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Multiplex real-time PCR and blood culture for identification of bloodstream pathogens in patients with suspected sepsis

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Abstract

Severe sepsis is increasingly a cause of death. Rapid and correct initial antimicrobial treatment reduces mortality. The aetiological agent(s) cannot always be found in blood cultures (BCs). A novel multiplex PCR test (Septi*Fast* (alpha version)) that allows identification of 20 bacterial and fungal species directly from blood was used, comparatively with BC, in a multicentre trial of patients with suspected bacterial or fungal sepsis. Five hundred and fifty-eight paired samples from 359 patients were evaluated. The rate of positivity was 17% for BC and 26% for Septi*Fast*. Ninety-six microorganisms were isolated with BC, and 186 microorganisms were identified with Septi*Fast*; 231 microrganisms were found by combining the two tests. Of the 96 isolates identified with BC, 22 isolates were considered to be contaminants. Of the remaining 74 non-contaminant BC isolates available for comparison with Septi*Fast*, 50 were identified as a species identical to the species identified with Septi*Fast* in the paired sample. Of the remaining 24 BC isolates for which the species, identified in the BC, could not be detected in the paired Septi*Fast* sample, 18 BC isolates were identified as a species included in the Septi*Fast* master list, and six BC isolates were identified as a species not included in the Septi*Fast* master list. With Septi*Fast*, 186 microorganisms were identified, 12 of which were considered to be contaminants. Of the 174 clinically relevant microorganisms identified with Septi*Fast*, 50 (29%) were detected by BC. More than half of the remaining microorganisms identified with Septi*Fast* (but not isolated after BC) were also found in routine cultures of other relevant samples taken from the patients. Future clinical studies should assess whether the use of Septi*Fast* is of significant advantage in the detection of bloodstream pathogens.

Keywords: Blood culture, LightCycler, multiplex, PCR, sepsis, Septi*Fast*
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Introduction

Sepsis represents a rising healthcare burden. The incidence of sepsis is increasing, as is the number of sepsis-related deaths [1]. There were 659 935 cases of sepsis reported in the USA in 2000, with a bias towards men (relative risk 1.3) and with an average mortality rate of 18%. Ten bacterial species (fungi were not investigated) found in the SENTRY Antimicrobial Surveillance Program (1997–2002) accounted for 89–92% of all isolates. The ranking of the presence of these species was very similar across North America, Latin America, and Europe [2]. In the USA, a nationwide hospital study of 24 179 nosocomial bloodstream infections showed that the nine most frequent bacterial pathogens were all included in the ten most frequent pathogens found in the SENTRY surveillance, with *Candida* being the fourth most common pathogen isolated [3].

The goal of blood culture (BC) in septic patients is to isolate a microorganism for identification, susceptibility testing, and typing, in order to optimize initial empirical therapy. Rapid and correct initial antimicrobial treatment is

crucial for successful treatment of septic patients [4–7]. Many years of research in BC technology has led to improved culture media and automated BC systems with increased sensitivity, inactivation of antimicrobial agents, rapid detection of microbial growth, and improved detection of fungi and fastidious microorganisms [8,9]. Despite such advances, the rates of positive BC from patients with different categories of sepsis vary greatly, depending on the degree of sepsis [10–12].

PCR assays developed for specific detection of pathogens in the blood were described as early as 1993 [13–16]. Further development led to broad-spectrum PCR assays, allowing more universal detection of microorganisms [17–20]. Such broad-spectrum PCR methodology has been hampered by problems of contamination. Contaminating microbial DNA can be introduced either during the sampling process or by handling in the microbiology laboratory [18,21–23]. In particular, PCR assay kit components, such as reagents for DNA extraction [24] and polymerases, are usually contaminated [25,26]. In addition, reservations have been voiced concerning the ability of PCR to achieve the required sensitivity, because of small sample volumes and the perceived necessity for an initial (and time-consuming) enrichment step involving microbial growth [27]. The diversity of the pathogens concerned necessitates the incorporation of multiple probes for multiple targets, once again prompting questions about the ease of use of the test and the time required to obtain results.

Septi*Fast* has recently been used in the molecular diagnosis of sepsis in neutropenic patients [28] and in emergency room, intensive-care unit and general medicine patients with suspected bloodstream infection [29]. We describe a large multicentre evaluation of Septi*Fast*, which was designed to be sensitive and rapid and to allow the identification of 20 species of bacteria and fungi that are responsible for up to 95% of all positive BCs.

Materials and Methods

Material

This multicentre study was initiated and performed in six centres; in each centre, 31–129 episodes were included between June and October 2004. An episode was defined as a BC and a simultaneously obtained blood sample for the Septi*Fast* test. All patients included were clinically suspected to have bacterial or fungal sepsis. Signs of the systemic inflammatory response syndrome (temperature, heart rate, respiratory rate, and white blood cell count) were recorded for all patients and registered in a case report form. Data registered in the case report form also included antimicrobial therapy and the suspected focus of infection. A patient could be included with more than one episode (one to three episodes per patient). The results of the Septi*Fast* test were not used to guide clinical treatment. The relevant institutional or regional review boards or ethics committees approved the research protocol, and participants gave written informed consent, except in one centre, where this was not required by the local ethics committee.

Methods

Blood culture, blood for the SeptiFast test and supplementary microbiological samples. Skin disinfection was performed twice, with ethanol (70%) or propanol (70%), and blood for the BC was drawn by a phlebotomist wearing sterile gloves. A single venipuncture was used to draw samples for 2×2 bottles of BacT/Alert (Biomerieux S.A., Marcy-l Etoile, France) (30–40 mL of blood) or three bottles of BACTEC (BD Diagnostics, Sparks, MD, USA) (25–30 mL of blood). Immediately after blood was drawn for BC (8–10 mL per BC bottle), 5 mL of whole blood was collected in sterile VACUETTE EDTA K2E tubes (Greiner Bio-One, Frickenhausen, Germany) for the alpha version of the Septi*Fast* test (see below). Each BC was performed in a pair of aerobic/anaerobic bottles. Blood for one or two additional BC sets was collected from each patient within a 24-h period and included in episode evaluation. The BCs were analysed using the semi-automated blood culture systems BACTEC or BacT/ALERT, according to laboratory-defined standard operating procedures, and time to culture positivity was registered. Systematic collection of samples from other body sites was not part of the protocol. Microbiological results from supplementary samples were obtained only when clinical indications were present. Identification of microorganisms from a suspected infectious focus within 48 h of the episode was used to resolve discrepancies in the results.

The SeptiFast kit. The internal transcribed sequences located between the bacterial 16S and 23S ribosomal RNA genes and the fungal 18S and 5.6S ribosomal RNA genes were selected as the targets for amplification and microorganism identification (Roche Diagnostics GmbH, Penzberg, Germany) [30,31]. Information concerning the sequences of primers and probes is proprietary. A Septi*Fast* test was taken to be positive when an internal hybridization probe emitted a fluorescent signal above a defined threshold level. The species identification of a positive Septi*Fast* test was based upon a subsequent melting curve analysis.

The analytical sensitivity of the assay as determined by the manufacturer is between 3 and 100 CFU/mL, depending on

TABLE 1. Multiplex PCR test Septi*Fast* master list; the bacteria and fungi listed can be detected by a three-capillary multiplex real-time LightCycler 2.0 system (limits of detection of microorganisms are described in the footnotes[a])

Gram-negative	Gram-positive	Fungi
Escherichia coli[b]	*Staphylococcus aureus*[c]	*Candida albicans*[c]
Klebsiella (pneumoniae[c]*/oxytoca*[c]*)*	Coagulase-negative staphylococci[d]	*Candida tropicalis*[c]
Serratia marcescens[b]	*Streptococcus pneumoniae*[c]	*Candida parapsilosis*[c]
Enterobacter (cloacae[c]*/aerogenes*[c]*)*	*Streptococcus* spp.[e]	*Candida glabrata*
Proteus mirabilis[b]	*Enterococcus faecium*[c]	*Candida krusei*[c]
Pseudomonas aeruginosa[b]	*Enterococcus faecalis*[c]	*Aspergillus fumigatus*[b]
Acinetobacter baumannii[c]	–	–
Stenotrophomonas maltophilia[c]	–	–

[a]Limit of detection of microorganisms as described in the package insert of the commercial assay: all microorganisms in the Septi*Fast* master list found at concentrations of 100 CFU/mL.
[b]Microorganisms found in 20/20; analysis at 3 CFU/mL.
[c]Microorganisms found in 20/20; analysis at 30 CFU/mL.
[d]The coagulase-negative staphylococci that can be identified with the commercial assay are described in the package insert as *S. epidermidis*, *S. hemolyticus*, *S. hominis*, *S. pasteuri*, *S. warneri*, *S. cohnii*, *S. lugdenensis*, *S. capitis*, *S. caprae*, *S. saphrophyticus*, and *S. xylosis*.
[e]The *Streptococcus* species that can be identified with the commercial assay are described in the package insert as *S. agalactiae*, *S. pyogenes*, *S. anginosus*, *S. bovis*, *S. constellatus*, *S. cristatus*, *S. gotdonii*, *S. intermedius*, *S. milleri*, *S. mitis*, *S. mutans*, *S. oralis*, *S. parasanguinis*, *S. salivarius*, *S. sanguinis*, *S. thermophilus*, *S. vestibularis*, and *S. viridans*.

the microorganism (Table 1). All reagents, instruments and disposables were obtained from Roche Molecular Diagnostics (Roche Diagnostics GmbH, Penzberg, Germany).

Sample preparation for the SeptiFast test and the PCR procedure The preparation of DNA and testing were performed as recommended by the manufacturer, using the alpha version of the Septi*Fast* lys kit, the Septi*Fast* prep kit, and the LightCycler Septi*Fast* kit, which were similar but not identical to the commercially available products. The principal difference between the alpha version of the Septi*Fast* kit and the commercial Septi*Fast* kit is the automated identification of species and controls by the Septi*Fast* identification software, where low concentrations of streptococci and coagulase-negative staphylococci (CoNS) are not displayed as positive results [30]. These 'low copy number positives' can be found manually by examining the amplification and melting curve data obtained from the 'Gram-positive' capillary.

All MGRADE reagents and disposables used in the Septi*Fast* test were produced using stringent DNA-depleting procedures as stated by the manufacturer. The mechanical lysis of the bacteria was performed using the Septi*Fast* lys kit and the MagNA Lyser instrument. After the MagNA Lyser procedure was performed, the internal control (IC) of the LightCycler Septi*Fast* kit was added to each sample and to the negative control (NC). Manual DNA extraction was performed according to the manufacturer's instructions, using the Septi*Fast* prep kit. Blood (in two aliquots of 1.5 mL) was lysed in the MagNA Lyser, using glass beads. Subsequently, total DNA was extracted from 2 mL and eluted in a final volume of 300 μL. Fifty microlitres was used for each LightCycler capillary. The amount of DNA available for amplification in each Septi*Fast* capillary originated from 1/3 mL of blood, as compared with the 30–40 mL of blood obtained for a BC set.

Potential amplicon contaminations were eliminated using AmpErase (Roche Diagnostics). The eluates were then subjected to multiplex real-time PCR analysis, using the LightCycler 2.0 instrument (Roche). LightCycler data were only considered valid if the corresponding assay controls (reagent control and the IC of the NC) were in the assigned T_m range and the NC was negative. A complete Septi*Fast* workflow included samples from seven patients and was analysed in 6 h.

Data analysis and interpretation A non-contaminant, positive BC result was assumed to represent a true infection according to previously published data [32–34]. Microorganisms contained in the Septi*Fast* master list (Table 1) were identified by characteristic peaks recognized with LightCycler software and by manual analysis of T_m values. The Septi*Fast* test was recorded as negative when the IC was positive and no other signals were detected. Septi*Fast* samples with a negative IC (as a sign of potential inhibition) were included in the study as negative results.

Whether microorganisms identified with the Septi*Fast* test represented a true infection was evaluated retrospectively by considering the identity of the microorganism and the focus of infection as diagnosed by the clinician, and by comparing the BC results with findings from other clinical specimens.

Evaluation of BC and SeptiFast test contaminants Typical BC contaminants (CoNS, *Streptococcus* spp., *Propionibacterium* spp., and *Bacillus* spp.) were identified by the local investigators. Generally, isolates were considered to be contaminants if only one positive BC result was available within 48 h. If two BC results were obtained with different samples from the same patient within 48 h, including one positive and one negative result, the positive BC was considered to be contaminated. However, if both results within this time period were positive, they were considered to indicate infection. In cases where three samples were drawn from the same patient within the same 48-h period, the patient was considered to have an infection if two of the three samples or all three samples yielded the same microorganism. The BC was considered to be contaminated if only one of three samples from the 48-h period was culture-positive [32–36].

Evaluation of a SeptiFast test result as contaminant was performed for CoNS and *Streptococcus* spp. on the basis of the following criteria: (i) BC bottles were negative; (ii) time to positive BC with contaminant was more than 24 h; (iii) microorganisms were not found in other culture specimens; and (iv) the crossing point (CP) was higher than 35 cycles.

This CP cut-off value was calculated from an in-house (Roche) experiment, in which a SeptiFast test was performed with samples from healthy participants and in which low copy numbers and high CP values were found for occasional CoNS and less frequent streptococci (data not shown). No other bacteria, or fungi, were found in these healthy participants.

Statistical methods The McNemar test was used for testing the differences between paired proportions. Comparisons of episodes and isolates/microorganisms were made using chi-square tests, with Yates' correction when the number of samples was <20.

Overall agreement between blood culture and SeptiFast Analyses of overall agreement between the findings from the SeptiFast test and BC were performed as follows: first, as an episode-to-episode comparison (Table 2)—positive episode agreement between the two tests could be achieved in spite of the identification of different clinical isolates/microorganisms with the two tests; and second, as an isolate-to-microorganism comparison (Table 3)—this could be considered to be a more direct comparison of the two tests than the episode-to-episode comparison. In the assessment of agreement, contaminant episodes or contaminant isolates/microorganisms were included, even though the two test systems could be contaminated at different phases and therefore could not be expected to find the same contaminant [37].

TABLE 2. Episode agreement between blood culture and SeptiFast test

	Blood culture			
	Positive	Negative	Contaminant	Total
SeptiFast				
Positive	58	77	3	138
Negative	16	382	16	414
Contaminant	0	3	3	6
Total	74	462	22	558

Significantly more episodes were positive by SeptiFast ($p < 0.0001$).
Overall percentage agreement, (58 + 382 + 3)/558: 79% (95% CI 76–83%).
Agreement of SeptiFast with positive blood culture, 58/74: 78% (95% CI 67–87%).
Agreement of SeptiFast with negative blood culture, 382/462: 83% (95% CI 79–86%).

TABLE 3. Isolate/microorganism agreement between blood culture and SeptiFast test

	Blood culture			
	Positive	Negative	Contaminant	Total
SeptiFast				
Positive	50	124	0	174
Negative	24	382	21	427
Contaminant	0	11	1	12
Total	74	517	22	613

Significantly more microorganisms were identified by SeptiFast, $p < 0.0001$.
Overall percentage agreement, (50 + 382 + 1)/613: 71% (95% CI 67–74%).
Agreement of SeptiFast with positive blood culture, 50/74: 68% (95% CI 56–78%).
Agreement of SeptiFast with negative blood culture, 382/517: 74% (95% CI 70–78%).

Results

Patients

Three hundred and fifty-nine patients were included in the study. From these patients, 558 episodes fulfilled the inclusion criteria of the study, with BC and SeptiFast samples obtained simultaneously. Of these, 382 episodes were negative in both BC and SeptiFast, and 176 episodes were positive in at least one test system (Table 2). The 176 positive episodes resulted in a total of 231 isolates/microrganisms found by either of the two methodologies (Table 4). Seventy episodes (12.5%) in which the IC was negative in the SeptiFast test were included in the study as SeptiFast negatives.

BC and SeptiFast episodes

For BC, the positive episode rate was 17% (96/558). Of the 96 positive episodes, 74 episodes contained clinical isolates and 22 episodes contained contaminant BC isolates only. The positive episode rate of SeptiFast was 26% (144/558). Of the 144 SeptiFast-positive episodes, 138 contained clinical microorganisms (six contaminants were found with clinical microorganisms), and six episodes contained contaminants only. The BC contamination rate was 3.9% (22/558), and the contamination rate for SeptiFast was 2.2% (12/558). Excluding contaminants, the positive rate of SeptiFast was twice as high as that of BC (25%, 138/558 vs. 13%, 74/558; Table 2).

BC and SeptiFast isolates/microorganisms

In this study, a single microorganism was detected in 74 non-contaminant positive BCs. Polymicrobial infection, however, was detected by SeptiFast at an average of 1.3 microorganisms per sample (112 episodes with one microorganism, 18 episodes with two microorganisms, six episodes with three microorganisms, and two episodes with four microorganisms). Fifty of the 74 positive BC isolates (68%) were

TABLE 4. Number of microorganisms/isolates detected with Septi*Fast* or blood culture

Pathogen	Any system	Both systems	Septi *Fast* only	Blood culture only	p[a]
Gram-positive					
Staphylococcus aureus	32	12	20	0	<0.0001
Streptococcus spp.	16	5	9	2	NS
Enterococcus faecalis	14	5	7	2	NS
Enterococcus faecium	14	2	8	4	NS
Staphylococcus spp.	11	8	2	1	NS
Streptococcus pneumoniae	3	0	2	1	NS
Gram-negative					
Escherichia coli	27	10	16	1	<0.0001
Stenotrophomonas maltophilia	12	2	10	0	<0.002
Klebsiella pneumoniae/oxytoca	11	0	10	1	<0.01
Enterobacter cloacae/aerogenes	8	0	8	0	<0.008
Pseudomonas aeruginosa	6	0	5	1	NS
Serratia marcescens	1	1	0	0	NS
Fungi					
Candida albicans	17	2	13	2	<0.008
Aspergillus fumigatus	12	0	12	0	<0.0005
Candida parapsilosis	7	2	2	3	NS
Candida tropicalis	1	1	0	0	NS
Not in Septi*Fast* master list	6	0	0	6	–
Subtotal	198	50	124	24	<0.0001
Contaminant microrganisms/isolates					
Staphylococcus spp.	19	1	2	16	–
Streptococcus spp.	10	0	9	1	–
Propionibacterium spp.[b]	3	0	0	3	–
Bacillus spp.[b]	1	0	0	1	–
Subtotal	33	1	11	21	–
Total	231				

NS, not significant.
[a]The McNemar test was used for testing the difference between paired proportions (Septi*Fast* only vs. blood culture only).
[b]Not included in the Septi*Fast* master list.

detected with both systems. Of the 24 BC-positive but Septi*Fast*-negative isolates, six isolates were not included and 18 isolates (ten different species) were included in the list of microorganisms that can be detected by Septi*Fast* (Table 1). The 174 Septi*Fast* clinical microorganisms (Table 3) were detected in 138 episodes. Of the 174 microorganisms, 50 (29%) microorganisms were also detected by BC. Of the 124 microorganisms detected only by Septi*Fast*, 67 (54%) could be confirmed as clinical pathogens by culture of the same microorganism/species from a relevant anatomical site within the same clinical time frame. The remaining 57 microorganisms found using Septi*Fast* only could not be confirmed, as the microorganism did not grow in culture from a clinically relevant site or because samples from such a site were not obtained. In total, 117/174 (67%) microorganisms found using Septi*Fast* could be confirmed by culture. The isolates/microorganisms found using BC and/or Septi*Fast* are shown in Table 4.

Episodes where *Staphylococcus aureus* was found by BC and using Septi*Fast* had a mean CP of 26.8 (standard deviation 3.8), whereas episodes that were Septi*Fast*-positive and BC-negative had a mean CP of 29.7 (standard deviation 3.8). This difference in CP suggests that the amount of *S. aureus* DNA present in Septi*Fast* samples where the paired BC sample is negative is significantly lower than the amount of *S. aureus* DNA present in Septi*Fast* samples where the paired BC sample is positive.

Low-level contamination in Septi*Fast*

Low-level contamination (included as a negative result) in the Septi*Fast* PCR (a CP higher than 35 cycles) was seen in 57 episodes due to CoNS and in two cases due to *Streptococcus* spp. (11%, in 558 episodes).

Agreement between BC and Septi*Fast* results

The overall episode-to-episode agreement (positives with positives plus negatives with negatives) between Septi*Fast* and BC was 79% (Table 2). For positive Septi*Fast* results, the agreement with BC was 78%, and for negative Septi*Fast* results, the agreement with BC was 83% (Table 2).

The overall microorganism-to-isolate agreement between Septi*Fast* and BC was 71% (Table 3). For positive Septi*Fast* results, the agreement with BC was 68%, and for negative Septi*Fast* results, the agreement with BC was 74% (Table 3).

In the absence of a laboratory reference standard for the diagnosis of sepsis, we compared the two tests in three different ways: (i) the BC result is 100% accurate; (ii) the Septi*Fast* result is 100% accurate; and (iii) the combined findings by BC or Septi*Fast*, excluding contaminant isolates/microorganisms, are 100% accurate. An analysis of the positive findings consequently leads to different sensitivity rates for BC and Septi*Fast*. On the basis of these definitions, the sensitivity of BC (ability to find a positive result) would be 29% if Septi*Fast* were used as the standard. On the other hand, the sensitivity of Septi*Fast* would be 68% if BC were the reference standard. If all non-contaminant findings by BC or Septi*Fast* were regarded as true positives, the sensitivity of BC would be 37%, and that of Septi*Fast* would be 88%.

Influence of antimicrobial therapy on BC and Septi*Fast* test results

Septi*Fast* detected 124 microorganisms in patients for whom the paired BC was negative. In 64 (52%, 95% CI 42–61%) of these episodes, at the time of sampling the patient had received antimicrobial therapy considered likely to be effective against the microorganism detected using Septi*Fast*. BC detected 74 isolates. In 27 (37%, 95% CI 27–49%) of these BC episodes, at the time of sampling the patient had received antimicrobial therapy effective against the microorganism.

Time to positive BC or Septi*Fast* test

The time to a positive result was documented in 36 of 50 episodes during which the same microorganism was detected using BC and Septi*Fast*. The median time to the first positive BC signal (equalling the time to Gram stain—not the time to the final species identification) was 2 days (range: 1–10 days). If Septi*Fast* had been performed on a once-daily basis (not as batched runs, as in this study), the average time from obtaining the sample to the Septi*Fast* result would be a median of 18 h (range: 6–30 h).

Discussion

The purpose (and design) of this study was to compare Septi*Fast* test results with BC results—not to assess the potential clinical value of the Septi*Fast* test when used in addition to BC. This latter issue can only be examined by controlled clinical trials evaluating the impact of Septi*Fast* test results on patient care and outcome variables.

We observed more episodes of circulating bacterial and/or fungal DNA detected using Septi*Fast* than episodes in which microorganisms were detected using BC, as observed in other studies [28,29]. In particular, we found more episodes with *S. aureus*, *Escherichia coli*, *Candida albicans*, *Aspergillus fumigatus*, *Stenotrophomonas maltophilia*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae*, and *Enterobacter aerogenes*. Although the numbers are small, it appears that, for *Candida* spp. and *A. fumigatus*, the Septi*Fast* methodology was more sensitive than conventional BC, suggesting that some fungal infections in intensive-care unit patients might currently be undiagnosed. The detection of *A. fumigatus* DNA was unexpected, as this microorganism is difficult to detect with current conventional technology [38,39]. In only two of five patients, *A. fumigatus* infection was confirmed by other diagnostic methods, autopsy and bronchoalvelolar lavage, as the cause of endocarditis and pneumonia, respectively. Diagnostic tests for *Aspergillus* were not performed for the other three patients. Recently, two neutropenic patients with haematological malignancies have also been found to be *A. fumigatus*-positive using Septi*Fast* and bronchoalvelolar lavage [28]. Specifically designed studies will be needed to investigate the clinical significance of positive Septi*Fast* results for *A. fumigatus* in suspected *A. fumigatus* infections. Patients who were positive for *S. aureus* by both BC and Septi*Fast* had lower CPs (more target) than did patients for whom BC was negative and Septi*Fast* positive for *S. aureus*. This may explain, in part, why BC was negative in these cases. The interpretation of *Streptococcus* spp. and CoNS as non-contaminant microorganisms was based on simultaneous findings of these microorganisms using Septi*Fast* and culture. This could be imprecise, as species determination cannot be performed for *Streptococcus* spp. and CoNS detected with the Septi*Fast* test. The contamination rate was slightly lower for Septi*Fast* (2.2%) than it was for BC (3.9%). This was primarily due to the CP cut-off value of 35 cycles defined in the software for CoNS and streptococci.

The most important advantage of BC over Septi*Fast* is that susceptibility testing of an isolate can be performed, allowing the implementation of specifically targeted antimicrobial or antifungal therapy. BC also has an advantage over Septi*Fast* with respect to microorganisms not included in the Septi*Fast* master list. In some cases, microorganisms were identified by BC that theoretically should have been found using Septi*Fast*. This probably happened in cases of low-level bacteraemia, where there was no target for Septi*Fast* in the sample tested. Unfortunately, IC DNA in the Septi*Fast* assay was not amplified in 12.5% of episodes; therefore, the Septi*Fast* test gave no information. This was due to either inhibition of the PCR reaction or inappropriate sample preparation, both of which must be addressed in future improvements of the assay [40].

Early appropriate antimicrobial treatment of sepsis has been demonstrated in several studies to improve survival [4–7,41]. The diagnosis of bacteraemia can be complicated in patients receiving antimicrobial treatment, and all current BC systems have been modified in an attempt to reduce the effect of antimicrobials in the BC bottle [42]. The advantage of a DNA-based detection system (as compared with BC) is that the microorganism causing sepsis does not have to be viable at the time of sampling. Although our data in this respect are limited, owing to the design of the study, Septi*Fast* may be particularly advantageous for patients receiving antibiotics.

One question that must be answered by future studies concerns the clinical relevance of microorganisms detected only by Septi*Fast*. It is not clear whether DNAaemia as revealed by the Septi*Fast* test reflects true infection. Analysis of BCs and other routine clinical microbiology samples revealed that 67% of the microorganisms detected using Septi*Fast* could be confirmed by culture. This is in agreement with the 69% confirmation rate found in a previous study [29]. The present study was not designed to evaluate the clinical significance of microbial DNAaemia, which is certainly not the same as, and therefore not directly comparable with, bacteraemia. We believe that further studies are needed to address this issue.

We observed an 'overall agreement' between Septi*Fast* and BC results of 79%. Similar imperfect overall agreements have previously been found when clinical microbiology methods, such as culture of *Chlamydia* or viruses, have been

replaced by much more sensitive nucleic acid amplification methods [43]. Finally, if any non-contaminant microorganisms found using BC or Septi*Fast* testing were viewed as true positives in septic patients, 37% of the microorganisms were found using BC as opposed to 88% using Septi*Fast* testing.

The Septi*Fast* technology could represent an advantageous addition to BC technology, and seems to hold promise for enhanced detection of bacteria and fungi in patients with suspected sepsis. This new test will not replace BC, which will still be required as a prerequisite for identification of microorganisms, and in particular for susceptibility testing.

Transparency Declaration

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