Authors: F a , Va a-S , E ab Pa a , Ma a Na a S a Z a , Pa La i , La a F i , * R a / S *

*O - a , a ,

Q b a A a S / M b , / C a a P b H a M b , / O , Lab a / P a S b , ,

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

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

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.

FAQ y I 1. AFB

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. O□-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.

FAQ I AFB

If changing the specimen collection device used for transporting blood to the laboratory to be directly inoc(tions.)]TJE

tory approves and employs a new container type, a di□erent container type, or a device provided by a di□erent vendor (64). Before using a di□erent 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.

FAQ

AFB

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 nonwaived, 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.

FAQ

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 the t5BDC BTiauf295 3BDC B. Be

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.

References

1. Archibald LK, Dobbie H, Kazembe P, Nwanyanwu O, McKnight C, Byrne T, Addison RM, Bell M, Reller LB, Jarvis WR. 2001. Utility of paired BACTEC MYCO/F LYTIC blood culture vials for detection of bacteremia, mycobacteremia, and fungemia. J Clin Microbiol 39:1960-2.
2. Fuller DD, Davis TE, Jr., Denys GA, York MK. 2001. Evaluation of BACTEC MYCO/F Lytic medium for recovery of mycobacteria, fungi, and bacteria from blood. J Clin Microbiol 39:2933-6.
3. Zheng S, Ng TY, Li H, Tan AL, Tan TT, Tan BH. 2016. A dedicated fungal culture medium is useful in the diagnosis of fungemia: a retrospective cross-sectional study. PLoS One 11:e0164668.
4. 2023. 9.4 Liquid Media Used for Isolation, Clinical Microbiology Procedures Handbook, 5th ed. ASM Press, Washington, DC.
5. Murray PR. 1991. Comparison of the lysis-centrifugation and agitated biphasic blood culture systems for detection of fungemia. J Clin Microbiol 29:96-8.
6. CLSI. Principles and Procedures for Blood Cultures. 2nd ed. CLSI guideline document M47, Wayne, PA: Clinical and Laboratory Standards Institute; 2022.
7. Salfinger M, Stool EW, Piot D, Heifets L. 1988. Comparison of three methods for recovery of Mycobacterium avium complex from blood specimens. J Clin Microbiol 26:1225-6.
8. Dorn GL, Burson GG, Haynes JR. 1976. Blood culture technique based on centrifugation: clinical evaluation. J Clin Microbiol 3:258-63.
9. Berlin OG, Zakowski P, Bruckner DA, Johnson BL, Jr. 1984. New biphasic culture system for isolation of mycobacteria from blood of patients with acquired immune deficiency syndrome. J Clin Microbiol 20:575 (yn0aE)0.5 (

fungi from blood. J Clin Microbiol 36:1176-9.
20. Farfour E, Le Brun C, Mizrahi A, Bargain P, Durieux MF, Boquel F, Corvec S, Jeddi F, Muggeo A, Huguenin A,

39. Reimer LG. 1994. Laboratory detection of mycobacteremia. Clin Lab Med 14:99-105.
40. Kiehn TE, Cammarata R. 1988. Comparative recoveries of Mycobacterium avium-M. intracellulare from isolator lysis-centrifugation and BACTEC 13A blood culture systems. J Clin Microbiol 26:760-1.
41. Fandinho FC, Grinsztejn B, Veloso VG, Lourenco MC, Werneck-Barroso E, Joao E, Nogueira SA, Fonseca Lde S. 1997. Diagnosis of disseminated mycobacterial infection: testing a simple and inexpensive method for use in developing countries. Bull World Health Organ 75:361-6.
42. Bille J, Stockman L, Roberts GD, Horstmeier CD, Ilstrup DM. 1983. Evaluation of a lysis-centrifugation sys-

document M40-A2, Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
66. Pathologists CoA. All Common Checklist, CAP Accreditation Program, Northfield, IL. College of American
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