DENOTES REVISED SECTION
See symbol glossary at end of insert.

INTENDED USE
BacT/ALERT® FA Culture Bottles are used with the BacT/ALERT® Microbial Detection System in qualitative procedures for enhanced recovery and detection of aerobic and facultative anaerobic microorganisms (bacteria and fungi) from blood and other normally sterile body fluids.

SUMMARY AND EXPLANATION
The BacT/ALERT Microbial Detection System 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. The BacT/ALERT System and 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. An inoculated bottle is placed into the instrument where it is incubated and continuously monitored for the presence of microorganisms that will grow in the BacT/ALERT FA bottle.

PRINCIPLE OF THE TEST
The BacT/ALERT Microbial Detection System utilizes a colorimetric sensor and reflected light to monitor the presence and production of carbon dioxide (CO₂) dissolved in the culture medium. If microorganisms are present in the test sample, CO₂ is produced as the organisms metabolize the substrates in the culture medium. When growth of the microorganisms produces CO₂, the color of the gas-permeable sensor installed in the bottom of each culture bottle changes from blue-green to yellow.¹ 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.

REAGENTS
For in vitro diagnostic use.

CAUTION: 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 your institution’s procedures.²

BacT/ALERT® FA (color-coded pale green) – BacT/ALERT FA disposable culture bottles contain 22ml of complex media and 8ml of a charcoal suspension with an average density of 1.0155 g/ml. The media component consists of soybean-casein digest (2.0% w/v), brain heart infusion solids (0.1% w/v), sodium polyanethol sulfonate (SPS) (0.05% w/v), pyridoxine HCI (0.001% w/v), menadione (0.000725% w/v), hemin (0.000725% w/v), L-cysteine (0.03% w/v) and other complex amino acid and carbohydrate substrates in purified water. Bottles contain an atmosphere of CO₂ in oxygen under vacuum. The composition of the media may be adjusted to meet specific performance requirements.

CAUTION: BacT/ALERT culture bottles contain polycarbonate. Not all disinfectants are intended for use with polycarbonate surfaces, and may cause bottle deterioration. Verify disinfectant compatibility with polycarbonate before use on BacT/ALERT culture bottle surfaces.

CAUTION: It is possible that certain rare, fastidious microorganisms will not grow or may grow slowly in the BacT/ALERT FA Culture Bottle growth media. If rare, fastidious organisms requiring specialized media and culture conditions are suspected, alternative methods or extended incubation time, should be considered for recovery.

CAUTION: BacT/ALERT FA Culture Bottles used to culture non-blood specimens (normally sterile body fluids) may require added blood or other supplements, such as sterile, defibrinated horse blood (5% v/v) to support growth, particularly for the recovery of fastidious organisms such as Haemophilus influenzae, Streptococcus pneumoniae, and Neisseria gonorrhoeae.³

CAUTION: On rare occasions, organisms may be encountered that grow in the BacT/ALERT FA Culture Bottle growth media but do not produce sufficient CO₂ to be determined positive. A factor that may lead to this situation is the presence of active antibiotics in a sample.

Additional materials required
BacT/ALERT® Microbial Detection Systems
Blood drawing device
Sterile Airway Needles/Subculture Units
Disposable gloves
Appropriate biohazard waste containers for materials potentially contaminated with infectious agents.

Materials available from bioMérieux
BacT/ALERT® Blood Collection Adapter Cap
BacT/ALERT® Microbial Detection Systems
Sterile Airway Needles/Subculture Units
**Storage instructions**

BacT/ALERT FA Culture Bottles are ready for use. Store protected from direct sunlight at room temperature (15-30°C). An expiration date is printed on each bottle label. Do not use the culture bottles beyond the expiration date indicated. If the bottles are exposed to temperatures less than 15°C, precipitates may form that will disappear when the bottles are warmed to room temperature. Bottles must be at room temperature before use.

**Chemical or physical indications of instability**

Prior to use, the BacT/ALERT FA Culture Bottles should be examined for evidence of damage or deterioration (discoloration). Bottles exhibiting evidence of damage, leakage, or deterioration should be discarded. The media in undisturbed bottles 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 slight opalescence or a trace of precipitate due to the anticoagulant SPS. Do not use a bottle which contains media exhibiting turbidity, a yellow sensor, or excess gas pressure; these are signs of possible contamination.

**INSTRUMENTS**

Review the appropriate BacT/ALERT Microbial Detection System User Manual before use.

**SPECIMEN COLLECTION AND PREPARATION**

**NOTE:** BacT/ALERT FA Culture Bottles should be utilized by trained healthcare personnel. Correct specimen collection is extremely important when obtaining blood culture specimens. Refer to Cumitech 1C5 for the proper specimen collection procedure.

**NOTE:** 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.

**NOTE:** Although not recommended by bioMérieux, blood may be drawn directly into collection tubes containing SPS. Tubes containing other anticoagulants should never be used for blood culture.

**NOTE:** bioMérieux recommends that inoculated culture bottles be placed into the BacT/ALERT Microbial Detection System as soon as possible after collection. Inoculated culture bottles delayed in entry should be maintained at room temperature until they can be loaded into the instrument.

**Bottle preparation**

1. Label the culture bottle with patient information. The icons on the bottle label (®, #, ☀️) can be defined by the user.
2. Remove plastic flip-top from culture bottle. Prior to inoculation, disinfect the culture bottle top with an alcohol swab or equivalent. Allow to air dry.
3. Clean the selected venipuncture site as recommended by your institution's approved procedure.

**Direct draw inoculation procedure**

**NOTE:** If inoculating more than one type of BacT/ALERT blood culture bottle using a butterfly blood collection set and direct draw adapter cap, inoculate first the aerobic culture bottle and then the anaerobic culture bottle so that any oxygen trapped in the tubing will not be transferred to the anaerobic bottle.

**NOTE:** Monitor the direct draw process closely at all times during collection to assure proper flow is obtained and to avoid flow of the bottle contents into the adapter tubing. 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. Collect the blood using a butterfly blood collection set and the BacT/ALERT Blood Collection Adapter Cap as recommended by your institution's approved procedure and inoculate directly into the culture bottle at the patient's bedside. Although lower sample volumes can be used, recovery may be improved using a sample volume closer to the recommended 10ml. To prevent over inoculation, monitor the blood volume intake into the culture bottle, using the 5ml incremental markings on the bottle label.

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

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

A contaminated culture bottle could contain positive pressure, and if used for direct draw, may cause reflux into the patient's vein. Culture bottle contamination may not be readily apparent. Monitor the direct draw process closely to avoid reflux. Do not use a bottle that contains media exhibiting turbidity, a yellow sensor, or excess gas pressure; these are signs of possible contamination.

**Syringe draw inoculation procedure**

**NOTE:** If inoculating more than one type of BacT/ALERT blood culture bottle using syringe draw, inoculate first the anaerobic culture bottle and then the aerobic culture bottle so that any oxygen trapped in the syringe will not be transferred to the anaerobic bottle. Line demarcations on the bottle label should be used to assist in estimating the sample volume.

a. Perform venipuncture and blood transfer to the BacT/ALERT culture bottle according to your institution's established procedures.

**CAUTION:** Never force the syringe plunger down during inoculation, as splashing of sample may occur. Remove the syringe when the fill amount is reached, as the vacuum automatically will draw more than the recommended maximum. Puncture the bottle stopper vertically to avoid releasing the vacuum; a bottle without a vacuum should not be inoculated.

4. Transfer the inoculated culture bottle promptly to the testing laboratory.
**BacT/ALERT FA CULTURE BOTTLE TEST**

**PROCEDURE**

**Preliminary comments and precautions**

1. Use disposable gloves and handle inoculated bottles cautiously as though capable of transmitting infectious agents. Consult a physician immediately if contaminated materials are ingested or come in contact with open lacerations, lesions, or other breaks in skin.

2. Immediately clean up any spillage of contaminated material using a 1:10 dilution of 5% sodium hypochlorite. Dispose of the cleaning material by an acceptable method.

3. All inoculated culture bottles, specimen collection needles, and blood-drawing devices should be decontaminated according to your institution’s procedures.

4. These bottles should be utilized by trained laboratory personnel.

**Procedural notes and precautions**

1. Great care must be taken to prevent contamination of the patient sample during venipuncture and during inoculation into the culture bottle since contamination could lead to a specimen being determined positive when a clinically relevant isolate is not actually present.

2. Obtain blood samples prior to initiating antibiotic therapy. If this is not possible, draw blood immediately before administering the next antibiotic dose.

3. If inoculated culture bottles have been delayed in their receipt into the laboratory or have been incubated prior to entry into the BacT/ALERT instrument, visually inspect for indications of microbial growth. If microbial growth is evident, treat the bottles as positive and do not place in the BacT/ALERT Microbial Detection System for monitoring.

**Laboratory procedure**

**CAUTION:** General caution should be taken when subculturing positive culture bottles as they could have been overfilled or contain high gas-producing organisms. Positive culture bottle contents may be under increased internal pressure. Positive culture bottles should be transiently vented before staining or disposal to release any gas produced during microbial metabolism.

1. Visually inspect bottles before testing. Do not use bottles with evidence of damage, leakage, or deterioration. Consider bottles with hemolysis, turbidity, excess gas pressure, yellow sensors, and/or evidence of growth as positive. Smear and subculture. Do not incubate unless smear is negative.

2. bioMérieux strongly recommends all bottles be scanned prior to loading into the BacT/ALERT instrument. Scanning the bottle barcode assures the correct algorithm is used with each bottle.

3. After culture bottles have been loaded into the instrument, incubate five to seven days or until designated positive.

4. Smear and subculture all positive bottles. If the smear is negative, indicating a possible false positive, the bottle should be reloaded into the instrument until growth of the subculture or redesignation as positive. Bottles that were initially determined false positive and were redesignated positive, should be smeared and subcultured.

5. Negative bottles may be checked by smear and/or subculture at some point prior to discarding as negative.

6. Procedures for loading and unloading culture bottles into the BacT/ALERT instrument are given in the User Manual.

7. **Do not reuse BacT/ALERT culture bottles.** Dispose of inoculated BacT/ALERT culture bottles according to your laboratory protocol. Autoclaving and/or incinerating inoculated BacT/ALERT bottles is appropriate.

8. Utilization of coring devices (i.e., blunt needle) to puncture the septum may result in bottle leakage.

**QUALITY CONTROL**

A Certificate of Conformance is available for each lot of culture bottles. If desired, individual laboratories can perform quality control testing of BacT/ALERT FA Culture Bottles. Refer to the BacT/ALERT User Manual and CLSI® document M22-A3.

**Instrument**

A BacT/ALERT Reflectance Standards kit is provided with each instrument for the QC and Calibration procedures. All quality control should be part of normal system maintenance. Refer to the BacT/ALERT User Manual for more information.

**RESULTS**

Positive or negative culture bottles are determined by decision-making software contained in the BacT/ALERT Microbial Detection System. No action is required until the BacT/ALERT instrument signals a culture bottle either positive or negative.

**LIMITATIONS OF THE TEST**

Many variables involved in blood culture testing cannot be practically controlled to provide total confidence that results obtained are due solely to proper or improper performance of any culture medium or detection system.

1. A sample volume of 10ml is recommended. Volumes greater than 10ml do not maintain the optimal blood to medium ratio.

2. Patient specimens determined positive by BacT/ALERT may contain organisms that are positive by smear that will not grow on routine subculturing media. When this is suspected, specimens should be subcultured on special media. Also, BacT/ALERT 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.
3. The presence of the activated charcoal appears as a black precipitate on the smear which may initially be difficult to interpret. Ease of interpretation improves with experience.

4. Certain strains of *Haemophilus influenzae*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, and *Peptostreptococcus anaerobius* may be sensitive to the anticoagulant SPS which may result in lack of growth or low production of CO₂ by these strains if an insufficient amount of sample is inoculated into the culture bottles.

5. Infrequently, if there is a very high number of white blood cells present in the sample, the BacT/ALERT may indicate a culture bottle positive. In this case, the smear and subculture results may be negative.

6. Organisms are often few in numbers and may appear intermittently in the bloodstream; therefore, consecutive blood samples should be collected from each patient.

7. Promptly remove positive culture bottles when they are signaled by BacT/ALERT to avoid possible non-viable cultures due to autolysis or other reasons. Certain strains of *Streptococcus pneumoniae* may be particularly prone to autolysis if they are not removed promptly after being signaled positive.

8. 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.

9. Anonymously loaded bottles are assigned a default algorithm. In some cases, depending on the bottle type, this default algorithm is not the correct algorithm for the bottle type. This use of an incorrect algorithm could lead to a false-positive result. Therefore, it is important to scan each bottle with the correct bottle barcode.

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**PERFORMANCE CHARACTERISTICS OF THE TEST**

In-house seeded studies were performed using the following organisms at levels of ≤ 10 CFU/bottle and ≤ 100 CFU/bottle in human blood from a healthy adult population.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Inoculum (CFU/bottle)</th>
<th>BacT/ALERT FA (Plastic)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram positives</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>(M. luteus, S. aureus, S. epidermidis, S. agalactiae, E. faecalis, S. pneumoniae, S. pyogenes, Group C streptococcus, L. monocytogenes, S. sanguis)</em></td>
<td>≤ 100</td>
<td>13.3 - 35.1</td>
</tr>
<tr>
<td><strong>Gram negatives</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>(E. coli, H. influenzae, N. meningitidis, P. aeruginosa, S. maltophilia, S. marcescens, A. baumanii, E. cloacae, A. faecalis, K. pneumoniae)</em></td>
<td>≤ 100</td>
<td>10.8 - 35.2</td>
</tr>
<tr>
<td><strong>Yeast</strong></td>
<td></td>
<td></td>
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<tr>
<td><em>(C. albicans, T. glabrata, C. tropicalis)</em></td>
<td>≤ 100</td>
<td>17.3 - 74.4</td>
</tr>
<tr>
<td></td>
<td>≤ 10</td>
<td>20.9 - 82.8</td>
</tr>
</tbody>
</table>

*Each organism was tested in triplicate and averages obtained. Values given are a range of these averages.*

**NOTE:** A list of rare and fastidious organisms recovered with BacT/ALERT culture bottles is available upon request from bioMérieux.
REFERENCES


7. Rare Organism Club, bioMérieux, Inc.

AVAILABILITY

bioMérieux

BacT/ALERT® FA

100/case REF 259791

For technical assistance in the USA, contact bioMérieux Customer Service at 1-800-682-2666. Outside the USA, contact your local bioMérieux Representative.

INDEX OF SYMBOLS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
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<tbody>
<tr>
<td>REF</td>
<td>Catalogue number</td>
</tr>
<tr>
<td></td>
<td>Manufacturer</td>
</tr>
<tr>
<td></td>
<td>Temperature limitation</td>
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<tr>
<td>LOT</td>
<td>Use by</td>
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<tr>
<td></td>
<td>Batch code</td>
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<tr>
<td></td>
<td>Consult Instructions for Use</td>
</tr>
<tr>
<td></td>
<td>Contains sufficient for &lt;n&gt; tests</td>
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<tr>
<td>EC/REP</td>
<td>Authorized representative in the European Community</td>
</tr>
<tr>
<td></td>
<td>Do not reuse</td>
</tr>
<tr>
<td></td>
<td>Latex-free</td>
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<tr>
<td>IVD</td>
<td>In vitro diagnostic medical device</td>
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</tbody>
</table>

Package insert provided in the kit or downloadable from www.biomerieux.com/techlib