, rabbit, or horse blood for use in isolating, cultivating, and determining hemolytic reactions of fastidious pathogenic microorganisms. Without enrichment, blood agar bases can be used as general purpose media. Comparative studies of horse, rabbit and sheep blood showed that sheep blood gave the clearest and most reliable colony and haemolysis characteristics at both 24 and 48 hours of incubation.[8] It can be used to prepare Chocolate Agar for the isolation of *Haemophilus* and *Neisseria* species. It can also be used for primary isolation of *Haemophilus* species, where horse blood is used for enrichment. Better results are obtained by spreading half of the horse blood agar plate with 2 drops of 10% saponin.[9] Liver extract and yeast extract helps enhance the growth and haemolytic reactions of fastidious organisms like *Streptococci* and *Pneumococci*.

Principles of the Procedure

Blood Agar Base No. 2 contains proteose peptone which serves as the nitrogen source while liver digest and yeast extract provide essential carbon, vitamin, nitrogen and amino acid sources. Sodium chloride maintains the osmotic equilibrium of the medium. Supplementation with blood (5-10%) provides additional growth factors and also serves as basis for determining haemolytic reactions. Haemolytic patterns may vary with the source of animal blood or type of base medium used.

Formula / Liter

Ingredients	Gms / Liter
Proteose peptone	15.00
Liver extract	2.50
Yeast extract	5.00
Sodium chloride	5.00
Agar	15.00
Final pH: 7.4 ± 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 21.25 grams in 500 ml distilled water.

2. Heat to boiling, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 40 - 50°C and aseptically add 7% v/vsterile defibrinated blood.
5. For *Brucella* species: Add rehydrated contents of 1 vial of Brucella Selective Supplement (MS043) to 500 ml sterile molten base.

6. For *Campylobacter* species : Add rehydrated contents of 1 vial of Campylobacter Supplement - I (MS004) or Campylobacter Supplement - II (MS005) or Campylobacter Supplement - III (MS007) or Campylobacter Growth Supplement (MS008) to 500 ml sterile molten base.
7. For *Streptococcus* species: Add rehydrated contents of 1 vial of Strepto Supplement (MS027) to 500 ml sterile molten base. Mix well and pour into sterile Petri plates

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Basal medium : Yellow coloured clear to slightly opalescent gel. After addition of 5-7% v/v sterile defibrinated blood: Cherry red coloured opaque gel forms in Petri plates.
Reaction of 4.25% solution	pH 7.4 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed with added 5-7% sterile defibrinated blood, after an incubation at 35-37°C for 18-48 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Haemolysis
1.	*Neisseria meningitidis ATCC 13090*	50-100	good-luxuriant	>=70%	none
2.	*Staphylococcus aureus ATCC 25923*	50-100	good-luxuriant	>=70%	beta
3.	*Streptococcus pneumonia ATCC 6303*	50-100	good-luxuriant	>=70%	alpha
4.	*Streptococcus pyogenes ATCC 19615*	50-100	good-luxuriant	>=70%	beta

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

Test Procedure

1. Process each specimen as appropriate, and inoculate directly onto the surface of the medium. Streak for oxygen-stable and oxygen-labile streptolysins.
2. Incubate plates aerobically, anaerobically, or under conditions of increased CO_2 (5 - 10%) in accordance with established laboratory procedures.
3. Refer to appropriate references for standard test procedures.

Results

Examine 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:

1. Alpha hemolysis (a) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This produces a green discoloration of the medium.
2. Beta hemolysis (ß) is the lysis of red blood cells, producing a clear zone surrounding the colony.

3. Gamma hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
4. Alpha-prime hemolysis (a') is a small zone of complete hemolysis surrounded by an area of partial lysis.

Refer to appropriate references and test procedures for interpretation of results.

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. Hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood. Such strains are beta-hemolytic on horse, human, and rabbit blood agar and alpha-hemolytic on sheep blood agar.
2. Incubation atmosphere can influence hemolytic reactions of beta-hemolytic streptococci. For optimal performance, incubate blood agar base media under increased CO_2 (5 - 10%), in accordance with established laboratory procedures.
3. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Blood Agar Base No.2
Product Code : DM041
Available Pack sizes : 100gm / 500gm

References

1. Norton C. F., 1986, Microbiology, 2nd Edition, Addison-Wesley Publishing Company.
2. Brown, J. H. 1919. The use of blood agar for the study of streptococci. NY Monograph No. 9. The Rockefeller Institute for Medical Research.
3. Association of Official Analytical Chemists. 1995. Bacteriological analytical manual, 8thed., App. 3.08-3.09. AOAC International, Gaithersburg, MD.
4. Vanderzant, C., and D. F. Splittstoesser (eds). 1992. Compendium of methods for the microbiological examination of food, 3rd ed., p. 1113. American Public Health Association, Washington, D.C.
5. Greenberg, A. E., L. S. Clesceri, and A. D. Eaton (eds.). 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
6. Hunter D. and Kearns M., 1977, Brit. Vet. J., 133:486.
7. Skirrow M. B., 1977, B.M.J., ii: 9.
8. Snavely and Brahier, 1960, Am. J. Clin. Pathol., 33:511.
9. Waterworth and Pamela M., 1955, Brit. J. Exp. Pathol., 36:186.

Further Information

For further information please contact your local MICROMASTER Representative.

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DM041PSS,QAD/FR/024,Rev.00/01.01.2018

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