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Microbiological Assay

REF M0602

20 tests

Anaerobic Blood Culture Bottle

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Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		date of manufacture

EC REP

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CE IVD

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Contact your local dealers for all product related questions in your local language

Intended use

Ready to use culture medium based assay for the qualitative detection of microorganisms in body fluids like blood, ascites, cerebrospinal fluid[1,2] etc, offering isolated strains to carry out susceptibility tests.
This assay is for professional use only.

Summary

Blood culture is one of the most important and critical procedures performed in the microbiology laboratory. Since blood is normally sterile, the isolation and identification of an organism has great diagnostic significance. Blood culture is of great importance in diagnosing such conditions as endocarditis, typhoid fever, pneumonia and other diseases characterized by bacteremia. The growth of microorganisms in a blood culture may be delayed or prevented if an anticoagulant is not used in the culture medium since the organisms may become trapped in the fibrin clot. However, some anticoagulants may be toxic for certain pathogens. In addition, many blood samples contain residual antibiotics, antibodies, ß-lysin and phagocytes which are natural bacterial inhibitors and greatly reduce chances of obtaining a positive culture. These obstacles may be overcome by the use of SPS (sodium polyanetholsulfonate), a nontoxic anticoagulant which enables bacterial growth by counteracting or absorbing those natural bacterial inhibitors in blood. Since SPS inhibits the activity of streptomycin, polymyxin B, kanamycin and gentamicin, therapy with these antibiotics should not interfere with microbial growth in blood cultures containing this anticoagulant.

Measurement Principle

All Blood Culture media will support the growth of a wide variety of clinically important pathogenic microorganisms, including fastidious organisms. There are hemin (x factor) and nicotinamide adenine dinucleotide (v factor) which can sustain *Haemophilu*[3], *Actinobacillus* and *Cardiobacterium* growing, pyridoxine HCl which is absolutely vital to *Streptococcus* depending on Vitamin B6, SPS which can counteract antimicrobial activity of residual antibiotics and immunity factors[4].

Components

1. 20 vials each containing 65 mL of culture broth in liquid state which is formulated by adding to each 977mL of purified water, 9.77 g of casein peptone, 4.89 g of sodium chloride, 2.93 g of dextrose, 0.98 g of L-Arginine, 0.24 g of SPS, 0.29 g of sodium bicarbonate, 1.564 g of Tris aminomethane, 1.954 g of cysteine hydrochloride, 4.4 g of yeast extract, 0.7mg Vit.K3 5.848mg of hemin, 58.48mg of NAD (nicotinamide adenine dinucleotide) The pH is 7.5.
2. 1 copy of instruction for use

Materials Required but not Provided

1. Incubator (35-37℃)
2. Butterfly blood collection set

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.
3. Consider all samples and their culturing wastes as potentially infectious materials. Dispose of waste material safely according to relevant local and national requirements.
4. Wear disposable gloves when dealing with samples and reagents. Wash hands after operations.
5. Conduct the assay away from bad ambient conditions. e.g. ambient air containing strong acid, strong alkali or volatile gas and so on.
6. Do not use bottles which show signs of contamination (risk of contamination to the patient in the event of reflux into the vein during sample collection).
7. Blood anticoagulant has already been applied in the culture bottle, so do not add more.
8. In order to detect septicemia with sufficient accuracy, it may be necessary to carry out 1 to 3 blood cultures at designated time intervals, depending on the clinical situations.
9. It is recommended that perform the collection of blood cultures at intervals and obtain samples at the first sign of fever.
10. When the sample needs to be inoculated into both Bi-state Blood Culture Bottle and Anaerobic Blood Culture Bottle, inoculate the anaerobic bottle before the bi-state one.
11. Do not use the reagents beyond the labeled expiry date.
12. Positive culture bottles for subculturing or staining etc.: before sampling it is necessary to release gas which often builds up due to microbial metabolism. Sampling should be performed in a biological safety cabinet. And appropriate protective clothing, including gloves and masks, should be worn.
13. To minimize the potential of leakage during inoculation of sample into culture bottles, use syringes with permanently attached needles or leakproof tips.
14. The sample must be collected using sterile techniques to minimize the chance of contamination.
15. Because blood can neutralize the toxicity of SPS towards organisms sensitive to SPS (such as P. anaerobius), the presence of optimum volumes of blood (5 – 10 mL) is a benefit in the recovery of these organisms.
16. The negative pressure in the blood culture bottle may vary in different regions. If the blood collection needle cannot be used, a syringe is required to draw blood.

17. Due to the presence of chemical additives in the culture bottle, it is important to prevent possible backflow and subsequent adverse reactions by following all steps below.

a) Hold the culture bottle at a position below the patient's arm with the bottle in an upright position (stopper uppermost).

b) Release the tourniquet as soon as the blood starts to flow into the culture bottle, or within 2 minutes of application.

c) Do not allow the culture bottle contents to touch the stopper or the end of the needle during the collection procedure.

18. Perform properly disinfection of skin and rubber plug to prevent contamination. Example of common skin contaminants are coagulase-negative *Staphylococcus species*, *Corynebacterium* species and *Cutibacterium acnes*[5].
19. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Storage

1. Store all components at 2-25℃. Store the Culture Bottles in an upright position protected from direct sunlight.
2. The Anaerobic Blood Culture Bottle may be used throughout the expiry date. Refer to the package label for the expiry date.

Reagent Preparation

Bring all reagents to room temperature (18-25℃) prior to use.

Sample Collection

1. Collect approximately 3-5 mL of adult patients' blood per bottle with a needle and syringe or 1-3 mL of infant patients' blood.
2. Collect samples prior to initiating antibiotic therapy. If this is not possible, draw the blood immediately before administering the next dose.
3. Collect samples before meals, since hyperlipemia may obscure visible evidence of bacterium growth in the liquid medium.
4. Since bacteremia is intermittent, collect samples at the proper time.
5. Test the samples immediately once received.

Note: Take care to prevent contamination during both bottle preparation and inoculation of the patient samples. Proper skin disinfection is an essential requirement to reduce the incidence of contamination.

Sample Processing

1. Test the samples immediately once received.
2. Prepare and label the appropriate blood culture bottle.
3. Do not unscrew cap. Remove the plastic top of the screw cap on the blood culture bottle.
4. Disinfect the visible part of the rubber plug with ethyl alcohol (75%) and allow drying.
5. Inject blood, ascites or cerebrospinal fluid into the culture bottle after being collected with aseptic operations. Then disinfect the rubber plug again and replace the plastic top.
6. After the inoculation, lean the bottle several times, and incubate it erectly at 35-37 ℃ for 24 hours.
7. If the blood culture bottle is not processed directly in the laboratory, it should be stored at room temperature (18-25 ℃) and never in cold chain (2-8 ℃).

Control Procedure

1. Positive Control

Test 100 μL of culture medium containing 1.5× 10^3 CFU/mL *Clostridium sporogenes* (CMCC (B)® 64941). The result is valid if evident turbidity appears.

Measurement Results

1. Report the positive results if turbidity in the culture broth, hemolysis of blood corpuscles, air bubbles or colonies is observed. Carry out susceptibility experiments and strain identification on positive samples.
2. Keep on incubating the bottle erectly if those phenomena do not occur, until the 7^{th} day.
3. If the abovementioned phenomena still do not occur after 7 days of culturing, report a negative result.

Limit of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. Some pathogenic bacteria, may have already grown in the bottles but do not appear so. In this case, the physician should pick some of the culture broth each day and streak on an anaerobic medium, then place into an anaerobic environment incubator calibrated at 35–37℃, until the 7^{th} day.
3. A gram-stained smear from culture medium may contain small numbers of nonviable organisms derived from media constituents, staining reagents, immersion oil, glass slides, and samples used for inoculation. In addition, the patient sample may contain organisms that will not grow in the culture medium or in medium used for subculture. Such media should be subcultured into special media as appropriate.

Performance Characteristics

1. Analytical Sensitivity

A panel member (100 μL of culture medium containing 1.5× 10^3CFU/mL *Clostridium sporogenes* (CMCC (B)® 64941)) was tested. Evident turbidity was observed.

2. Measurement Accuracy by Correlation

A study was performed where samples were tested using this assay and the bioMérieux ® assay. Data were analyzed and are summarized in the following table.

Product	Positive	Negative	Positive rate
This assay	12	190	5.9%
bioMérieux	13	189	6.4%

The agreement is 92.3%. In x^2 method, P > 0.05, there is no obvious difference between the 2 methods.

Literature References

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