OXALIS Diagnostics Pvt. Ltd.

Technical Data

Dual Performance medium- Blood Agar / Brain Heart Infusion Broth 20/30 mL

Recommended for qualitative detection of microorganisms in blood. Combination of solid (20mL) and liquid (30mL) media in single bottle.

Product Presentation:

Cat No.	Product description	Pack Size
22020022030	Dual Performance medium- Blood Agar/ Brain Heart Infusion Broth 20/30 mL	1 Bottle.

Blood Agar

Principle

Hemolysins are exotoxins produced by bacteria that lyse red blood cells. The hemolytic reaction can be visualized on blood agar plates. On blood agar plates colonies of hemolytic bacteria may be surrounded by clear, colorless zone where the red blood cells have been lysed and the hemoglobin destroyed to a colorless compound. This is beta hemolysis. Other types of bacteria can reduce hemoglobin to methemoglobin which produces a greenish zone around the colonies and is called alpha hemolysis. Gamma hemolysis is no hemolysis where no change in the medium is observed. Blood gar Base supplemented with sheep blood is used to study hemolytic reactions (patterns) of organisms. But this gave mixed hemolytic reactions due to the physiological differences between sheep blood and horse blood. Sheep Blood Agar Base with added sheep blood was developed to allow maximum recovery of organisms without interfering with their hemolytic reactions. Sheep Blood Agar Base was formulated to be compatible with sheep blood and give improved hemolytic reactions of organisms. Tryptone, peptone and yeast extract provide nitrogen, carbon, amino acids and vitamins. Sodium chloride maintains the osmotic balance. Sheep Blood Agar Base showed considerable improvement and the expected beta hemolytic reactions with S.pyogenes in comparison to other blood agar bases supplemented with blood.

Composition

Ingredients	Grams / Liter
Tryptone	14.0
Peptone	4.5
Yeast extract	4.5
Sodium chloride	5.0
Agar	13.5
After sterilization.	
Defibrinated sterile blood at (45°C-50°)	50 mL

*Formula adjusted, standardized to suit performance parameters

Brain Heart Infusion Broth

Principle

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BHI Medium is useful for cultivating a wide variety of microorganisms since it is a highly nutritive medium. It is also used to prepare the inocula for antimicrobial susceptibility testing. BHI Broth is also the preferred medium for anaerobic bacteria, yeasts and molds This medium is nutritious and well buffered to support the growth of wide variety of organisms. With the addition of 10% defibrinated sheep blood, it is useful for isolation and cultivation of *Histoplasma capsulatum* and other fungi. For selective isolation of fungi, addition of gentamicin and/or chloramphenicol is recommended. Proteose peptone, Calf Brain, Infusion from 200g and Beef Heart, Infusion from 250g serve as sources of carbon, nitrogen, essential growth factors, amino acids and vitamins. Dextrose serves as a source of energy. Disodium phosphate helps in maintaining the buffering action of the medium whereas sodium chloride maintains the osmotic equilibrium of the medium. SPS acts as an anticoagulant and as an inhibitor of the bacteriostatic and bactericidal effects of blood cells and plasma factors.

Composition

Ingredients	**Grams / Liter**
Calf Brain, Infusion from 200g	7.70
Beef Heart, Infusion from 250g	9.80
Proteose Peptone	10.00
Dextrose	2.00
Sodium Chloride	5.00
Disodium Phosphate	2.50
SPS	0.50

**Formula adjusted, standardized to suit performance parameters

Type of specimen

Clinical samples:- Blood.

Specimen Collection and Preparation

- ✓ No special preparation of the patient is required prior to sample collection by approved techniques.
- ✓ The specimen must be collected using sterile techniques to reduce the chance of contamination.
- ✓ Collect 1:10 ratio blood to broth, 3 mL of patient's blood for 30 mL BHI broth.
- ✓ Samples should be stored at 2°C-8°C if not tested immediately.
- ✓ Avoid using hemolyzed samples for testing.

Directions

- ✓ Label the ready to use Dual Performance medium- Blood Agar/ Brain Heart Infusion Broth 20/30 mL.
- ✓ Remove the Aluminum foil cap. Disinfect the part of the rubber stopper which is now exposed.
- ✓ Draw patient's blood with the sterile or disposable needle and syringe as explained in specimen collection and disposable column.
- ✓ Transfer the blood sample immediately into the culture bottle by puncturing the rubber stopper with the needle and injecting the blood. Venting: Use sterile venting needle. Keep the bottle in an upright position preferably in a biological safety cabinet, place an alcohol swab over the rubber stopper and insert the venting needle with filter through it. Insertion and withdrawal of the needle should be done in a straight line.
- ✓ Incubate the bottle for 4-6 hours at 30 -35°C. For adsorption on solid surface. DO NOT SHAKE OR HOLD

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- ✓ MORE THAN 15 SECONDS.
- ✓ Do Not vent the bottle for anaerobic cultures.
- ✓ Revert into an upright position and incubate for 18-24 hours at 30-35°C or longer
- ✓ if necessary
- ✓ Discard the needle and mix the contents by gently inverting the bottle 2-3 times.

Storage and Stability

- ✓ Store the ready to use Dual Performance medium- Blood Agar/ Brain Heart Infusion Broth 20/30 mL.,
- ✓ dry place at 2°C-8°C away from direct light.
- ✓ Stability as per the expiry date mentioned on the label.

Quality Control

Appearance: In a sterile glass bottle combination of broth and one agar coated surface.
Color of Agar Medium : Cherry red colored opaque medium slightly opalescent gel.
Color of Broth Medium : Amber coloured clear solution.
Appearance on Addition of Blood: Cherry red colored opaque solution without any blood clot.

Quantity of medium

20ml of Agar medium in glass bottle & 30ml of Broth medium in glass bottle

pH of Agar medium	**pH of liquid medium**
7.10- 7.50	7.20- 7.60

Cultural Response: Cultural response is observed after an incubation of 18-24 hours at 35°C-37°C.

Organisms	Type Culture	Growth on Liquid Medium	Growth on Agar Medium	Haemolysis
Staphylococcus aureus	ATCC 25923	Good	Good	Beta Haemolysis
Streptococcus pyogenes	ATCC 19615	Good	Good	Beta Haemolysis
Bacteroides vulgatus	ATCC 8482	Good	Good	-

Interpretation of Results

Growth in the broth medium is indicated by the presence of turbidity. The bottles should be held for 7 days before reporting a negative blood culture.
Inoculam for good growth <100 CFU.
Clearly visible growth of the test microorganisms is comparable to that previously tested and approved batch.

Warranty

- ✓ This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

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Disposal

Disposal of infectious material and material that comes in to contact with clinical sample must be decontaminated and dispose of by autoclaving or incineration or established laboratory procedures. User must be ensure safe disposal of used or unusable preparation of the products.

Reference

1. Atlas R. M., 1993, Handbook of Microbiological Media, 147-153, CRC Press, Boca Raton, FL.
2. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
3. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
4.Diagnostic Microbiology , Baily & Scott, 9th Edition , Ellen jo Baron, etal. , Mosby 1994.

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