Blood Agar Base Nº2 ISO

Cat. 1328

For the cultivation and detection of hemolytic activity of fastidious microorganisms.

Practical information

Aplications	Categories
Detection	Fastidious microorganisms

Industry: Clinical

Regulations: ISO 11290 / ISO 7932

CE
IVD

Principles and uses

Blood Agar Base Nº 2 is a base medium rich in nutritional properties, used for the preparation of blood agar plates. It is used for the isolation, cultivation and recovery of fastidious microorganisms to study hemolysis activity.

Liver extract and yeast extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride maintains the osmotic equilibrium. The blood is an additional source that provides growth factors for the microorganisms and is the basis for determining hemolytic reactions. Hemolytic patterns may vary with the type of blood or base medium used. For example, defibrinated sheep blood gives best results for Group A streptococci. Bacteriological agar is the solidifying agent.

This medium can be used to prepare a selective medium for Brucella spp or Campylobacter spp by adding an antibiotic supplement. It may also be used for the primary isolation of Haemophilus spp.

This medium has been recommended by ISO normative 7932 for the confirmation of Bacillus cereus. The Bacillus cereus has positive reaction of ß-hemolysis. The width of the hemolysis zone may vary.

It is also a medium recommended by ISO normative 11290-1 for the confirmation of Listeria monocytogenes. . A zone of ß- hemolysis is considered a positive reaction.

Results:

1. Alpha-hemolysis: greenish discoloration of medium.
2. Beta-hemolysis: clear zone surrounding colony.
3. Gamma-hemolysis: no change.

Formula in g/L

Bacteriological agar	12	Sodium chloride	5
Yeast extract	5	Proteose peptone	15
Liver extract	2,5		

Preparation

Suspend 39,5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 ºC for 15 minutes. Cool to 45-50 ºC and aseptically add 5-7% of sterile defibrinated blood, homogenize and pour intro Petri dishes. Be careful to avoid bubble formation when adding the blood to the cooled medium and rotate the flask or bottle slowly to create a homogeneous solution.

Instructions for use

» For clinical diagnosis, the type of sample comes from the throat and/or the genitals.

- Inoculate on the surface. Parallel striae with the handle or swab.
- Incubate in aerobic conditions at 35±2 ºC for 24-48 hours.
- Reading and interpretation of the results.

» For other uses not Covered by the CE marking:

Confirmation of Bacillus cereus according to ISO 7932:
- Incubate at 30 °C for 24±2 h.
- Interpret the hemolysis reaction.

Confirmation of Listeria monocytogenes according to ISO 11290-1:
- Incubate at 35 ºC or 37 ºC for 18-24 hours.
- Interpret the hemolysis reaction.

Primary isolation of Haemophilus spp:
- Add horse blood to enrich the medium.
- Incubate at 35±2 ºC and observe after 24-48 hours.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Opaque red	7,0±0,2

Microbiological test

Incubation conditions: (41,5±1 ºC / 44±4 h).

Microrganisms	Specification
Campylobacter jejuni ATCC 29428	Good growth
Campylobacter jejuni ATCC 33291	Good growth
Campylobacter coli ATCC 43478	Good growth

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

WATERWORTH, P.M.: BRIT. J. Exp. Pathol., 36(02); 186-194 (1955)
ISO 7932 Horizontal Method for the enumeration of Bacillus cereus
ISO 11290-1 Microbiología de los alimentos para consume humano y para animales. Método horizontal para la detección y el recuento de Listeria monocytogenes Parte 1 Método de detección