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Evaluation of BacT/Alert 3D SA Bottles for Accurate Detection of Mycobacteremia with Special Reference to *Mycobacterium abscessus*

E. Kasuga[1], T. Matsumoto[1], K. Oana[2], M. Shiohara[1], T. Okabe[1], K. Yamauchi[1], T. Honda[1], H. Ota[2], Y. Kawakami[2]

[1]Department of Laboratory Medicine, Shinshu University Hospital,
[2]Department of Biomedical Laboratory Sciences, School of Health Sciences, Shinshu University School of Medicine, Matsumoto, Japan

Abstract
Bacteremia due to *Mycobacterium abscessus*, a rapid grower, belonging to the Runyon group IV, occurred in an inpatient with fever of unidentified origin in Shinshu University Hospital. To the best of our knowledge, this is the first documented case of *M. abscessus* bacteremia in Japan. The organism initially grew on Sheep blood agar plates after terminal-subculturing from the BacT/Alert SA aerobic blood culture bottles with no positive signal, and was subsequently identified as *M. abscessus* using 16S rRNA sequence analysis. We evaluated the BacT/Alert SA bottles for the detection of *Mycobacterium* species, with special reference to the rapid growers including *M. abscessus* by seeding experiments and obtained the following findings; 1) The BacT/Alert system shows the positive sign when the bacterial cell counts reach around 10^6 to 10^7CFU/ml. 2) The System requires around 6 to 7 days of incubation to obtain a sufficient bacterial growth for the positive signal. 3) The System may result in false negative under the 5-day-culture method recommended by American Society for Microbiology in cases of using automated blood culture systems. 4) So-called the blind- or terminal-subcultures from the bottles are inevitable to perform for precluding the false negative cases.

Key words: BacT/Alert 3D, SA Bottle, *Mycobacterium abscessus*

Introduction

Bacteremia is an extremely important and not to be left untreated clinical condition. The prompt detection of the causative microorganism is a prerequisite for accurate identification and effective antimicrobial chemotherapy in case of bacteremia. In recent years, many automated blood culture instruments, including BacT/Alert 3D system, have been widely adopted to many clinical microbiology laboratories all over the world for the rapid diagnosis of bloodstream infections. Indeed, the introduction of such automated systems into clinical microbiology laboratories has brought to the marked reduction in intervals required to detect blood stream pathogens. However, false-positive and false-negative results still occur in routine blood culturing, although automated continuous blood culture systems have been improved successively [1-5].

In recent years, MB/BacT 240 and BacT/Alert 3D MB and FA bottles for the detection of mycobacteria have already been evaluated [6-8]. However, no evaluation study has been conducted concerning the SA bottles for the detection of mycobacteria. The objective of this study was to evaluate the BacT/Alert 3D SA bottles for detecting mycobacteria, especially belonging to the Runyon group IV, the rapid growers, focusing on the incubation period required to signal positive cultures.

A case of mycobacteremia due to Mycobacterium abscessus: We encountered the first case in Japan of *M. abscessus* mycobacteremia from peripheral blood of a 75-year-old female patient with high fever of unidentified origin in Shinshu University Hospital in December 2004. Blood culture samples were successively submitted to our laboratory with a pair of 2 bottles, the SA for obligate aerobes and facultative anaerobes and the SN for obligate anaerobes, for bacteriological examination, 27 times during 4 months. The positive blood cultures were repeatedly observed only in SA bottles 11 out of 27 times at the 5.3 to 6.9 days of incubation, and recovered acid-fast bacteria after sub-culturing techniques on Sheep Blood agar (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan) plates in an atmosphere with enhanced 5% CO_2. Among the positive blood cultures, gram-positive cocci and gram-negative rods were simultaneously isolated in addition to the acid-fast bacteria only once out of 11 positive times. Negative results were obtained at the remaining 16 times after 7 days of incubation. However, it should be noted that all of the 16 negative SA bottles with no positive signal uniformly demonstrated positive for acid-fast stain and yielded the growth of acid-fast bacteria without exception after terminal-subcultures on Sheep Blood Agar (Nippon Becton Dickinson Ltd., Tokyo, Japan) plates. The acid-fast bacteria were consequently identified as *Mycobacterium abscessus* using commercially available kit system DDH-mycobacteria (Kyokuto Seiyaku Co. Ltd., Tokyo, Japan), confirming with 16S rRNA sequence [9-10] analysis in our laboratory. The bacteria simultaneously isolated in SA bottles only once were *Stenotrophomonas maltophilia* (identi-

fied by MicroScan WalkAway System; Dade Behring, Inc., Tokyo, Japan) and methicillin-resistant *Staphylococcus ureolyticus* (identified by ID-Test SP-18 System; Nissui Seiyaku, Co. Ltd., Tokyo, Japan), respectively.

Materials and Methods

The blood culture system and culture bottles used:
Evaluation studies were conducted with the BacT/Alert 3D system ver. 4.00D (bioMérieux Japan Ltd., Tokyo, Japan) using SA and MB blood culture bottles.

Bacterial strains tested:
A strain of *M. abscessus* was used for the evaluation study, which was isolated from peripheral blood culture of an inpatient with fever of unknown origin. In addition, a *M. fortuitum* isolate from sputum was also used as the evaluation investigation, which were recovered and identified in our laboratory and stored in Micro-Bank vials (Pro-Lab Diagnostic, Ontario, Canada) at -83 °C in a deep freezer.

Seeding experimental procedures:
In order to avoid the false negative results in SA blood culture bottles, seeding experiments were carried out as follows. Respective 1ml. of a mycobacterial saline suspension (adjusted approximately to 10^1CFU/ml) of *M. abscessus* and/or *M. fortuitum* strain was inoculated into SA bottles together with MB bottles for comparison. The inoculated SA and MB bottles were placed in BacT/Alert 3D system as indicated in the manufacture's manual. Viable cells in the SA and MB bottles were counted as follows. Samples (100µl) for viability measurement were taken at various points of time, that is, very day inoculated, 2 days, 4 days, 6 days, 8 days, 10 days, 12 days, and 14 days of incubation, respectively. Viability was determined by the plate colony count technique. After serial 10-fold dilution technique with saline solution, 100µl of each diluent's sample was plated duplicate onto Sheep Blood agar plates. Appearing colonies were counted after 5 days of incubation at 37 °C in an atmosphere with enhanced 5% CO_2. We evaluated the BacT/Alert 3D SA bottles from the incubation intervals require to signal positive cultures and the growth curves based on the periodical viable cell counts.

Results

Shown in Figures 1 and 2 are the growth curves of *M. abscessus* and *M. fortuitum* including the days showing positive blood culture signals of the BacT/Alert 3D SA bottles. Proliferation of both of the strains in MB bottles were apparently enhanced in comparison with

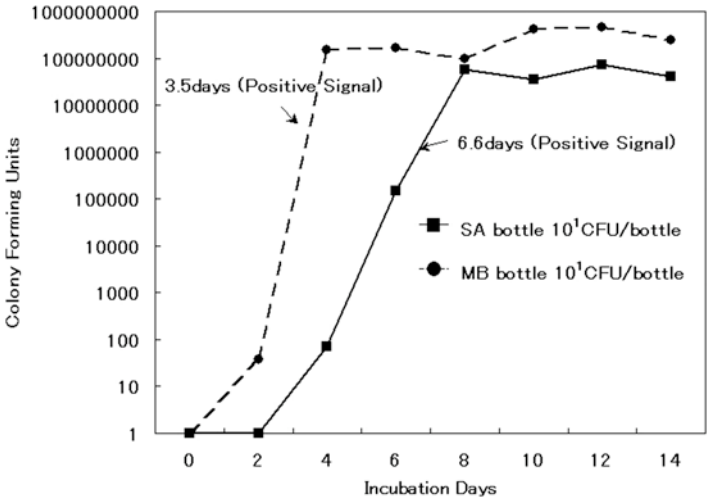

Fig. 1. Growth curves of *Mycobacterium abscessus*.

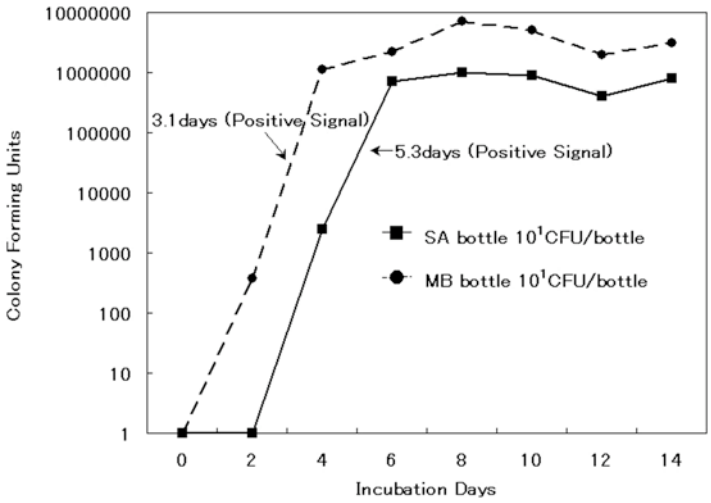

Fig. 2. Growth curves of *Mycobacterium fortuitum*.

that in SA bottles. Therefore, the time for growth detection in the MB bottle was shorter than that in the SA bottle. That is, in the case of *M. abscessus* strain, 3.2 days of incubation is needed as for MB bottles and 6.6 days of incubation as for SA bottles, respectively. In addition, in case of *M. fortuitum* strain, although the time for detection is somewhat short, almost the same results were obtained as shown in Fig. 2.

Discussion

Among the bacteremia, cases of mycobacteremia have infrequently been reported to date. In fact, this case of mycobacteremia we encountered in 2004 due to *M. abscessus* was, to the best of our knowledge, actually the first case in Japan. To be sure, this case had been given no consideration of mycobacteremia, however, *M. abscessus* was successfully recovered from BacT/Alert 3D SA aerobic bottles with no positive signals after terminal subcultures in an atmosphere with enhanced 5% CO_2.

Although the conventional BacT/Alert FA [8] or FN [11] blood culture bottles indeed supported the ample growth of *Mycobacterium* species, no evaluation study, thus far, has been documented as for SA bottles. Our case serves to reinforce the need for a high index of clinical suspicion of infections caused by unusual microorganisms during the course of routine blood cultures especially in cases beyond the consideration of mycobacteremia, and found that BacT/Alert 3D SA bottles actually support the growth of *Mycobacterium* species, belonging to the Runyon group IV.

As the positive bacterial detection by automated blood culture system assay only upon terminal subculture may occur in a very small number of patients, in cases of using automated blood culture systems subculture from the bottles of automated systems has little clinical utility for the recovery of blood stream pathogens. In fact, the reference standard in routine clinical microbiology laboratories was that every bottle with no positive signal should be discarded after 5 days of incubation without employing the blind- or terminal-subcultures [12-13]. Nevertheless, as shown in Figs. 1 and 2, our seeding experimental study clearly indicated that 5 days of incubation is indeed inadequate and that it is crucial that even the automated blood culture bottle specimens should be subcultured prior to discard in order to lessen the likelihood of missing a microbial pathogen especially in patients with fever of unidentified origin or with successive submission of blood culture samples without detecting any microbial pathogens. More to the point, in cases of taking no consideration into mycobacteremia, unless performed blind- or terminal-subcultures, our results also demonstrated that the majority of such cases be brought to false negative results.

Furthermore, we also considered that acid-fast stain, when negative for gram's stain, is extremely favorable to avoid false negative results.

In conclusion, our finding makes the blind- or terminal-subculture necessary for BacT/Alert 3D SA bottles after the incubation period to lessen the false negative results especially in cases of mycobacteremia.

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Address for correspondence:
Prof. Yoshiyuki Kawakami, Ph.D.
Division of Clinical Microbiology
Department of Biomedical Laboratory Sciences
School of Health Sciences
Shinshu University School of Medicine
Matsumoto, 390-8621
Japan
Phone: +81-263-37-2381
Fax: +81-263-37-2370;
E-mail: yk23724@gipac.shinshu-u.ac.jp