Mary Jane Ferraro
Microbiology Laboratory
Massachusetts General Hospital
Boston, Massachusetts 02114

Janet Hindler
Microbiology Laboratory
UCLA Medical Center
Los Angeles, California 90024

James Jorgensen
Department of Pathology
University of Texas Health Science Center
San Antonio, Texas 78284

Patrick Murray
Division of Laboratory Medicine
Washington University School of Medicine
St. Louis, Missouri 63110

BACTEC High-Blood-Volume Fungal Medium versus Isolator Comparison Misleading

Scientific investigation must be evaluated by using actual experimental conditions. Wilson et al. (4) mislead the reader by their global conclusion that BACTEC high-blood-volume fungal medium (HBV-FM) is equivalent and Bactec Plus 26 (BP26) is superior to Isolator. Tenets of microbial nutrition and experimental design were not followed, yet conclusions were drawn from flawed testing.

Concerning microbial nutrition, the media and incubation conditions used for Isolator are not entirely those suggested by the manufacturer, and, as such, they create additional variables for simultaneous evaluation of both fungal and bacterial recovery.

Regarding temperature, two of the four plates (Isolator) were incubated at 25°C (not optimal for growth of streptococci), whereas all BACTEC media were at 35°C.

Regarding media, Why was Sabouraud dextrose agar selected rather than SABHI or BHI (suggested by manufacturer), both of which more closely resemble the formulation of BACTEC FM? Sabouraud (one plate) is neither conducive for bacterial growth nor recommended for recovery of fungi from clinical specimens such as blood (3). Although an option, Sabouraud was devised for dermatophytes (1). The type of Sabouraud (4% dextrose, pH 5.6) or Emmons medium (2% dextrose, pH neutral) used was not indicated. Media selected for recovery of fungi can influence yield (2), and one could postulate quite different results had optimal media been used.

Dating back at least to the early 1940s, the literature is replete with examples of interrelationships between temperature, pH, nutritional requirements, and microbial growth. Such blatant omissions and inequalities by design are difficult to understand.

Concerning the experimental design, basic principles of investigation, and especially those for blood cultures, dictate that the volume of blood used in each system be equal. This evaluation (4) does not accurately compare systems for both bacteria and fungi, but rather, fragments of each; i.e., for bacterial growth, 50% of the inoculum was "wasted" by suboptimal incubation at 25°C. As a result, the original objective (fungal recovery) and the extrapolated results (bacterial recovery) suffered from inadequate experimental design.

Regarding data management, the ambiguity of the data presented can be illustrated by trying to determine the total number of isolates of *Candida albicans* per system. Consistency in the boxheads of Tables 4 and 6 as well as an indication of the total numbers of isolates would have been helpful. This style, used by recent authors (2), is much clearer.

Somewhere in the midst of controlled evaluations, the patient has been lost. Perhaps future studies should reflect bacteremia-fungemia detection per patient per system rather than isolates. Also, data on polymicrobial recovery were lacking and, with a selective fungal medium, should have been addressed.

Since the experimental design so seriously compromised basic conditions for growth, it is superfluous to attempt comparisons of speed of detection.

Regarding the supporting data, the discussion should be pertinent and current. Perpetuation of outdated information is a disservice. For example, references supporting high contamination rates with Isolator were, with one exception, prior to the release of Isostat processing and before Occupational Safety and Health Administration (OSHA) mandates (1992). Contamination in the 1990 report was 1%. Such discussion in 1992–1993 misrepresents Isolator and is analogous to reporting that a drawback of current BACTEC systems is the use of radioisotopes. Similarly, the recovery of certain bacteria is a function of how the system is used.

While a perfect blood culture system does not exist, and while Wilson et al. (4) noted that Isolator is acceptable, it must be emphasized that the routine clinical laboratory can utilize Isolator much more effectively than portrayed in their article.

Systems may be customized for patient populations, and customization is a distinct advantage of Isolator. However, if in the name of customization one abases a system, as was done here, and goes against basic scientific principles and the manufacturer's suggestions, it must be remembered that differences detected could quite likely be the result of the customization and not the system.

In summary, the results of this evaluation cannot be applied to Isolator as currently recommended and utilized. If so, then one could just as easily conclude that Isolator at 50% capacity (two plates suboptimal for bacteria) performs (Table 2) as well as BP26 for all bacteria recovered except viridans streptococci, and even when compromised by media selection, it is at least equivalent to HBV-FM (Table 6). However, the real problem with this comparison is that someone, during a cursory reading, might actually believe the global implications. Also, the article illustrates the need for more editorial and referee responsibility in ascertaining the application of scientific principles and reflection by manuscripts of research limitations as well as current uses of systems.

REFERENCES

1. **Becton Dickinson Microbiology Systems.** 1992. Sabouraud Dextrose Agar. BBL quality control and product information manual

for plated media. Becton Dickinson Microbiology Systems, Cockeysville, Md.
2. **Guerra-Romero, L., C. E. Musial, D. M. Ilstrup, C. D. Horstmeier, and G. D. Roberts.** 1992. Evaluation of fungal culture media using the Isolator and the Septi-Chek fungal blood culture systems (BC) for the recovery of yeast (YS) and filamentous fungi (FF) from blood, abstr. F-85, p. 512. Abstr. 92nd Gen. Meet. Am. Soc. Microbiol. 1992. American Society for Microbiology, Washington, D.C.
3. **Merz, W. G., and G. D. Roberts.** 1991. Detection and recovery of fungi from clinical specimens, p. 591, 593. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
4. **Wilson, M. L., T. E. Davis, S. Mirrett, J. Reynolds, D. Fuller, S. D. Allen, K. K. Flint, F. Koontz, and L. B. Reller.** 1993. Controlled comparison of the BACTEC high-blood-volume fungal medium, BACTEC Plus 26 aerobic blood culture bottle, and 10-milliliter Isolator blood culture system for detection of fungemia and bacteremia. J. Clin. Microbiol. **31**:865–871.

Katherine M. Hunter, Ph.D.
Clinical Microbiologist
BMC Pathology Group
Birmingham, Alabama 35213

Author's reply:

Dr. Hunter comments on the controlled comparison of the new BACTEC HBV-FM with the BACTEC BP26 and 10-ml Isolator (IS) tube for recovering fungi and bacteria from blood (2).

In response to the comments regarding temperature of incubation, BACTEC bottles were incubated at 35°C because that is the manufacturer's recommendation. Two of the four plates inoculated with sediment from IS tubes were inoculated at 25°C and the other two were incubated at 35°C to optimize recovery of both fungi and bacteria; there is no single temperature at which one can optimize the recovery of all microorganisms. Dr. Hunter asks, "Why was Sabouraud dextrose agar selected rather than SABHI or BHI. . . ." As stated in the Materials and Methods section of the paper, a BHI plate was used for all IS cultures. Sabouraud medium was also selected because it still is widely used in clinical microbiology laboratories. Whether or not Sabouraud medium is "conducive for bacterial growth" is irrelevant; there was no intention of using fungal media to recover bacteria. The cited chapter in the *Manual of Clinical Microbiology* (1) merely provides a list of acceptable media and does not exclude Sabouraud medium for primary recovery of fungi.

In response to the paragraph on experimental design, the volume of blood used in each system was carefully controlled, as stated in Materials and Methods. It is true that we divided the sediment from Isolator tubes into "fungal" and "bacterial" cultures, but this is the way that IS is used in many clinical laboratories, often with the addition of mycobacterial cultures. The study design required a 30-ml blood draw; this is the maximum volume that has been recommended for many years, and no institutional review board that we are aware of will allow larger volumes of blood to be drawn. Hence, one cannot perform a study simultaneously comparing the HBV-FM, BP26, and two IS tubes. I do not understand the statements regarding data management; the boxheads of Tables 4 and 6 are identical (other than the obvious difference that they deal with two different comparisons). Including total numbers of isolates would have been inappropriate; these two comparisons were not intended to include bacteria (the HBV-FM bacterial data were included only to demonstrate that the medium does inhibit bacterial growth and therefore cannot be used to recover bacteria). Furthermore, table styles are determined by the American Society for Microbiology and not by the authors. Dr. Hunter further states that "data on polymicrobial recovery were lacking"; the Results section clearly states that only 5 of 79 (6.3%) patients had fungemia caused by more than one organism. I also do not understand Dr. Hunter's statement that "the patient has been lost" in the midst of controlled evaluations. The sole purpose of evaluations such as this is to ensure that the performance characteristics of commercial products are established in such a way that physicians and clinical microbiologists can optimize the recovery of microbial pathogens from the blood of bacteremic or fungemic patients. Moreover, it was because of the "lost" patient that we divided the sediment from IS tubes and included a BP26 bottle to ensure that each patient had both fungal and bacterial blood cultures.

With regard to supporting data, Dr. Hunter must be unaware that the radiometric BACTEC system is still in use in the United States, both for routine and for mycobacterial cultures, and that the radioactive waste generated by the system, albeit low, continues to be a problem for some users. Processing IS tubes with the Isostat and/or in biological safety cabinets does reduce contamination rates when processing Isolator tubes; our statement regarding this matter reads "has been reported," not "continues to be" (2). Dr. Hunter does not address the issue of inferior recovery of certain microorganisms from Isolator tubes, which does continue to be a concern.

In summary, it must be emphasized that controlled clinical evaluations of blood culture products must be performed in such a way as to reflect how products are used in actual laboratory practice. We neither made nor intended any "global conclusions" in our article.

REFERENCES

1. **Merz, W. G., and G. D. Roberts.** 1991. Detection and recovery of fungi from clinical specimens, p. 591, 593. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and J. G. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
2. **Wilson, M. L., T. E. Davis, S. Mirrett, J. Reynolds, D. Fuller, S. D. Allen, K. K. Flint, F. Koontz, and L. B. Reller.** 1993. Controlled comparison of the BACTEC high-blood-volume fungal medium, BACTEC Plus 26 aerobic blood culture bottle, and 10-milliliter Isolator blood culture system for detection of fungemia and bacteremia. J. Clin. Microbiol. **31**:865–871.

Michael L. Wilson
Microbiology Laboratory
Denver General Hospital
Denver, Colorado 80204