Blood Agar Base w/Nalidixic Acid

M1904

Recommended for the differentiation of haemolytic activity of Streptococci.

Composition**

Ingredients	Gms / Litre
Heart infusion	10.000
Meat peptone	10.000
Sodium chloride	5.000
Nalidixic acid	0.040
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.04 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 40 - 50°C and aseptically add 5% v/v sterile defibrinated blood. Mix well and pour into sterile Petri plates.

Principle And Interpretation

A fastidious organism is one with complete nutritional requirements, needing additional cellular building-block molecules in order to survive (1) Blood Agar Base is a highly nutritive medium. Microorganisms producing haemolysin give visible haemolytic zones on this medium. Blood Agar Base is modified with addition of Nalidixic acid which acts as an inhibitor for the accompanying flora and to support the growth of Staphylococci, haemolytic Streptococci and Enterococci when supplemented with 5% blood. Nalidixic acid blocks the DNA replication of susceptible bacteria and acts against many Gram-negative bacteria(2).

Heart infusion and Meat Peptone are rich source of nitrogen, vitamins, minerals and amino acids. Sodium chloride maintains the osmotic balance. Blood is an additional source that provides growth factors for the microorganisms and is the basis for determining haemolytic reactions. Haemolytic patterns may vary with the source of animal blood or type of base base medium used (3).

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of 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
Reaction of 4.25% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

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

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis

Cultural Response

Staphylococcus epidermidis ATCC 12228	50-100	good	>=50%	
Staphylococcus aureus ATCC 25923	50-100	none-poor	<=10%	beta
Streptococcus pneumoniae ATCC 6303	50-100	good-luxuriant	>=70%	alpha
Streptococcus pyogenes ATCC 19615	50-100	good-luxuriant	>=70%	beta
Escherichia coli ATCC 25922	$>10^3$	inhibited		

Storage and Shelf Life

Store the dehydrated and prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Norton C. F., 1986, Microbiology, 2nd Edition, Addison-Wesley Publishing Company.
2.Cruikshank.R(1972),Medical Microbiology ,11th edition, for differentiation of Haemolytic activity of Streptococci.
3. Murray P. R,, Baron E. J., JorgensenJ. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.

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