

# Microbiological Assay











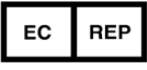

**REF** MC0301



20 tests

## Aerobic Culture Bottle FA

*Aerobic Culture Bottle FA 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.*

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Key to Graphical Symbols Used			
	batch code		use by
	manufacturer		contains sufficient for <n> tests
	<i>In vitro</i> diagnostic medical device		temperature limitation
	catalogue number		consult instructions for use
	latex-free		do not reuse
	authorized representative in the European Community		keep away from sunlight

	OBELIS S.A. Bd. Général Wahis, 53 1030 Brussels Belgium
	AUTOBIO DIAGNOSTICS CO., LTD. No.87 Jingbei Yi Road National Eco & Tech Development Area Zhengzhou China 450016



For any technical assistance please contact us in English at:

Email: [customerservice@autobio.com.cn](mailto:customerservice@autobio.com.cn)

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

## Introduction

Blood is normally a sterile environment until it was infected by the bacteria<sup>[1]</sup>. 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/fungemia 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<sup>[2]</sup>. Bacteria/fungi 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<sup>[3]</sup>.

Blood culture is a microbiological culture of blood to detect infections that are spreading through the bloodstream<sup>[4]</sup>. 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<sup>[5]</sup>. The blood culture bottles are incubated for a prescribed period of time and signal audibly and/or visually if growth is detected<sup>[6]</sup>.

## Measurement Principle

The **Automated Blood Culture System** utilizes a colorimetric sensor to monitor the presence and production of carbon dioxide (CO<sub>2</sub>) 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<sub>2</sub> and other acid gases, 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

20 vials Aerobic Culture Bottle FA each containing 30mL of culture broth. The culture broth is formulated by Tryptone, Gelatin Peptone, Yeast Extract, sodium chloride, Sodium Polyanethol Sulfonate, Sodium pyruvate, Hemin, Nicotinamide adenine dinucleotide, Resin, assay buffer and other Multivitamins, amino acids and carbohydrates. The culture bottles contain a vacuum environment with CO<sub>2</sub> and oxygen. 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 culture bottles. 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 FA** 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. A possible factor that may lead to this situation is the presence of active antibiotics in a sample.
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°C) 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 FA** 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 leave to air dry.
5. Aseptically inject or draw directly 8-10ml of sample per culture bottle. **Aerobic Culture Bottle FA** 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 (according to local regulations, CLSI or Cumitech 1C) are essential requirements to reduce the chance of contamination.
3. The typical specimen volume is 8-10ml.
4. If using a needle and tubing set, carefully observe the direction of blood flow when starting collection.
5. The vacuum in the culture bottle will usually exceed 10ml; the user should monitor the volume collected by means of the 5ml graduation marks on the bottle label.
6. When the desired 8-10ml has been drawn, the flow should be terminated by crimping the tubing and removing the tubing set from the culture bottle.
7. Generally, culturing higher volumes of blood containing small numbers of bacteria will improve recovery of bacteria by culture.

## 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. The **Automated Blood Culture System** can determine the color difference in the bottles. And positive culture bottle will be determined by BC120 instrument (See BC120 User's manual).
3. After culture bottles have been loaded into the instrument, they should remain there for 5-7 days or until designated positive.
4. 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 FA** 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 available for each lot of culture bottles. If desired, quality control testing can be performed on **Aerobic Culture Bottle FA**, referring to the CLSI® document M22-A3<sup>[7]</sup>.

## Performance Characteristics

### 1. Quality Control

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

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### **Standard Strains**

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*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 meningitidis* ATCC 13090

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### 2. Performance Comparison

A study was performed where samples at the level of 5 CFU/ bottle were tested using this assay, marked assay A and marked assay B. Data were analyzed and are summarized in the following table.

Strains	Average Time to detect(hours)	
	Autobio	Marked assay A
		Marked assay B

<i>Staphylococcus aureus</i>	16	15.96	13.93
<i>Staphylococcus epidermidis</i>	24.12	32.04	20.57
<i>Pseudomonas aeruginosa</i>	17.44	19.92	18.63
<i>Acinetobacter baumannii</i>	11.52	15.84	11.27
<i>Escherichia coli</i>	12.72	15.96	14.18
<i>Klebsiella pneumoniae</i>	12.88	16.92	14.3
<i>Enterobacter cloacae</i>	13.44	15.24	14.62
<i>Enterococcus faecium</i>	12.4	15.72	11.81
<i>Proteus mirabilis</i>	13.92	14.76	14.93
<i>Streptococcus agalactiae</i>	9.92	12.72	9.51
<i>Streptococcus pneumoniae</i>	24.32	16.32	11.88
<i>Streptococcus sanguis</i>	18.56	23.28	21.77
<i>Streptococcus pyogenes</i>	14.28	N	12.88
<i>Serratia marcescens</i>	11.2	13.8	11.58
<i>Citrobacter freundii</i>	11.2	14.64	11.84
<i>Haemophilus influenza</i>	14.08	20.4	15.1
<i>Haemophilus parainfluenzae</i>	33.76	N	61.91
<i>Eikenella corrodens</i>	25.68	37.92	28.98
<i>Alcaligenes faecalis</i>	24.64	26.28	22.8
<i>Candida albicans</i>	29.84	28.44	30.71
<i>Candida glabrata</i>	36.32	66.36	50.56

Note: N means no positive result.

### 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. 10ml specimen volume is recommended. Volumes greater than 10ml do not fit the optimal ratio to medium, may result in false positive.
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*, resulting in lack of growth or low production of CO<sub>2</sub> 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 pneumoniae* 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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