Clinical evaluation of FAPlus/FNPlus bottles compared with the combination of SA/SN and FA/FN bottles in the BacT/Alert blood culture system

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INTRODUCTION

Bloodstream infections are associated with a high morbidity and mortality (1). Blood culture is still essential for detecting bloodstream infections, although direct molecular detection methods have been developed in recent years (2). Advancements in blood culture techniques occurred in the 1990s following the introduction of automated incubators with continuous monitoring and enrichment of culture media (3). The BacT/Alert automated blood culture system (BioMérieux Co., Ltd., Tokyo, Japan) is one of the main systems used worldwide for the detection of bloodstream infections (4).

BioMérieux Co., Ltd. initially introduced standard aerobic (SA) and standard anaerobic (SN) culture bottles, followed by fastidious aerobic (FA) and anaerobic antibiotic neutralization (FN) bottles. SA/SN bottles, which contain supplemented soy-bean-casein digest broth medium, use 1 : 9 blood : broth dilution ratio. Because of this low dilution, these bottles were shown to have low detection rates and false-negative results in hospitalized patients who had received antimicrobial therapy before collection of blood (5). In fact, approximately 50%-90% of inpatients had already received antimicrobial therapy at the time of blood culture (6, 7), and the presence of antibiotics in the blood might inhibit the growth of microorganisms, particularly in SA/SN bottles. Unlike SA/SN bottles, FA/FN bottles contain absorbent charcoal and were developed to avoid the effect of antimicrobial agents and other substances in the blood that could inhibit bacterial growth (8). However, the presence of charcoal represents a major limiting factor for the application of Gram-staining, direct mass spectrometry (MS), and molecular methods (9, 10).

FAPlus/FNPlus bottles, which contain adsorbent polymeric beads and thus prevent difficulty in interpreting Gram-staining results, became available in December 2011. Several clinical studies have already demonstrated the advantages of FAPlus/FNPlus bottles over the earlier blood culture bottles (90) in terms of TTP. J. Med. Invest. 67:90-94, February, 2020

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RESULTS

The microorganism detection rate was significantly higher in the later period than in the earlier period (11.2% vs. 9.6%, \( P < 0.001 \)), particularly for Enterococcus and Streptococcus species, nonfermentative Gram-negative bacilli, and Helicobacter cinaedi. TTP for pathogens was longer when FAPlus/FNPlus bottles were used than when a combination of SA/SN and FA/FN bottles was used (14.9 vs. 13.3 h, \( P = 0.014 \)), particularly, in the case of Gram-negative bacilli including Escherichia coli.

CONCLUSION

The microorganism detection rate was improved with the use of FAPlus/FNPlus bottles compared with the combination of SA/SN and FA/FN bottles; however, FAPlus/FNPlus bottles seemed to be inferior to SA/SN and FA/FN bottles in terms of TTP.
infections were cultured as directed by the physicians as part of routine patient care. Throughout the study, we collected data on the types of blood culture bottles, bacterial identification results, and TTPs using the Laboratory Information System.

Blood culture bottles were incubated at 37°C under aerobic and anaerobic conditions in an automated BacT/Alert 3D system until a positive result was obtained or for up to 6 days. Microorganisms from positive blood cultures were further identified by using the Vitek MS system (BioMérieux Co., Ltd.) according to our routine procedures (14) and were further classified as pathogens or contaminants. When a blood culture yielded microorganisms commonly considered to be contaminants (e.g., coagulase-negative staphylococci, Corynebacterium species, Bacillus species, or Cutibacterium acnes), the culture was considered to be contaminated as in previous studies (15-17). The TTP was defined as the interval from loading bottles into the automated blood culture system until the growth signal was obtained, and it was automatically recorded by the blood culture system. If multiple species of microorganisms were detected in one bottle, which was defined as a polymicrobial culture, the first positive result was used to determine the TTP. Both clinical and laboratory blood culture procedures were unchanged during the study period, except the introduction of FAPlus/FNPlus bottles.

The ethics committee of our hospital waived the need for ethical approval and informed consent because of the retrospective and anonymized nature of the study.

**Statistical analysis**

Differences of nominal data were evaluated using the χ²-test. If a patient had multiple sets of positive blood cultures, the shortest TTP was used. The normality of the distribution of numerical data was examined by the Kolmogorov-Smirnov test, and the Mann-Whitney U test was performed if normality was not confirmed. All the tests were two-tailed, and P < 0.05 was considered to be statistically significant. Statistical analyses were performed with StatView 4.5 software (Abacus Concepts, Berkeley, CA) or modified R software (The R Foundation for Statistical Computing, Perugia, Italy).

**RESULTS**

**The microorganism detection rate**

During the first and second consecutive 12-month periods, 8771 and 8035 blood culture sets were obtained from 3362 and 2802 patients, respectively. Among them, the overall positive rates were 9.6% and 11.2%, respectively (Figure 1A). The microorganism detection rate was significantly higher when FAPlus/FNPlus bottles were used than when a combination of SA/SN and FA/FN bottles was used (P < 0.001). When pathogens and contaminants were assessed separately (Figure 1B), the detection rate of pathogens was significantly higher when FAPlus/FNPlus bottles were used (9.6%) than when SA/SN and FA/FN bottles were used (7.9%, P < 0.001). However, no significant difference was found in the detection rate of contaminants between the two sets of bottles (1.7% vs. 1.6%, P = 0.515). Further analysis revealed that a significantly higher detection rate of Gram-positive cocci including Enterococcus and Streptococcus species, nonfermentative Gram-negative bacilli (e.g., Pseudomonas aeruginosa and Serratia marcescens), Helicobacter cinaedi, and polymicrobial cultures was observed with FAPlus/FNPlus bottles than with the combination of SA/SN and FA/FN bottles (Table 1). Interestingly, H. cinaedi, which was included with other Gram-negative bacilli, was not detected when SA/SN and FA/FN bottles were used, but it was found in nine culture sets when FAPlus/FNPlus bottles were used (P < 0.001).

**Time to positivity**

The TTP data for the two sets of bottles are compared in Table 2; overall TTP was not significantly different in both the sets (median, 15 vs. 16 h; P = 0.145), whereas the TTP for pathogens was significantly longer with FAPlus/FNPlus bottles than with SA/SN and FA/FN bottles (median, 14.9 vs. 13.3 h; P = 0.014). Further analysis revealed that the TTP for Gram-negative bacilli including Escherichia coli, Aeromonas species, Aggregatibacter segnis, Capnocytophaga ochracea, Capnocytophaga sputigena, Eikenella corrodens, Haemophilus influenzae, Brevibacillus laterosporus, and non-identifiable Gram-negative bacilli were significantly longer.
when FAPlus/FNPlus bottles were used. The median TTP for *H. cinaedi* was 90 h [95% confidence interval (CI); range, 79.6-136.7 h]. After excluding *H. cinaedi*, TTP for pathogens was also longer when FAPlus/FNPlus bottles were used (median, 14.8 h; 95% CI; range, 12.8-14.2 h; *P* = 0.036).

### DISCUSSION

This study showed that the microorganism detection rate was higher and the TTP for pathogens was significantly longer when FAPlus/FNPlus bottles were used than when SA/SN and FA/FN bottles were used.

Some researchers have already reported the superiority of FAPlus/FNPlus bottles over either SA/SN bottles or FA/FN bottles (4, 11, 12); however, the comparison of the performance of FAPlus/FNPlus bottles and combination of SA/SN and FA/FN bottles is not yet reported. Interestingly, our study showed that FAPlus/FNPlus bottles might be superior for detecting Gram-positive cocci including *Enterococcus* and *Streptococcus* species, nonfermentative Gram-negative bacilli, and *H. cinaedi*. Furthermore, polymicrobial cultures were significantly more often found in FAPlus/FNPlus bottles. Because the mortality rate was reported to be 2.15 times higher in patients with polymicrobial bloodstream infections than in those with monomicrobial infections (18), the increased detection rate for polymicrobial cultures could have a profound clinical impact.

It is also noteworthy that nine cases of *H. cinaedi* infection were detected by the FAPlus/FNPlus bottles. *H. cinaedi* causes enteric or bloodstream infections, and bacteremia seems to be more common in Japan (19). Reports of the detection of *H. cinaedi* using the BacT/Alert blood culture system have been very limited (20); however, to the best of our knowledge, the present study is the first to show that the detection rate of *H. cinaedi* was increased when FAPlus/FNPlus bottles were used. Better detection of *H. cinaedi* is important and has a great clinical impact, particularly in immunocompromized patients. Lee et al. reported that FAPlus/FNPlus bottles detected more pathogens, although a lower mean volume of blood was inoculated into FAPlus/FNPlus bottles than into SA/SN bottles (12). Considering all our results together, the threshold of FAPlus/FNPlus bottles for positive blood culture is potentially lower than that of SA/SN or FA/FN bottles.

An increase of microorganism detection may be caused at the expense of a higher contamination rate (21, 22). However, our results showed that there was no significant difference in the contamination rates between the two sets of bottles. The contamination rate in our study (1.6%-1.7%) was below the optimal contamination rates described in CLSI guidelines (23). The reason for this is not clear, but a possible explanation is good compliance of phlebotomists with the blood culture procedure throughout the two study periods with different sets of bottles.

TTP for pathogens is important with regard to patient management. Several studies have demonstrated a significant decrease in TTP with FAPlus/FNPlus bottles compared with FA/FN or SA/SN bottles (11, 12). However, our findings were different; a significantly longer TTP was observed with pathogens, particularly Gram-negative bacilli including *E. coli*, in FAPlus/FNPlus bottles than in SA/SN and FA/FN bottles. Indeed, a previous study investigated a small number of samples (11), and the other study did not comply with the recommended blood inoculation volume (12). Our results show that FAPlus/FNPlus bottles might be inferior to SA/SN or FA/FN bottles in terms of TTP.

### Table 1. Microorganisms detected in blood cultures comparing the two periods

| Microorganism(s)                        | Sep 2012-Aug 2013 (SA/SN and FA/FN) | Sep 2013-Aug 2014 (FAPlus/FNPlus) | *P* value |
|-----------------------------------------|-------------------------------------|-----------------------------------|-----------|
| Pathogens                               | 694 (7.9)                           | 772 (9.6)                         | <0.001    |
| Gram-positive cocci                     | 228 (2.6)                           | 272 (3.4)                         | <0.001    |
| *Staphylococcus aureus*                 | 89 (1.0)                            | 90 (1.1)                          | 0.506     |
| *Enterococcus* species                  | 32 (0.4)                            | 50 (0.6)                          | 0.017     |
| *Streptococcus* species                 | 97 (1.1)                            | 124 (1.5)                         | 0.013     |
| Other Gram-positive cocci               | 10 (0.1)                            | 8 (0.1)                           | 0.775     |
| Gram-negative bacilli                   | 354 (4.0)                           | 374 (4.7)                         | 0.048     |
| *Enterobacteriales*                     | 311 (3.5)                           | 280 (3.5)                         | 0.830     |
| Nonfermentative Gram-negative bacilli   | 27 (0.3)                            | 73 (0.9)                          | <0.001    |
| Other Gram-negative bacilli             | 16 (0.2)                            | 21 (0.3)                          | 0.275     |
| *Helicobacter cinaedi*                  | 0 (0.0)                             | 9 (0.1)                           | 0.001     |
| Gram-negative cocci                     | 5 (0.1)                             | 0 (0.0)                           | 0.064     |
| Gram-positive bacilli                   | 4 (0.0)                             | 7 (0.1)                           | 0.296     |
| Anaerobes                               | 36 (0.4)                            | 33 (0.4)                          | 0.998     |
| Fungi                                   | 18 (0.2)                            | 20 (0.2)                          | 0.551     |
| Polymicrobial cultures                  | 49 (0.6)                            | 66 (0.8)                          | 0.039     |
| Contaminants                            | 151 (1.7)                           | 128 (1.6)                         | 0.515     |
| Coagulase-negative *Staphylococci*      | 106 (1.2)                           | 85 (1.1)                          | 0.357     |
| *Bacillus* species                      | 25 (0.3)                            | 24 (0.3)                          | 0.870     |
| *Corynebacterium* species               | 6 (0.1)                             | 12 (0.1)                          | 0.109     |
| *Cutibacterium acnes*                   | 14 (0.2)                            | 7 (0.1)                           | 0.184     |
| All microorganisms                      | 845 (9.6)                           | 900 (11.2)                        | <0.001    |
This study had some limitations. The first was its before-vs.-after design, which introduces some confounders and is less powerful than a direct, synchronous comparison. It was also impossible to exclude selection bias such as changes in hospital care, patient characteristics, and infectious diseases. However, the two study periods were consecutive, and there were no changes in the blood culture procedures of our hospital. Indeed, the contamination rate was extremely low during both the periods. Second, we did not investigate whether patients received antimicrobial therapy before blood collection, so we could not assess the microorganism detection capacity of the FAPlus/FNPlus bottles for patients taking antimicrobial therapy. Kirn et al. reported an improved performance of FAPlus/FNPlus bottles compared with FA/FN bottles regardless of antimicrobial treatment (11). The superior performance of FAPlus/FNPlus bottles may be related to the inactivation of antibiotics as well as the inactivation of toxic compounds and cytokines. Finally, we did not record the blood volumes of each bottle. Blood volume is known to be the most important factor affecting the quality of a blood culture (24). Accordingly, a further study including blood volume information is warranted.

In conclusion, the pathogen detection rate was higher with FAPlus/FNPlus bottles than with the combination of SA/SN and FA/FN bottles. In particular, there was a significant increase in the detection of *Enterococcus* and *Streptococcus* species, nonfermentative Gram-negative bacilli, *H. cinaedi*, and polymicrobial cultures. However, FAPlus/FNPlus bottles might be inferior to SA/SN and FA/FN bottles in terms of TTP. Our study suggests a lower threshold for positive blood cultures and lower bacterial growth rates in FAPlus/FNPlus bottles than in SA/SN and FA/FN bottles.

### Table 2. Time to positivity in blood cultures comparing the two periods

| Microorganism(s)                  | Sep 2012-Aug 2013 (SA/SN and FA/FN) | Sep 2013-Aug 2014 (FAPlus/FNPlus) | P value |
|-----------------------------------|-----------------------------------|-----------------------------------|---------|
|                                   | No. Median 95% CI                  | No. Median 95% CI                  |         |
| Pathogens                         | 493 13.3 12.8-14.2                 | 541 14.9 14.3-15.9                 | 0.014   |
| Gram-positive cocci               | 163 14.1 13.2-15.5                 | 185 14.8 13.7-15.8                 | 0.407   |
| *Staphylococcus aureus*           | 59 14.6 13.2-18.4                  | 60 17.5 14.8-18.3                  | 0.288   |
| *Enterococcus* species            | 27 15.5 12.6-16.7                  | 40 15.7 14.3-18.8                  | 0.247   |
| *Streptococcus* species           | 68 12.9 11.1-14.0                  | 80 12.1 11.2-13.8                  | 0.668   |
| Other Gram-positive cocci         | 9 24.7 20.1-27.7                   | 5 38.8 17.4-76.5                   | 0.364   |
| Gram-negative bacilli             | 239 11.8 11.1-12.4                 | 252 14.0 12.9-14.6                 | <0.001  |
| *Enterobacteriales*               | 205 11.1 10.8-11.9                 | 185 12.3 11.9-13.0                 | 0.032   |
| *Escherichia coli*                | 98 10.9 10.1-11.8                  | 112 12.0 11.3-13.0                 | 0.043   |
| *Klebsiella pneumoniae*           | 59 10.8 9.6-13.7                  | 29 12.2 10.5-15.8                  | 0.303   |
| *Klebsiella oxytoca*              | 11 12.8 9.7-24.9                  | 5 10.2 NA 0.743                    |         |
| *Proteus mirabilis*               | 7 17.4 8.2-59.2                   | 6 13.1 6.2-14.3                   | 0.352   |
| *Enterobacter cloacae* complex    | 10 11.9 6.3-13.0                  | 5 11.3 NA 0.667                    |         |
| Other *Enterobacteriales*         | 20 14.2 13.0-28.9                  | 28 15.2 14.0-23.7                  | 0.917   |
| Nonfermentative Gram-negative bacilli | 22 21.6 18.4-29.1                | 51 21.1 19.6-22.3                  | 0.568   |
| Other Gram-negative bacilli       | 12 21.1 10.0-52.5                  | 16 79.6 46.1-90.0                  | 0.013   |
| *Helicobacter cinaedi*            | 0 90.0 79.6-136.7                 | 112 22.0 19.6-25.1                 | 0.972   |
| Other Gram-negative bacilli       | 12 21.1 10.0-51.1                  | 9 39.8 21.5-69.4                  | 0.345   |
| Gram-negative cocci               | 4 22.1 15.0-62.2                  | 0 0 0.057                          |         |
| Gram-positive bacilli             | 3 22.9 17.4-25.2                  | 5 39.4 20.9-60.4                  | 0.393   |
| Anaerobes                         | 27 51.5 28.8-64.8                 | 28 36.6 31.1-41.3                  | 0.386   |
| Fungi                             | 17 38.3 33.4-52.1                 | 16 36.1 24.3-59.1                  | 0.829   |
| Polymicrobial cultures            | 40 15.2 12.1-20.6                  | 55 14.1 12.8-17.8                  | 0.684   |
| Contaminants                      | 131 22.8 20.2-24.2                 | 101 22.5 19.6-25.1                 |         |
| Coagulase-negative *Staphylococci*| 94 23.1 21.7-24.7                 | 69 21.8 19.4-24.4                  | 0.241   |
| *Bacillus* species                | 23 12.2 11.6-13.2                 | 16 12.0 10.8-18.7                  | 0.710   |
| *Corynebacterium* species         | 6 43.9 29.1-69.8                  | 10 36.0 32.0-45.6                  | 0.492   |
| *Cutibacterium* acnes             | 8 118.3 111.3-133.8               | 6 130.7 114.1-137.0               | 0.491   |
| All microorganisms                | 624 15.0 14.1-15.8                 | 642 16.0 14.9-17.3                 | 0.145   |

*NA, not applicable because of insufficient number of samples*

*a Other *Enterobacteriales* includes *Klebsiella aerogenes*, *Citrobacter koseri*, *Citrobacter freundii*, *Citrobacter amalonaticus*, *Serratia marcescens*, *Cronobacter sakazakii*, *Cronobacter malonaticus*, *Morunella morganti*, *Pantea dispersa*, *Salmonella group*, *Proteus vulgaris*, *Raoultella planticola*, *Raoultella ornithinolytica*, *Edwardsiella hoshinae*, *Edwardsiella tarda*, *Hafnia alvei*, and *Leclercia adenoscarborylata*.

*b Other Gram-negative bacilli excluding *H. cinaedi* includes *Aeromonas* species, *Aggregatibacter segnis*, *Capnocytophaga ochracea*, *Capnocytophaga spautigena*, *Elkenella corrodens*, *Haemophilus influenzae*, *Brevibacillus laterosporus*, and non-identifiable Gram-negative bacilli.*
CONFLICT OF INTEREST
None

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