Blood Agar Base No. 2 (NCM0040)

Revision: 0 Effective Date: 12/1/2021

Intended Use
Blood Agar Base No. 2 is used with blood for the isolation and cultivation of a wide variety of fastidious microorganisms. Blood Agar Base is not intended for use in the diagnosis of disease or other conditions in humans.

Description
A very rich agar base which, with the addition of blood, is capable of growing fastidious organisms. The medium gives colonial appearances, hemolysis patterns and pigment production of indicative value. When the blood is 'chocolated' the medium gives good recovery of *Haemophilus* spp. The medium can be made selective for various groups by the addition of appropriate antibiotic mixtures eg:

*Streptococc*us spp.– Colistin/Oxolinic acid (X013)
Gardnerella spp. – Colistin/Oxolinic acid (X011)
C. perfringens – Neomycin (X015) (X016)
Staphylococcus/Streptococcus spp. – Colistin/Naladixic acid (X012)

Typical Formulation

Tryptose	15.0 g/L
Soy Peptone	2.5 g/L
Yeast Extract	5.0 g/L
Sodium Chloride	5.0 g/L
Agar	12.0 g/L

pH: 7.4 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution
Refer to SDS

Preparation
1. Suspend 39.5 grams of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C.
5. Aseptically add 5-7% sterile, defibrinated horse or sheep blood.
6. Mix well before pouring.

Test Procedure
1. Process each sample 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.

Quality Control Specifications
Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium without blood is light yellow beige, and trace to slightly hazy. With 5% sheep or horse blood, medium is red and opaque.

Expected Cultural Response: Cultural response on Blood Agar Based No. 2 with 5-7% sheep or horse blood incubated at appropriate atmosphere and temperature and examined for growth after incubation.

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Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Reactions
Enterococcus faecalis ATCC® 29212	4Q streak	Good growth	β hemolysis
Escherichia coli ATCC® 25922	4Q streak	Good growth	β hemolysis
Staphylococcus aureus ATCC® 25923	4Q streak	Good growth	Weak β hemolysis
Pseudomonas aeruginosa ATCC® 27853	4Q streak	Good growth	NA
Campylobacter jejuni ATCC® 29428	50-200	≥70%	NA
Campylobacter jejuni ATCC® 33291	50-200	≥70%	NA
Campylobacter coli ATCC® 43478	50-200	≥70%	NA
Streptococcus pyogenes ATCC® 19615	50-200	≥70%	β hemolysis
Streptococcus pneumoniae ATCC® 6305	4Q streak	Good growth	α hemolysis

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

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

Expiration

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

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. 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.
3. 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.

Storage

Store dehydrated culture media at 2 – 30°C away from direct sunlight. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

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