BLOOD AGAR BASE Nº2 ISO 7932

CAT Nº: 1328 For the cultivation and detection of hemolytic activity of fastidious microorganisms, confirmation of *Bacillus cereus* (ISO 7932) and *Listeria monocytogenes* (ISO 11290-1)

FORMULA IN g/l

Proteose peptone	15.00	Liver Extract	2.50
Yeast Extract	5.00	Bacteriological Agar	12.00
Sodium Chloride	5.00		

Final pH 7.0 ± 0.2 at 25ºC

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 121ºC for 15 minutes. Cool to 45-50ºC 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. The prepared medium should be stored at 8-15ºC. The color is opaque red without hemolysis.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

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 the 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.* Add horse blood to enrich the medium.

Incubate at 35 ± 2ºC and observe after 24-48 hours.

This medium has been recommended by ISO normative 7932 for the confirmation of *Bacillus cereus*. Incubate at 30°C for 24±2 h and interpret the hemolysis reaction. 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*. The normative recommends incubation at 35°C o 37°C for 18-24 hours. 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

MICROBIOLOGICAL TEST

The following results were obtained adding 5% of defibrinated sheep blood in the performance of the medium from type culture after incubation at a temperature of 35 ± 2ºC and observed after 24-48 hours.

Microorganisms	Growth	Hemolysis
Neisseria meningitidis ATCC 13090	Good	-
Streptococcus pneumoniae ATCC 6303	Good	Alpha
Streptococcus pyogenes ATCC 19615	Good	Beta
**Bacillus cereus* ATCC 11778	Good	Beta
***Listeria monocytogenes* ATCC 11778	Good	Beta

* Incubate at 30ºC for 24 ± 2 hours according to ISO 7932

** Incubate at 35 or 37ºC for 18-24 hours according ISO 11290-1

According to ISO 11133 (44±4)h/41,5±1°C

Microorganisms	Inoculum (cfu/ml)	Productivity Quantitative	Recovery Rate (%)
Campylobacter jejuni ATCC 29428	10^2	$P_R \geq 0.7$	
Campylobacter jejuni ATCC 33291	10^2	$P_R \geq 0.7$	
Campylobacter coli ATCC 43478	10^2	$P_R \geq 0.7$	

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

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STORAGE

Once opened keep powdered medium closed to avoid hydration.