**INTENDED USE**

BD BACTEC™ Myco/F Lytic culture medium when used with the BD BACTEC fluorescent series instruments is a nonselective culture medium to be used as an adjunct to aerobic blood culture media for the recovery of mycobacteria, yeast and fungi from blood. This medium may also be used for the culture of sterile body fluids when yeast or fungi are suspected.

**SUMMARY AND EXPLANATION**

Since the mid-1980s and expanding size of the immunocompromised patient population, the incidence of septicemia caused by opportunistic pathogens such as yeast, fungi and mycobacteria has risen. Mycobacterium tuberculosis (MTB) and mycobacteria other than tuberculosis (MOTT), especially Mycobacterium avium complex (MAC), have become resurgent. From 1985 to 1992, the number of MTB cases reported increased 18%. Between 1981 and 1987, AIDS case surveillances indicated that 5.5% of the patients with AIDS had disseminated nontuberculous mycobacterial infections, e.g., MAC. By 1990, the increased cases of disseminated nontuberculous mycobacterial infections had resulted in a cumulative incidence of 7.6%. It has also been noted that the incidence of fungemia has steadily increased since the early 1980s. This has increased the need for the clinical laboratory to have effective diagnostic procedures for fungemia and mycobacteremia.

The Centers for Disease Control and Prevention (CDC) have recommended that every effort must be made for laboratories to use the most rapid methods available for diagnostic mycobacteria testing. These recommendations include the use of a liquid medium for mycobacterial culture.

The BD BACTEC fluorescent series instruments are designed for the rapid detection of microorganisms in clinical specimens. BD BACTEC Myco/F Lytic Culture medium is a Middlebrook 7H9 and Brain Heart Infusion broth formulation for the recovery of mycobacteria from blood specimens and yeast and fungi from blood and sterile body fluids. Specific modifications were made to enhance the growth and recovery of mycobacteria, yeast and fungi. These modifications include ferric ammonium citrate to provide an iron source for specific strains of mycobacteria and fungi, the addition of saponin as a blood lysing agent and the addition of specific proteins and sugars to provide nutritional supplements. Each vial contains a sensor which can detect decreases in oxygen concentration in the vial resulting from microorganism metabolism and growth. The sensor is monitored by the BD BACTEC fluorescent series instrument for increasing fluorescence which is proportional to the decrease in oxygen. A positive determination indicates the presumptive presence of viable microorganisms in the vial.

**PRINCIPLES OF THE PROCEDURE**

The BD BACTEC Myco/F Lytic culture vial is designed for the rapid detection of mycobacteria in blood, and yeast and fungi in blood and sterile body fluids. Specimens are inoculated into the BD BACTEC Myco/F Lytic vial either with a syringe or direct draw with a needle and tubing. The vial is placed into the BD BACTEC fluorescent series instrument and is continuously agitated and incubated at 35 °C for maximum recovery. The default testing protocol is 42 days. The recommended testing protocol for the following organisms are 7 days for yeast, 30 days for fungi and 42 days for mycobacteria. Each vial contains a sensor which can detect decreases in oxygen concentration in the vial resulting from microorganism metabolism and growth. The sensor is monitored by the BD BACTEC fluorescent series instrument every ten minutes. Analysis of the rate of oxygen decrease as measured by increasing fluorescence enables the BD BACTEC fluorescent series instrument to determine if the vial is instrument positive. A positive determination indicates the presumptive presence of viable microorganisms in the vial.

**REAGENTS**

Each BD BACTEC Myco/F Lytic culture vial contains the following active ingredients prior to processing:

<table>
<thead>
<tr>
<th>List of Ingredients</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processed Water</td>
<td>40 mL qs</td>
</tr>
<tr>
<td>7H9 Middlebrook Broth Base without phosphate salts</td>
<td>0.12% w/v</td>
</tr>
<tr>
<td>Brain Heart Infusion Broth</td>
<td>0.5% w/v</td>
</tr>
<tr>
<td>Casein Hydrolysate</td>
<td>0.10% w/v</td>
</tr>
<tr>
<td>Suppiment H</td>
<td>0.10% w/v</td>
</tr>
<tr>
<td>Inositol</td>
<td>0.05% w/v</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0.10% w/v</td>
</tr>
<tr>
<td>Sodium Polyanetholsulfonate</td>
<td>0.025% w/v</td>
</tr>
<tr>
<td>Polysorbate 80</td>
<td>0.0025% w/v</td>
</tr>
<tr>
<td>Pyridoxal HCl</td>
<td>0.0001% w/v</td>
</tr>
<tr>
<td>Ferric Ammonium Citrate</td>
<td>0.006% w/v</td>
</tr>
<tr>
<td>Potassium Phosphate</td>
<td>0.024% w/v</td>
</tr>
<tr>
<td>Saponin</td>
<td>0.24% w/v</td>
</tr>
<tr>
<td>Antifoam</td>
<td>0.01% w/v</td>
</tr>
</tbody>
</table>

Composition may have been adjusted to meet specific performance requirements.

This BD BACTEC medium is dispensed with added CO₂ and O₂.
BD BACTEC Myco/F Lytic medium requires no supplement addition. Each 40 mL vial of BD BACTEC Myco/F Lytic is ready for use when received. The appearance of the media upon receipt should be clear and light amber in color.

**Warnings and Precautions**
*For in vitro diagnostic Use.*

This Product Contains Dry Natural Rubber.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. “Standard Precautions”5-8 and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids.

If recovery of mycobacteria is intended, CDC-NIH guidelines strongly recommend that the test instrument be placed in the mycobacteria laboratory where the additional safety issues that the recovery of mycobacteria present can be addressed.6

BD BACTEC Myco/F Lytic vials will accept more than the recommended maximum of 5 mL of specimen volume. Monitoring of fill should be conducted.

For activities involving the propagation and manipulation of *Mycobacterium tuberculosis* or *Mycobacterium bovis* grown in culture, Biosafety Level 3 practice, containment equipment and facilities are recommended.9

Prior to use, each vial should be examined for evidence of contamination such as cloudiness, bulging or depressed septum, or leakage. **DO NOT USE** any vial showing evidence of contamination, leakage or damage. Vial contamination may not be readily apparent. A contaminated vial could contain positive pressure. If a contaminated vial is used for direct draw, gas or contaminated culture media could be refluxed into the patient's vein. On rare occasions, the glass bottle neck may be cracked and the neck may break during removal of the flip-off cap or in handling. Also, on rare occasions, a vial may not be sealed sufficiently. In both cases the contents of the vials may leak or spill, especially if the vial is inverted.

To minimize the potential of leakage during inoculation by syringe of specimen into culture vials, use syringes with BD Luer-Lok™ brand tips. A one-handed inoculation technique and a suitable vial holder should be employed to prevent accidental needle stick injury.

Before discarding, sterilize all inoculated BD BACTEC Myco/F Lytic vials by autoclaving.

**Positive culture vials for subculturing or staining, etc.:** Before sampling it is necessary to release gas which often builds up due to microbial metabolism. Sampling and venting of vials must be performed in a biological safety cabinet, and appropriate protective clothing, including gloves and masks, should be worn. See PROCEDURE Section for more information on subculturing.

**Leaking or Broken Vials**

**CAUTION:** Because an inoculated leaking or broken vial may produce an aerosol of mycobacteria, including *M. tuberculosis*, or other bacteria, appropriate handling should be observed.

If an inoculated vial is found to be leaking or is accidentally broken during collection or transport, use the established procedure in your facility for dealing with mycobacterial spills. As a minimum, “Standard Precautions” should be employed. Vials should be discarded in an appropriate manner.

If a vial is found to have leaked contents into the instrument proper, or if a vial is accidentally broken, turn off the instrument immediately. Vacate the affected area. Contact your facility’s Safety or Infection Control Officer(s). Determine the necessity of turning off or modifying the settings of the air handling units serving the affected area. Do not return to the area until any potential aerosols have settled or have been removed by appropriate ventilation. BD Life Sciences should be notified by calling 1.800.638.8663 in the USA or the appropriate BD representative in your area. Guidelines for proper handling of accidental mycobacterial contamination due to breakage of culture tubes or broth suspensions have been issued by the CDC.9

**Storage Instructions**

Store at 2–25 °C in a dry location out of direct light.

DO NOT use after expiration date.

**SPECIMEN COLLECTION**

**NOTE:** It is recommended that this procedure be reviewed with the appropriate personnel prior to use of medium to ensure proper specimen collection techniques as described in this section.

The specimen must be collected using sterile technique to reduce the chance of contamination. The range of blood volume which can be cultured is 1 mL to 5 mL, with optimum recovery obtained at 3 mL to 5 mL. It is recommended that the specimen be inoculated at bedside. Most commonly, a syringe with a BD Luer-Lok® brand tip is used to draw the specimen. If appropriate, a BD Vacutainer® brand Needle Holder and a BD Vacutainer brand Blood Collection Set, BD Vacutainer Safety-Lok™ Blood Collection Set or other tubing “butterfly” set may be used. If using a needle and tubing (direct draw), carefully observe the direction of the blood flow when starting sample collection. Prior to inoculation, the medium fill volume should be noted on the label with a pen or marker to indicate the starting point of specimen collection. The vacuum in the bottle will usually exceed 5 mL, so the user should monitor the volume collected by means of the 5 mL graduation marks on the vial label. When the desired 1–5 mL of blood has been drawn, the flow should be stopped by crimping the tubing and removing the needle from the BD BACTEC vial. The BD BACTEC vial should be transported as quickly as possible to the laboratory and placed in the BD BACTEC instrument. A yellow-top BD Vacutainer Brand Tube containing SPS may also be used to collect the blood sample from the patient. The tube should be transported to the laboratory as quickly as possible for transfer into the BD BACTEC culture vial.

**PROCEDURE**

**Materials Provided:** BD BACTEC Myco/F Lytic Culture Vials.

**Materials Required But Not Provided:** Biological Safety Cabinet, autoclave, venting unit, mycobacterial disinfectant, 70% isopropyl alcohol. Quality Control Organisms (*Mycobacterium intracellulare*, ATCC® 13950; *Candida glabrata*, ATCC 15545; *Cryptococcus neoformans*, ATCC 13690), microscope and materials for staining slides and subculturing vials.
Inoculation of BD BACTEC Myco/F Lytic Culture Vials

1. Remove the flip-off cap from the BD BACTEC vial top and inspect the vial for cracks, leaks, contamination, excessive cloudiness and bulging or indented septum. DO NOT USE if any defect is noted.

2. Label culture vial with specimen identification and mark medium fill graduation line on vial label.

3. Before inoculating, swab the septum with alcohol. Aseptically inject with a syringe or draw directly with the aid of the graduation lines on the vial label 1–5 mL of specimen per vial (see the section on Limitations of the Procedure). Inoculated vials should be placed into the BD BACTEC fluorescent series instruments as soon as possible for incubation and monitoring.

4. Vials entered into the instruments will be automatically tested for the duration of the testing protocol. The default testing protocol is 42 days. The recommended testing protocol for the following organisms are 7 days for fungi and 42 days for mycobacteria. See the appropriate BD BACTEC User’s Manual, to set protocol length. If at the end of the protocol, a negative BD BACTEC Myco/F Lytic vial appears visually positive (i.e., bulging septum), it should be subcultured, AFB and Gram-stained and treated as a presumptive positive.

5. Positive vials will be identified by the BD BACTEC fluorescent series instrument. The sensor inside the vial may not appear visibly different in positive or negative vials; however, the BD BACTEC fluorescent series instrument can determine a difference in sensor fluorescence. All positive vials should be handled in a Biological Safety Cabinet. Biosafety Level 3 practices, containment equipment and facilities are recommended.

Positive vials should be subcultured and an appropriate smear prepared.

Processing an instrument-positive vial

- a) Remove the vial from the instrument and place in a biological safety cabinet.
- b) Invert vial to mix contents.
- c) Vent the vial to equilibrate vial pressure with atmosphere.
- d) Remove aliquot from vial (approx. 0.1 mL) for stain preparations (AFB and Gram).
- e) Inspect smear and report preliminary results only after smear evaluation.

Subculturing: Subculturing should be performed in a biological safety cabinet, and appropriate clothing, including gloves and masks, should be worn. Prior to subculturing, place the vial in an upright position, and place an alcohol wipe over the septum. To release any positive pressure in the vial which could be caused by growth of possible contaminants, insert a sterile 25-gauge (or smaller) needle equipped with an appropriate filter or pledget through the alcohol wipe and septum. The needle should be removed after any pressure is released and before sampling the vial for subculture. The insertion and withdrawal of the needle should be done in a straight-line motion, avoiding any side-to-side motions which could permanently damage the septum. Do not re-cap the needle. Discard needles and syringes in a puncture-resistant biohazard container.

QUALITY CONTROL

Each lot of media has been tested using appropriate quality control organisms and testing meets product specifications and CLSI standards, where relevant. As always, QC testing should be performed in accordance with applicable local, state, federal or country regulations, accreditation requirements, and/or your laboratory’s standard quality control procedures.

Quality Control Certificates are provided with each carton of media. Quality Control Certificates list test organisms, including ATCC cultures specified in the CLSI Standard M22, (Quality Control for Commercially Prepared Microbiological Culture Media), that are applicable to this type of culture medium.

Quality Control (optional) for BD BACTEC Myco/F Lytic media: ATCC control organisms identified in the following chart are positive controls and an uninoculated vial is used as a negative control.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Range of Time-to-detection (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycobacterium intracellulare, ATCC 13950</td>
<td>8 to 16</td>
</tr>
<tr>
<td>Candida glabrata, ATCC 15545</td>
<td>&lt; 3</td>
</tr>
<tr>
<td>Cryptococcus neoformans, ATCC 13690</td>
<td>&lt; 3</td>
</tr>
</tbody>
</table>

The positive vials should be inoculated using a 1:100 dilution of a McFarland #1 suspension grown on solid medium. Inoculate the vial with 0.1 mL of the diluted culture. The vials and an uninoculated control vial should be scanned into the instrument and tested. The inoculated vial should be detected as positive by the instrument within the test protocol. The negative control should remain negative. If expected results for Quality Control are not obtained, do not use the medium and contact BD Technical Services (in the U.S only: 1.800.638.8663) or your local BD representative for further assistance.

For information on quality control for the BD BACTEC System, refer to the appropriate BD BACTEC User’s Manual.

Reporting of RESULTS

An instrument positive vial must be confirmed by acid-fast smear or Gram stain. A positive result indicates the presumptive presence of viable microorganisms in the vial.

If AFB smear or Gram stain positive, subculture to solid media and report as: instrument-positive, AFB or Gram stain positive, ID pending.

If no microorganisms are present on the smears, subculture to solid media, re-enter the vial into the instrument as an ongoing negative vial and allow to complete test protocol. No reportable result.

Perform subcultures from the BD BACTEC Myco/F Lytic vial for identification and susceptibility testing.
LIMITATIONS OF THE PROCEDURE

BD BACTEC Myco/F Lytic vials are not selective and will support the growth of other aerobic organisms besides mycobacteria, yeast and fungi. Positive vials may contain one or more species of mycobacteria and/or other non-mycobacterial species. If present, fast growing organisms may mask the detection of slower growing mycobacteria, yeast and fungi. Subculture and additional procedures are required. The consistency of microscopic morphology in BD BACTEC Myco/F Lytic has not been established.

Inoculation of blood volumes of 1–5 mL are acceptable, but optimum recovery is obtained with 3–5 mL. During internal studies with less than 3 mL of blood, *M. intracellulare*, *M. malmoense*, *M. haemophilum* and *M. xenopi* exhibited detection delays and/or compromised recovery with BD BACTEC Myco/F Lytic. False positivity most likely will increase when the blood volume is above 5 mL.

Care must be taken to prevent contamination of the sample during collection and inoculation into the BD BACTEC vial. A contaminated vial will give a positive instrument reading, but will not indicate a relevant clinical result. Such a determination must be made by the user, based on such factors as stain results, type of organism recovered, occurrence of the same organism in multiple cultures, patient history, etc.

Mycobacteria may vary in acid-fastness depending on strain, age of culture and other variables.

Blood may contain antimicrobials or other inhibitors which may slow or prevent the growth of microorganisms.

BD BACTEC Myco/F Lytic vials are incubated at 35 °C potentially precluding the recovery of mycobacteria requiring other incubation temperatures (e.g., *M. marinum*, *M. ulcerans*, *M. haemophilum*). Recovery of such organisms requires additional culture methods.

Penicillium purpurescens and Blastomycyes dermatitidis were not detectable in the BD BACTEC Myco/F Lytic culture medium. Hansenula anomala, Exophiala jeanselmei, Actinomyces bovis, Rhodotorula rubra, and Mucor ramosissimus exhibited inconsistent results at low inoculum levels (<10 CFU/vial) with seeded culture studies. Recovery of such organisms may require additional culture methods.

The following isolates were detected as positive in the BD BACTEC 9240 instrument using BD BACTEC Myco/F Lytic medium during internal seeded studies and/or clinical trials:

- *Mycobacterium terrae*
- *Mycobacterium tuberculosis*
- *Mycobacterium avium*
- *Mycobacterium kansasii*
- *Mycobacterium fortuitum*
- *Mycobacterium intracellulare*
- *Mycobacterium gordonae*
- *Mycobacterium szulgai*
- *Mycobacterium simiae*
- *Mycobacterium celatum*
- *Cryptococcus neoformans*
- *Histoplasma capsulatum*
- *Aspergillus flavus*
- *Aspergillus fumigatus*
- *Nocardia asteroides*
- *Malassezia furfur*
- *Trichophyton rubrum*

EXPECTED RESULTS

1,488 blood cultures obtained from patients suspected of mycobacterial, yeast or fungal infections were evaluated in the BD BACTEC Myco/F Lytic culture vial with the BD BACTEC 9240 Blood Culture System. There were 315 positive cultures with 243 clinically significant organisms recovered, of which 131 (53.9%) were mycobacteria, 35 (14.4%) were yeast or fungi and 77 (31.7%) were other bacteria. Of the 1,488 blood specimens tested in the clinical study, eleven BD BACTEC Myco/F Lytic culture vials (0.7%) were determined to be false positive (instrument-positive, smear and/or subculture-negative). Of the 315 instrument positive Myco/F Lytic vials, 11 (3.5%) were determined to be false positive. Of the 1,488 blood specimens tested in the clinical study, one (1) BD BACTEC Myco/F Lytic culture vial (0.07%) was determined to be false negative (instrument-negative, smear and/or subculture-positive). Of the 1,173 instrument negative BD BACTEC Myco/F Lytic culture vials, one (0.08%) was determined to be false negative. The contamination rate during this evaluation was 3.3%.

Frequency distribution of clinical trial specimens positive in the BD BACTEC Myco/F Lytic culture vials with the BD BACTEC 9000 Blood Culture System are illustrated in FIGURE 1 for times to detection (TTD) for mycobacteria and FIGURE 2 for TTD for yeast and fungi.

FIGURE 1
PERFORMANCE CHARACTERISTICS

The BD BACTEC Myco/F Lytic medium was evaluated with the BD BACTEC 9240 instrument at two clinical sites considered large tertiary care teaching hospitals in geographically diverse areas. The site populations included patients infected with HIV, immunocompromised patients, transplant patients, and patients suspected of a mycobacterial infection. The BD BACTEC Myco/F Lytic medium was compared to the BD BACTEC 13A medium for the recovery and detection of mycobacteria from blood. A total of 1,100 blood culture specimens were tested during the evaluation. The total number of pathogenic mycobacteria isolates recovered in the study was 111 (See TABLE 1). Of these positives, ten (9%) were recovered in the BD BACTEC Myco/F Lytic medium only and three (3%) were recovered by BD BACTEC 13A medium only.

TABLE 1: Summary of Myco/F Lytic Culture Medium Isolate Recovery During Clinical Trials

<table>
<thead>
<tr>
<th>Organism</th>
<th>Total Isolates</th>
<th>Myco/F Lytic Medium Only</th>
<th>13A Medium Only</th>
<th>Both</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Pathogenic Mycobacteria:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mycobacterium avium</td>
<td>108</td>
<td>10</td>
<td>3</td>
<td>95</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Mycobacterium celatum</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>111</td>
<td>10</td>
<td>3</td>
<td>98</td>
</tr>
</tbody>
</table>

The BD BACTEC Myco/F Lytic medium was evaluated with the BD BACTEC 9240 instrument at four clinical sites considered large tertiary care teaching hospitals. The site populations included patients infected with HIV, immunocompromised patients, transplant patients, and patients suspected of a fungal infection. The BD BACTEC Myco/F Lytic medium was compared to the ISOLATOR™ System (Wampole Laboratories, Cranbrook, NJ) for the recovery and detection of yeast and fungi from blood. BD BACTEC Myco/F Lytic vials were inoculated with 1–5 mL of blood and ISOLATOR tubes were inoculated with 3–10 mL of blood. The ISOLATOR sediment was plated to Chocolate Agar, Brain Heart Infusion Agar with 5% sheep blood, and Sabouraud Dextrose Agar. A total of 748 specimens were tested during the evaluation. The total number of pathogenic yeast and fungal isolates recovered in the study was 32 (See TABLE 2). Of these positives, seven (22%) were recovered in the BD BACTEC Myco/F Lytic medium only and six (19%) were recovered in the ISOLATOR system only.

TABLE 2: SUMMARY OF MYCO/F LYTIC MEDIUM ISOLATE RECOVERY DURING CLINICAL TRIAL

<table>
<thead>
<tr>
<th>Organism</th>
<th>Total Isolates</th>
<th>Myco/F Lytic Medium Only</th>
<th>ISOLATOR Only</th>
<th>Both</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Pathogenic Fungi:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>10</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Candida krusei</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Candida parapsilosis</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Fusarium species</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Histoplasma capsulatum</td>
<td>11</td>
<td>0</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>7</td>
<td>6</td>
<td>19</td>
</tr>
</tbody>
</table>
AVAILABILITY
Cat. No. Description
442288 BD BACTEC™ Myco/F Lytic Culture Vials, case of 50 vials

REFERENCES


Change History

<table>
<thead>
<tr>
<th>Revision</th>
<th>Date</th>
<th>Change Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>(07)</td>
<td>2019-08</td>
<td>Converted printed instructions for use to electronic format and added access information to obtain the document from BD.com/e-labeling. Deleted Cat. No. 442003 from Availability section.</td>
</tr>
</tbody>
</table>