Blood Agar Base CE (NCM2014)

Intended Use
Blood Agar Base is used with blood for the isolation and cultivation of a wide variety of fastidious microorganisms.

Description
An inexpensive, general purpose agar base which, with the addition of 5-7% sterile blood, can be used to cultivate a wide range of microorganisms of indicative significance. Typical hemolysis patterns are obtained with this medium.

Typical Formulation

Beef Extract	10.0 g/L
Balanced Peptone No. 1	10.0 g/L
Sodium Chloride	5.0 g/L
Agar No. 2	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. Disperse 37g in one liter of deionized water.
2. Soak for 10 minutes, swirl to mix and sterilize by autoclaving for 15 minutes at 121°C.
3. Cool to 45-50°C and add 5-7% of defibrinated blood, mix before pouring into Petri dishes and then dry the agar surface.

Test Procedure
1. Process each sample as appropriate, inoculate directly onto the surface of the medium. Streak for isolation with inoculating loop, stab agar several times to deposit beta-hemolytic streptococci beneath agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to activity of both 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 light beige to beige.

Prepared Appearance: Prepared medium without blood (plain) is trace to slightly hazy and yellow-beige. With 5-7% horse blood, medium is red and opaque.

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Expected Cultural Response: Cultural response on Blood Agar Base with 5-7% horse or sheep blood at the appropriate time, temperature and environment in compliance with ISO 11133:2014.

Microorganism	Approx. Inoculum (CFU)	Recovery	Reactions
Enterococcus faecalis ATCC® 29212	4Quad Streak	Growth	NA
Escherichia coli ATCC® 25922	4Quad Streak	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
Pseudomonas aeruginosa ATCC® 27853	4Quad Streak	Growth	NA
Staphylococcus aureus ATCC® 25923	4 Quad Streak	Growth	NA
Streptococcus pneumoniae NCIMB® 13286	4 Quad Streak	Growth	Alpha hemolysis
Streptococcus pyogenes NCTC® 8198	50-200	≥ 70%	Beta hemolysis

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 (ά) 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 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.6 For optimal performance, incubate blood agar base media under increased CO_2 (5 - 10%).

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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