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Microbiological Assay

REF **MC0303**

20 tests

Aerobic Culture Bottle PF

Aerobic Culture Bottle PF is used with Automated Blood Culture System for the qualitative recovery and detection of aerobic and facultative anaerobic microorganisms from blood and other normally sterile body fluids.

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
LATEX	Latex-free		Do not reuse
EC REP	Authorized representative in the European Community		Keep away from sunlight

EC REP

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CE IVD

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Introduction

Blood is normally a sterile environment until it was infected by the bacteria [1]. Bacteria and fungi can enter the bloodstream as a severe complication of infectious (like pneumonia or meningitis), during surgery or due to catheters and other foreign bodies entering the arteries or veins. It is called bacteremia/fungemia when there is presence of bacteria/fungi in the blood.

Bacteremia can have several important health consequences. The immune response to the bacteria/fungi can cause sepsis and septic shock, which has a high mortality rate [2]. Bacteria can also spread via the blood to other parts of the body (which is called hematogenous spread), causing infections away from the original site of infection, such as endocarditis or osteomyelitis [3].

Blood culture is a microbiological culture of blood to detect infections that are spreading through the bloodstream [4]. The specimens should be collected from independent venipuncture sites from infants, children or adult and are entered into blood culture bottles through an incubation protocol on a continuously monitored blood culture device [5]. The blood culture bottles are incubated for a prescribed period of time and signal audibly and/or visually if growth is detected [6].

Measurement Principle

The **Automated Blood Culture System** utilizes a colorimetric sensor to monitor the presence and production of carbon dioxide (CO_2) dissolved in the culture medium. If microorganisms (bacteria and fungi) are present in the test sample, carbon dioxide is produced as the organisms metabolize the substrates in the culture medium. When growth of the microorganisms (bacteria and fungi) produces CO_2, the color of the gas-permeable sensor installed in the bottom of each culture bottle changes from blue-green to yellow. The lighter color results in an increase of reflectance units monitored by the system. Bottle reflectance is monitored and recorded by the instrument every 10 minutes.

Components

1. Blood Culture Bottle

The Aerobic Culture Bottle PF disposable culture bottle contains 20ml of media and an internal sensor that detects carbon dioxide as an indicator of microbial growth. Ingredients of media are as follows:

Ingredient	Percentage
Tryptone	1% w/v
Gelatin Peptone	1% w/v
Yeast Extract	0.45% w/v
Glucose	0.3% w/v
Sodium Polyanethol sulfonate	0.025% w/v
Resin	0.5% w/v

And other amino acids as components. Bottles are prepared with an atmosphere of CO_2 in oxygen under vacuum. The composition of the media may be adjusted to meet specific performance requirements.

2. 1 copy of instruction for use

Materials Required but not Provided

1. Blood drawing apparatus
2. Subculture units
3. Smear units
4. Disposable gloves

Instrumentation

BC120 Automated Blood Culture System. Review the appropriate BC120 User Manual before use.

Warnings and Precautions

1. For professional use only. Cannot be reused.
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. Wear protective clothing and disposable gloves when dealing with strips. Wash hands after operations. Handle the potentially contaminated materials safely according to local requirement.
4. Standard guidelines for the safe handling and disposal of infectious organisms should be observed throughout all procedures.
5. Do not smoke, drink, eat or use cosmetics in the working area.
6. Avoid contamination during both bottle preparation and inoculation of the sample. Proper skin disinfection is a critical requirement to reduce the incidence of contamination.
7. The inoculated culture bottles should be placed into the **Automated Blood Culture System** as soon as possible. Inoculated culture bottles delayed in entry should be maintained at ambient temperature until they can be loaded into the instrument.
8. Fastidious microorganisms (bacteria and fungi) will not grow or may grow slowly in the **Aerobic Culture Bottle PF** growth medium, which are rare, requiring alternative methods or extended incubation time for recovery.
9. In rare occasions that the microorganisms (bacteria and fungi) may not produce sufficient carbon dioxide to be determined positive.
10. Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
11. General caution should be taken when subculturing positive culture bottles as they could contain increased internal pressure which is caused by gas produced during microbial metabolism. Therefore, positive culture bottle should be transiently vented before subculturing or disposal.
12. Decontaminate the dispose of bottles and potentially contaminated materials as if they were infectious waste, in a biohazard container.

Storage

1. Store culture bottle at ambient temperature (2-25℃) and protected from direct sunlight.
2. The culture bottle is stable throughout the expiration date printed on each bottle. It cannot be used beyond the expiration date.

Prior to Use

1. The **Aerobic Culture Bottle PF** should be examined for evident damage, deterioration (discoloration), excessive cloudiness or bulging. Culture bottles exhibiting with mentioned defects should be discarded. The media should be clear, but there may be a slight opalescence or a trace of precipitate due to the anticoagulant SPS. Do not confuse opalescence with turbidity. Do not use a culture bottle which contains media exhibiting turbidity, a yellow sensor, or excess gas pressure.
2. Mark the culture bottle with patient information. The icons on the bottle label can be defined by the user.
3. Remove the flip-off cap from the culture bottle and inspect it for damage, deterioration, excessive cloudiness or bulging.
4. Before inoculating, swab the culture bottle top with alcohol and leave to air dry.

5. Aseptically inject or draw directly 1-5ml of sample per culture bottle. **Aerobic Culture Bottle PF** should be placed in the **BC120** as soon as possible, if placement of an inoculated culture bottle into the instrument has been delayed and visible growth is apparent, it should not be tested in the instrument, instead, it should be subcultured, smeared and treated as a presumptively positive culture bottle.

Specimen Collection

1. Collect specimens in accordance with correct medical practices.
2. Prevent contamination during both bottle preparation and inoculation of the sample. Proper skin disinfection and sterile techniques are essential requirements to reduce the chance of contamination.
3. The typical specimen volume is 1-5ml.
4. If using a needle and tubing set, carefully observe the direction of blood flow when starting collection.
5. The user should monitor the volume when performing direct draw collection of blood from young children, infants, and neonates where total blood volume is a concern.
6. When the desired 1-5ml has been drawn, the flow should be terminated by crimping the tubing and removing the tubing set from the culture bottle.
7. Volumes as low as 1ml can be used, however, recovery will not be as great as with larger volumes.

Direct Draw Inoculation Procedure

a) Hold the culture bottle at a position below the patient's arm with the bottle in an upright position.
b) Collect the blood by using a butterfly blood collection set and inoculate directly into the culture bottle.
c) Release the tourniquet as soon as the blood starts to flow into the culture bottle, or within 2 minutes of application.
d) If inoculating more than one type of culture bottle, first inoculate the aerobic culture bottle and then the anaerobic culture bottle to avoid any oxygen trapped in the tubing transferring to the anaerobic bottle.

Syringe Draw Inoculation Procedure

a) Perform venipuncture and blood transfer to the culture bottle according to the established procedures.
b) To minimize the potential of leakage during inoculation of sample into culture bottles, use syringes with permanently attached needles.
c) If inoculating more than one type of culture bottle, first inoculate the anaerobic culture bottle and then the aerobic culture bottle to avoid any oxygen trapped in the syringe transferring to the anaerobic bottle.

8. Transfer the inoculated culture bottle immediately to the testing laboratory.

Measurement Procedure

1. Culture bottles entered into the instrument will be automatically tested every 10 minutes for the duration of the testing protocol period.
2. Positive culture bottle will be determined by **BC120** instrument.
3. The sensor inside the bottle will not have evidently visible differences in positive and negative culture bottle; the **Automated Blood Culture System** can determine a difference. (See **BC120** User's manual).
4. After culture bottles have been loaded into the instrument, they should remain there for 5-7 days or until designated positive.
5. Procedures for loading and unloading culture bottles into the instrument are given in the instrument User Manual.

Result

Positive or negative culture bottles are determined by decision-making software contained in the **Automated Blood Culture System.** No action is required until the instrument signals positive or negative culture bottles.

Interpretation of Result

1. Positive culture bottle should be subcultured and smeared.
2. Negative culture bottle is recommended to be checked by smear and/or subculture at some point prior to discarding as negative.
3. If the instrument signals positive, yet smear is negative, indicating a possible false positive, the culture bottle should be reloaded into the instrument until growth of subculture or re-designation as positive. Cultures which were initially determined false positive and were re-designated positive should be smeared and subcultured.
4. If at the end of the testing period a negative **Aerobic Culture Bottle PF** appears visually positive, it should be subcultured, smeared and treated as presumptively positive.

Subculturing:

1. Before sampling it is necessary to release gas which often builds up due to microbial metabolism.
2. Sampling should be performed in a biological safety cabinet if possible, and appropriate protective clothing, including gloves and masks, should be worn.
3. Prior to sampling, put the culture bottle upright position and disinfect the top with alcohol swab or equivalent. Allow to air dry.
4. Insert a sterile needle through the culture bottle top after being disinfected.
5. The needle should be removed after the pressure is released and before sampling.
6. The insertion and withdrawal of the needle should be done in a straight-line motion, avoiding any twisting motions.
7. For maximum yield of isolated, negative culture bottles are recommended checked by smear and/or subcultured at some point to discarding as negative.

Control Procedure

A Certificate of Analysis is provided with each box of culture bottles. If desired, quality control testing can be performed on **Aerobic Culture Bottle PF**, referring to the CLSI® document M22-A3 [7].

Performance Characteristics

The operation was performed as in **Measurement Procedure** on the following ATCC® and CMCC® strains at levels of 1000 CFU/bottle, which were detected as positive in culture bottle within 72 hours.

Standard Strains
Micrococcus Luteus CMCC(B)28001
Streptococcus Pneumoniae ATCC 6305
Pseudomonas Aeruginosa ATCC 27853
Alcaligenes faecalis ATCC 8750
Streptococcus Pyogenes ATCC 19615
Candida Albicans ATCC 18804
Haemophilus Influenzae ATCC 19418
Escherichia coli ATCC 25922
Staphylococcus Aureus ATCC 25923
Neisseria meningitides ATCC 13090

Limitations of the Procedure

1. Contamination should be prevented during venipuncture and inoculation in the culture bottle, which could lead to a positive result when a clinically relevant isolate is not actually present.
2. Use of lower or higher volumes may adversely affect recovery and/or detection times. 1-5ml specimen volume is recommended. Volumes greater than 5ml do not fit the optimal ratio to medium.
3. Blood may contain antimicrobials or other inhibitors which may slow or prevent the growth of microorganisms (bacteria and fungi). In such case, to obtain blood specimens prior to initiating antibiotic therapy is necessary.
4. Visually inspect for indications of microbial growth, especially when the culture bottle have been delayed loaded or incubated prior to entry into the instrument, treat the culture bottles as positive and do not place in the system for monitoring.
5. Some strains maybe sensitive to the anticoagulant SPS contained in the medium, such as *Haemophilus influenzae*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, and *Peptostreptococcus anaerobius*, resulting in lack of growth or low production of CO_2 to trigger the sensor. Hence, sufficient amount of blood sample is inoculated into the culture bottles is a key factor to the result.
6. Infrequently, positives may occur due to a very high number of white blood cells being present in the blood sample.
7. Organisms are often few in numbers and may appear intermittently in the blood stream; therefore, several consecutive blood specimens should be collected from each patient.
8. If organisms that are positive by smear that will not grow on routine subculturing media, specimens should be accordingly subcultured on special media. Also, positive specimens may contain organisms that are not seen with routine smear methods and may require both specialized smears and subculturing media for detection and recovery.
9. Certain strains of *Streptococcus pneumonia* may be particularly prone to autolysis if they are not removed promptly after being signaled positive.
10. A Gram-stained smear from a negative bottle may sometimes contain a small number of non-viable organisms that were derived from culture medium components, staining reagents, immersion oil or glass slides, therefore, false-positive results are indicated.

Literature References

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