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Clinical Laboratory Considerations after Discontinuation of the Isolator™ Tube Lysis-Centrifugation System for Fungal and Mycobacterial Blood Cultures

Frequently Asked Questions (FAQ) for Clinical Laboratories

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Background

In 2023, the Isolator™ Tube Blood Culture System (Abbott Laboratories, Abbott Park, IL), a commonly used collection and specimen preparation device for culturing blood, was discontinued in the United States. This product may also be known as the Wampole Isolator™ system and its specimen collection tubes and will be referred to as “Isolator™ Tubes” in this document. Among other applications, the Isolator™ Tubes were used for whole blood lysis-centrifugation processing before inoculating primary media for the recovery of mycobacteria and/or fungi. With the discontinuation of Isolator™ Tubes, many laboratories must adjust their specimen collection and culturing approaches. In this FAQ, we summarize other blood or bone marrow and blood culture collection devices that may be considered, their reported performance, and regulatory considerations when changing test methods.

FAQ 1: With discontinuation of the Isolator™ Tubes, what are alternative methods for clinical laboratories to adopt?

Various alternatives for blood cultures of fungi and acid-fast bacilli (AFB) are available to clinical laboratories. These include automated blood culture systems, classical manual methods, and modification of FDA-approved or cleared systems.

Laboratories should inquire with their blood culture system manufacturers if AFB or fungal specific bottles are available. In the U.S., FDA-approved or cleared, automated continuous monitoring blood culture systems for mycobacteria and/or fungi are available from some manufacturers. They include the BACTEC™ Myco/F Lytic bottle used in conjunction with the BACTEC™ series of blood culture instruments (Beckton Dickinson (BD) Diagnostic Systems, Sparks, MD) and the VersaTREK™ instrument used in conjunction with the Myco Media bottle (Thermo Scientific). The BACTEC™ Myco/F Lytic bottle system is FDA-approved or cleared for mycobacteria from blood and fungi from blood and sterile body. Advantages of the BD system include a single bottle that may be used for both AFB and fungal culture and acceptance of blood either drawn directly into the Myco/F Lytic vial or transferred directly to the Myco/F Lytic vial from a sodium polyanethol sulfonate (SPS) vacutainer tube. Although there is concern that each Myco/F Lytic bottle only requires 3-5 mL of blood and that relatively low volumes may result in suboptimal organism recovery, this does not appear to be the case in published studies when compared to other methods that used increased blood volumes (1, 2). The VersaTREK™ Myco Media bottle system is designed for mycobacterial and not fungal cultures and is FDA-approved or cleared for blood and sterile body specimens as well as digested-decontaminated specimens. Five to 10 mL of blood is collected and must first undergo a manual lysis-centrifugation step (which in the absence of Isolator™ Tubes may be performed using sterile water for lysis before centrifugation) or buffy coat preparation prior to inoculating the VersaTREK Myco bottle. Both the BD Myco/F Lytic vial and Versa-

TREK™ Myco bottle are incubated longer than routine blood cultures (but also at 35-37°C), commonly for at least 6 weeks, which must be taken into account when considering instrument capacity. Outside of the U.S., BACTEC™ Mycosis IC/F Culture vials, used with BACTEC™ blood culture systems, may be available and are intended for recovery of fungi from blood. The BACTEC™ Mycosis IC/F Culture vial has been found to substantially increase detection of fungemia in immunocompromised patients (3). At this time, the BacT/ALERT® system (bioMérieux, Durham, NC) offers the MP bottle for AFB cultures of sterile, non-blood specimen types, but the MB bottle for AFB blood cultures has been discontinued. The BacT/ALERT® MP Reagent System is FDA-cleared or approved for use with BacT/ALERT®.

Manual methods that were used prior to introduction of the Isolator™ Tube system may be considered for blood AFB or fungal cultures (4). Use of a lytic agent to process blood is recommended prior to inoculation to culture media (5, 6). In the lysis-centrifugation method, blood is first collected in anticoagulant (see FAQ3 below) and is transferred to a secondary container to be lysed with distilled water. The specimen then undergoes centrifugation and the resulting pellet is inoculated to appropriate culture media. Variations with enhancements have been used, such as lysis with 0.3% sodium desoxycholate solution instead of water (7). The benefit of manual lysis-centrifugation is that it requires only standard laboratory reagents and media and the laboratory can flexibly choose the media used for inoculation. Downsides include that it is labor-intensive and prone to contamination as an open system involving transfer and aliquoting steps (8). Biphasic systems such as using brain heart infusion media, may be used for subsequent inoculation for AFB and fungal recovery with acceptable yield (5, 9) but are not readily commercially available. Recovery of fungi in biphasic culture is enhanced with agitation of the bottle in the initial 24 hours (5).

Modification of FDA-approved or cleared systems for the blood culture of AFB or fungi has been described. For example, off-label use of the BD MGIT™ automated monitoring system for blood specimens that have undergone lysis-centrifugation (10), prolonged incubation of BacT/ALERT® aerobic bottles for detection of AFB (11, 12) or terminal subculturing of routine blood culture bottles to increase yield of fungi or AFB (12, 13) have been reported. Alternatively, routine blood culture media may support the growth of mycobacteria but is not optimal for expeditious growth without enhancements such as lysis of host cells, addition of antimicrobial agents, and supplementation with lipids and other nutrients (6). While modifications may allow laboratories to utilize their existing instrumentation, any modification to FDA-approved or cleared assays makes them laboratory developed tests (LDT), requiring the laboratory to perform full validation studies and must comply with LDT-related regulations.

FAQ2. What is the comparative clinical utility between the Isolator™ Tube Blood Culture System and other fungal and AFB blood culture methods?

Relative recovery of mycobacteria and fungi from blood varies between organism types. Several studies have examined the comparative yield of Isolator™ Tubes versus another method; example findings are summarized here.

Candida spp.

Detection of invasive candidiasis remains challenging, with estimates that blood cultures are only ~50% sensitive, although this figure may be closer to 75% after excluding cases of deep-seated infection unlikely to have active candidemia (14). Several studies have shown no significant differences in yields for the recovery of *Candida* spp. using standard blood culture bottles with the BACTEC™ and BacT/ALERT® systems compared to the Isolator™ Tube system (15, 16). Studies have also found that the recovery of *Candida* spp. using BACTEC™ Myco/F Lytic fungal blood culture bottles was comparable to that using a combination of aerobic and anaerobic standard bacterial blood culture bottles (17-20). Similarly, newer versions of automated blood culture systems, such as BACTEC™ FX, VersaTREK™, and BacT/ALERT® Virtuo®, using standard bacterial culture bottles and a 5-day incubation period, have been evaluated and found to have comparable recovery of *Candida* spp. to the Isolator™ Tube system (21-24). These results demonstrate that, when culturable, episodes of candidemia can generally be detected by standard blood culture media in automated blood culture systems without the need for a separate fungal blood culture bottle.

Other yeasts

As a lipophilic yeast, *Malassezia furfur* requires media supplemented with long-chain fatty acids for optimal growth. Routine blood culture broth may not reliably support the propagation of *M. furfur* without lipid supplementation particularly as blood may be inhibitory to its growth (6, 25). Recovery with the Isolator™ Tube system was comparably superior especially if the sediment were plated to media enriched with lipids, e.g. fungal media with olive oil overlay (26, 27). One referenced procedure for when *M. furfur* fungemia is suspected is to directly plate patient blood to lipid rich media as an alternative to use of a commercial system (28). Data are sparse for the BACTEC™ Myco F/Lytic and Mycosis IF/C culture systems but they appear to be able to support growth of *M. furfur* based on manufacturer's product insert and rare published data (29).

For *Cryptococcus* spp., routine blood cultures can generally support their growth, but some strains require more than the standard 5-day incubation period to signal positivity, or else result in falsely negative signals when blind terminal subcultures result in growth (30). Recovery of *Cryptococcus* spp. yield is boosted with the addition of dedicated fungal blood culture as shown in several studies including the BACTEC™ Myco/F Lytic and Isolator™ systems and should be considered when cryptococemia is suspected (27, 29, 31).

Dimorphic fungi and molds

While standard blood culture is comparable to the Isolator™ Tube system for *Candida* spp., recovery rate is low across methods and thus other approaches are needed to recover dimorphic fungi and molds causing fungemia. In a study published in 2001, of 6,108 blood cultures inoculated using the Isolator™ Tube and three BACTEC™ bottles – Myco/F Lytic, Plus Aerobic/F, and Anaerobic Lytic/10, two isolates of *Histoplasma capsulatum* were recovered from the Isolator™ Tube system only; *Histoplasma* was not detected by any other method in this study and no other molds were isolated by any method (31). A recent review of diagnostic methods for the laboratory diagnosis of fungal diseases identified diagnostic approaches for mold blood cultures as suboptimal (32). Optimal culture methods for recovery of dimorphic fungi and molds from blood remain elusive.

Mycobacteria

With respect to the isolation of mycobacteria, studies have shown comparable sensitivity for *M. tuberculosis* and *M. avium* complex (MAC) between the BACTEC™ Myco/F Lytic bottle and the Isolator™ Tube system, and faster detection of MAC bacteremia with automated systems than with the Isolator™ Tube method using culture on solid mycobacterial media (18, 19, 33, 34). For mycobacterial blood culture, in centers with a low prevalence of disseminated tuberculosis, a dedicated bottle should be used mostly for immunocompromised, particularly acquired immunodeficiency syndrome (AIDS), patients suspected of or at risk for disseminated MAC infection. The optimal specimen is typically 5 mL of blood inoculated directly into AFB specific blood culture bottles (35), or other volume as directed by the manufacturer.

Taken together, conventional blood culture methods are adequate for the recovery of *Candida* spp. Limited available evidence suggests that BACTEC™ Myco/F Lytic bottles offer an acceptable method for culture of dimorphic and filamentous fungi as well as mycobacteria from blood.

FAQ3: How does choice of anticoagulant for blood collection impact fungal and AFB blood cultures?

Older versions of the Manual of Clinical Microbiology indicate that for the recovery of mycobacteria, blood should be collected in tubes containing SPS, heparin, or citrate anticoagulants (36); however, there is sparse scientific data on the effect of anticoagulants on the growth of fungal and mycobacterial organisms. SPS is the only anticoagulant that has been widely used clinically and for which clinical evaluation of the detection of mycobacteria and fungi has been reported (5, 10, 34, 37-44). Inconsistent findings on the usefulness of the Isolator™ Tube system have made the added value of SPS uncertain in the recovery of fungi and mycobacteria, with some reports even finding slowing of growth (2, 26, 31). Only few publications examine the impact of heparin on microbial growth; of those, an older article concluded that the growth of microorganisms including *Candida* species may be suppressed in the presence of heparin (45), with newer literature confirming the notion that heparin is a microbial growth inhibitor (46). Likewise, the use of specimens collected in sodium citrate or other citrate containing anticoagulants has long been determined to be unsuitable for bacterial and

fungal cultures (47). Because acid-citrate-dextrose (ACD) contains citrate, it is not a suitable anticoagulant for the collection of blood specimens destined for microbial culture. Similarly, EDTA is known to inhibit bacterial growth (48, 49).

The culture of uncoagulated blood collected in tubes may be useful in the diagnosis of certain bacterial infections (50); however, there are no studies comparing its use for fungal or mycobacterial culture against specimens collected with SPS or specific culture media. The traditional notion is that organisms present in the blood specimen become entrapped in the blood clot and are not able to grow in media (51). Consequently, the collection of blood with anticoagulants other than SPS or in the complete absence of them is not recommended as replacement for the Isolator™ Tube.

FAQ4: We previously used Isolator™ 1.5 Tubes for collecting bone marrow for culture. What are other options for fungal and AFB cultures of bone marrow aspirates?

Bone marrow aspirates collected in a preservative-free, sterile container are acceptable for culture if immediately delivered to the laboratory for culture processing. Direct bone marrow smears for calcofluor white and/or AFB stains are recommended before inoculating to primary culture media for fungal and/or AFB culture (52). Clotted bone marrow specimens are not acceptable for testing (53).

To prevent coagulation, bone marrow aspirates may alternatively be collected in sodium heparin-containing devices. Although it is not recommended for peripheral blood culture collections (6, 54), some references include heparin as an option for bone marrow aspirate collection for fungal culture, either in a heparin vacutainer tube (55) or into a heparinized syringe for bedside inoculation of culture media (56). For AFB culture of bone marrow aspirates, another anticoagulant option is SPS (in 10 mL tube) (57).

Bone marrow aspirates from these collections may be directly inoculated to fungal and AFB media with acceptable yield of mycobacteria (58). Manual lysis-centrifugation may likewise be applied to bone marrow as for peripheral blood (59). Data are lacking for comparisons between lysis-centrifugation and direct media inoculation for recovery of fungi from bone marrow specimens. Note that histopathologic and cytologic examination of bone marrow specimens may provide more rapid and similarly sensitive detection of fungal and AFB pathogens (60, 61).

With regards to automated culture systems, the VersaTREK™ Myco Media bottle system (see FAQ1) is FDA-cleared for mycobacterial culture of bone marrow. Off-label usage of automated systems and blood culture bottles for the purpose of bone marrow AFB and fungal cultures has been described but published data are limited. Examples from clinical laboratories include the BD MGIT™ system for AFB culture (62, 63) and Myco F/Lytic bottle on the BACTEC™ system for mycobacterial culture. However, testing a non-FDA-approved or cleared specimen type is a modification of the FDA-approved test, requires validation, and must comply with relevant LDT-related regulations.

FAQ5: What validation or verification studies are clinical laboratories required to perform when changing from the Isolator Tubes™ to a different specimen collection device or container for conventional fungal or AFB culture?

If changing the specimen collection device used for transporting blood to the laboratory to be directly inoculated to culture media, the testing typically still falls within the conventional culture category as addressed by CLIA '88 and accrediting agencies. CLIA '88 is not prescriptive about the requirements for verification of conventional culture testing. Each laboratory should confer with their accrediting agency's requirements to ensure compliance. At minimum, the potential of the specimen collection device to inhibit or reduce organism recovery should be considered. For example, the College of American Pathologists (CAP) requires through checklist item GEN.40942 that the laboratory evaluate significant changes to specimen containers to ensure that they do not contribute to analytic interference in the assays to be performed, including before a labora-

tory approves and employs a new container type, a different container type, or a device provided by a different vendor (64). Before using a different device for clinical testing, the laboratory should evaluate available clinical literature and all information provided by the manufacturer and determine if additional verification studies are needed. Based on this requirement, for example, if a laboratory chooses to transition to collecting blood for AFB or fungal culture in SPS tubes instead of the Isolator™ Tubes for conventional culture, they must evaluate the literature and manufacturer's information for use as well as any manufacturer bulletins or other documentation related to the SPS tubes that may be relevant to microbial culturing. They may find that SPS has been relatively well studied in the literature and consider the fact that SPS was the anticoagulant component of the Isolator™ Tube. Depending on the individual laboratory director or designee assessment, laboratories must determine if additional in-house verification of blood collection in SPS tubes for culture should be performed before employing this device for AFB or fungal culture.

When verification or validation studies are deemed necessary, they may be achieved in a number of ways as determined by the laboratory. One common method is to seed a set of mycobacterial or fungal organisms to culture media at concentrations near the expected limit of detection and in the presence or absence of anticoagulant (or any other tube additives) (6, 65). Recovery of microorganisms in the culture system, including any interference in organism recovery by anticoagulants or additives in the collection tube, is then evaluated against expected outcomes. Alternatively, seeded specimens may be inoculated to the Isolator™ Tubes, if still available, and to the alternative collection container, then each cultured to compare organism recovery.

It is notable that conventional culture methods are not always treated like most LDTs, nor are they an FDA-approved or cleared test, regarding verification or validation requirements. For example, the CAP checklist item COM.40350 addressing the broad validation requirements for modified-FDA approved or cleared tests and LDTs notes that the requirement does not apply to conventional culture (66). However, if a laboratory incorporates an FDA-approved or cleared test medium within their culture techniques, such as some chromogenic media, a change in specimen type or processing may then be a modification of an FDA-approved or cleared method and validation and compliance with relevant LDT-related regulatory rules would be required.

FAQ6: What validation or verification studies are clinical laboratories required to perform when changing to an automated blood culture system for AFB and fungal blood cultures?

For FDA-approved or cleared commercial systems, such as fungal or mycobacterial culture vials incubated in automated blood culture systems, laboratories should follow accreditation standards on verification of non-waived, FDA-cleared/approved tests. For example, per the CAP checklist item COM.40250, if modification is being made to the acceptable container type or to the manufacturer's methods, a validation as an LDT is required (66). Whereas, if the manufacturer indicates that alternative collection and transport devices are acceptable (such as SPS tubes), before transitioning from one approved collection device to another on an already verified system, the laboratory should perform a similar assessment as described in FAQ5 to determine the need for additional in-house verification.

FAQ7: What should our laboratory consider when determining the best alternative to the Isolator™ Tubes?

When navigating a discontinuation or change in any specimen collection device, it is prudent to conduct a review of alternative collection devices including those that may use the existing systems in the laboratory. Because AFB and fungal blood cultures have highest utility in certain immunocompromised patient populations, a review of the current volume of tests requests, institutional detection rate of mycobacteremia or fungemia, contamination rate, and patient population being tested are useful factors to assess. This information will help in determining the demand, the type of organisms most frequently recovered from the population served and clinical need for testing. A utilization review may furthermore shed light on ordering practices and appropriateness of test ordering. For example, there are no current guidelines regarding the specific indications for performing a fungal blood culture, which can result in overuse and low diagnostic yield. Stewardship initiatives have recommended that fungal blood cultures only be ordered for patients with suspected disseminated infection due to mold or dimorphic fungi (67). Thus, fungal blood culture utilization for diagnosis of candi-

demia is unnecessary and may comparably be achieved by routine bacterial blood culture conditions. This can then lead to final considerations for the type of method to pursue.

A laboratory's instrumentation and workflow, biosafety requirements, and overall investment (financial and time) to implement alternative culture methods must also be calculated. Commonly, low volume, specialized tests are sent to reference laboratories in lieu of performing verification or validation studies for continuation of in-house testing. When considering outsourcing of testing, the potential increase in turnaround time, cost, order and result interfacing capabilities, ease of information flow, and specimen stability limits including specimen transport times should also be evaluated.

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