Blood Culture Medium Bottles are medical laboratories test used for detection of aerobic and facultative anaerobic microorganisms (bacteria and fungi) from human blood.

**Blood Culture Medium**

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| REF: V/BS04.080 4 Tests for Adult REF: V/BS05.020 5 Tests for Pediatrics |  REF: V/BS06.008 6 Tests for Neonate  |

# CLINICAL SIGNIFICANCE

The Blood Culture Media is used to determine if microorganisms are present in blood or other normally sterile body fluid samples taken from a patient suspected of having bacteremia/fungemia. culture bottles provide both a microbial detection system and a culture media with suitable nutritional and environmental conditions for organisms commonly encountered in blood infections and other normally sterile body fluid infections.

# METHOD PRINCIPLE

Blood samples are collected from patients, using strict aseptic technique and sterile equipment. The samples are inoculated into the blood culture bottles and mixed with the medium. The formulation of the medium encourages the growth of aerobic, anaerobic and micro-aerophilic organisms. The medium is also designed to create pressure in the sealed bottle when organisms are growing. The detection of positive pressure is by means of a growth indicator device which is connected to the bottle after the blood sample is added. A positive pressure in the bottle displaces a

quantity of blood/broth mixture into the chamber as a sign of microbial activity. A positive result is indicated when the blood/broth mixture rises above the green locking sleeve of the growth indicator device.

# MEDIA COMPOSITION

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| **Item**  | **Formula in g/L**  |
| Tryptone Soya Broth Gelatin peptone Yeast extract Meat extract Sodium chloride Potassium nitrate Glucose L-arginine Sodium pyruvate Gelatin Sodium thioglycollate Cysteine HCl Sodium bicarbonate Phosphate buffer Sodium polyanethol sulphonate Dithiothreitol Adenine sulphate Sodium succinate Ammonium chloride Magnesium sulphate Menadione  | 10.0 10.0 5.0 5.0 8.0 2.0 1.0 1.0 1.0 1.0 0.5 0.4 0.4 0.3 0.3 0.2 0.01 0.01 0.008 0.008 0.005  |

## pH 7.0 ± 0.2 at 25°C

Sodium polyanethol sulphonate (SPS), 0.03% is added because it inhibits clotting, neutralizes the bactericidal effect of human serum, prevents phagocytosis and partially inactivates certain antibiotics (streptomycin, kanamycin, gentamicin and polymyxin B. SPS may be inhibitory to some strains of Peptostreptococcus anaerobius, Neisseria meningitidis and Neisseria gonorrhoeae; therefore gelatin is added to the medium to neutralize this inhibition. When human blood is added to this medium, CO2 produced can be detected at 2.5 to 5% v/v in the bottle head-space.

# PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person.

Good Laboratories practices using appropriate precautions should be followed in:

* Wearing personnel protective equipment (overall, gloves, glasses,..).
* Do not pipette by mouth.
* In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
* Handle specimens and inoculated culture bottles as though capable of transmitting infectious agents. All inoculated culture bottles, specimen collection needles, and blood drawing devices should be decontaminated according to country requirement for waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment.

For further information, refer to the Blood Culture medium

material safety data sheet.

# MEDIA PREPARATION, STORAGE AND STABILITY

Blood Culture media are ready to use and stable until expiration date stated on label when properly stored 10-30°C.

## Deterioration

The Blood Culture medium is clear liquid, media should not be used if there is signs of deterioration (turbidity or discoloration), and contaminations.

# SPECIMEN COLLECTION AND PRESERVATION

## Blood

Take care to prevent contamination during both bottle preparation and inoculation of the patient sample. Proper skin disinfection is an essential requirement to reduce the incidence of contamination. Although not recommended, blood may be drawn directly into collection tubes containing SPS. Tubes containing other anticoagulants should never be used for blood culture. Note: for patient preparation follow Medical laboratory instruction

# EQUIPMENT REQUIRED NOT PROVIDED

* Sterile syringe or other means of obtaining blood.
* Alcohol solutions, or other suitable skin disinfection material.
* Incubator equipment to maintain 36 ± 1°C.
* Orbital shaker (for optimal results Chemical or physical indications of instability

# PROCEDURE

## Inoculation Procedure

1. Examine the bottle of broth before taking the blood sample and discard it if any evidence of contamination can be seen.
2. Prepare the bottle for inoculation before taking the blood sample by removing the green plastic `flip-off’ cap and disinfect the exposed part of the rubber stopper.
3. Aseptically inject a blood volume of 8-10 ml for adult patients, 2-3 ml for pediatrics and 1-2 ml for neonate patients through the central ring of the rubber stopper.
4. Thoroughly mix the blood with the broth in the bottle.
5. Identify the bottle by writing patient’s information on the label, and Immediately transfer the inoculated blood culture bottle to the laboratory..

## Laboratory Procedure

1. Incubate the inoculated bottle at 36±1°C for approximately 1 hour.
2. Remove from the incubator and place the bottle in an incubation tray.
3. Disinfect the rubber stopper of the bottle by swabbing, e.g. with alcohol.
4. Remove the growth indicator device from its sterile package and ensure that the needle and cap are fully tightened. (Hold the clear plastic body of the device with the covered needle pointing downwards. Tighten the needle by turning the needle cover anticlockwise. Tighten the cap by turning it clockwise.)
5. Slide the plastic shield from the needle. Do not touch the needle.
6. Aseptically insert the needle through the center of the rubber stopper. Push the needle shaft as far as it will go through the rubber stopper.
7. Slide the green locking sleeve of the growth indicator device downwards until it fully locks on to the neck of the blood culture bottle. Press down the chamber to ensure full contact with the rubber seal of the bottle.
8. It is recommended to use shaker/incubator at 150 orbits/minute, or a bench top integrated, at 36±1°C. Otherwise manual shaking in specified interval, at least 4 times during first 24 hours.
9. At the end of the 24 hour period, remove the system from the shaking apparatus and place on the shelf of an incubator preset at 36±1°C.
10. Examine the system on the incubator shelf twice daily for 7 days, consider vigorously agitation if negative results in order to resuspend the erythrocytes. For positive result, remove for further examination.
11. Positives results: Mix the contents of the chamber, unscrew the green cap and aseptically remove a sample of blood/broth mixture for subculture, microscopy and susceptibility testing. The vent in the cap contains a 0.2 micron hydrophobic membrane which ensures that the chamber is not under pressure. After sampling replace the cap on the chamber.

# QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control values are found outside the defined range, check the system performance. If control still out of range please contact the technical support.

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# PERFORMANCE CHARACTERISTICS

The following organisms are used by us as part of the quality assurance of the product. The total inoculum challenge for each test organism per bottle is 10 to 50 colony forming units (CFU’s).

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| **Test Organisms** | **ATCC ® Number** |
| Bacillus cereus | 10876 |
| Bacteroides fragilis | 25285 |
| Clostridium perfringens | 13124 |
| Escherichia coli | 25922 |
| Fusobacterium nucleatum | 10953 |
| Haemophilus influenzae | 19418 |
| Klebsiella pneumoniae | 29665 |
| Neisseria meningitidis | 13077 |
| Peptostreptococcus anaerobius | 27337 |
| Staphylococcus aureus | 25923 |
| Streptococcus pneumoniae | 6303 |
| Candida albicans | 10231 |

# REFERENCES

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2. Hinder S. M., Sawhney D. and Swaine D. 2nd European Congress of Clinical Microbiology 1985, Abstract 12/2.
3. King A., Bone G. and Phillips I. 2nd European Congress of Clinical Microbiology 1985, Abstract 12/4.
4. King A., Bone G. and Phillips I. (1986) J. Clin. Pathol. 39. 661665.
5. Sawhney D., Hinder S., Swaine D. and Bridson E. Y. (1986) J.

Clin. Pathol. 39. 1259-1263.

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| **SYMBOLS IN PRODUCT LABELLING**  |
|   IVD For in-vitro diagnostic use  |   Number of <n> test in the pack  |
|  LOT Batch Code/Lot number  |  Caution  |
|  REF Catalogue Number  | Do not use if package is damaged   |
|   Temperature Limitation   Expiration Date   Manufactured by  |  Consult Instruction for use      |