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Performance of the LightCycler SeptiFast Test M^{grade} in Detecting Microbial Pathogens in Purulent Fluids$^{\nabla}$

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The performance of the LightCycler SeptiFast (SF) assay was compared to that of culture methods in the detection of microorganisms in 43 purulent fluids from patients with pyogenic infections. The SF assay was more sensitive than the culture methods (86% versus 61%, respectively), irrespective of whether the infections were mono- or polymicrobial.

The LightCycler SeptiFast (SF) test is a commercially available multiplex real-time PCR assay able to detect a wide range of bacterial and fungal organisms commonly involved in systemic infections (10). The SF assay has been shown to be a valuable ancillary method for the etiological diagnosis of bacteremia, sepsis, endocarditis, and more recently, periprosthetic joint infections, particularly in patients who have received antibiotics prior to diagnostic testing (1–3, 5, 6, 9, 10, 13–17). Molecular methods can allow the rapid detection of the microorganisms involved in severe pyogenic infections, thus leading to a more efficient and targeted early therapeutic intervention, which could translate into major clinical benefits for patients. To the best of our knowledge, there is no published experience on the performance characteristics of the SF assay for the detection of microorganisms in purulent fluids.

A total of 43 purulent fluids obtained from 41 patients (29 male and 12 female; mean age, 64.1 years) over a period of 5 months (July to November 2010) were included in this study, as follows: 11 biliary fluid samples from patients with acute cholangitis, 7 pleural fluid samples from patients with empyema secondary to mesothelioma, lung cancer, or community-acquired and nosocomial pneumonia, 7 peritoneal fluid samples from patients with secondary peritonitis, 7 dialysis effluents from 6 patients with continuous ambulatory peritoneal dialysis (CAPD)-related peritonitis, 5 purulent liquid samples from 4 patients with intra-abdominal abscesses secondary to abdominal surgery, 4 purulent (>250 polymorphonuclear leukocytes/μl) ascites fluid samples from cirrhotic patients with spontaneous bacterial peritonitis (SBP), and 2 intra-articular fluid samples from patients with acute septic arthritis. A total of 31 of the 41 patients had been treated with broad-spectrum antibiotics prior to sampling, as follows. All patients (n = 11) undergoing biliary surgery, 3 patients subjected to thoracocentesis for empyema, and 3 patients undergoing laparotomy for secondary peritonitis received perioperative antimicrobial prophylaxis with amoxicillin-clavulanic. All patients with intra-abdominal abscesses and 4 patients with secondary peritonitis were empirically treated with ciprofloxacin plus metronidazole for a median of 2 days (range, 1 to 4 days) prior to specimen sampling. Four patients with secondary empyema had been treated with imipenem, imipenem plus metronidazole, or imipenem plus vancomycin for a median of 1 day prior to specimen sampling (range, 1 to 3 days). One patient with CAPD, one patient with SBP, and one patient with septic arthritis were treated with vancomycin or ceftriaxone (the two latter patients), respectively, 1 day prior to sampling. Six patients with CAPD, 3 patients with SBP, and one patient with septic arthritis did not receive antimicrobial therapy prior to sampling.

Direct microscopic examination (DME) by Gram staining was performed for all but two samples. The results were immediately reported to the clinician in charge. Clinical specimens were cultured aerobically in 5% sheep blood agar containing nalidixic acid, chocolate blood agar, and MacConkey agar (at 37°C in 5% CO_2) and in Sabouraud dextrose agar (at room temperature) and anaerobically in Schaedler K-V agar with 5% sheep blood at 37°C. All specimens were inoculated in parallel into thioglycolate broth (TGB) tubes, which were screened daily for the occurrence of turbidity. TGB tubes were subcultured on chocolate blood agar and Schaedler agar as soon as turbidity was detected or at 96 h if turbidity was not observed. The dialysis effluents (50 ml), ascites fluids (10 to 20 ml), and the remaining purulent fluids (1 to 2 ml) were centrifuged at 3,000 × g for 15 min, and the pellet was used for culturing and DME. Twenty-nine specimens were simultaneously inoculated into a pair of bottles of Bactec media for aerobic and anaerobic bacteria (BD Diagnostics, Sparks, MD), which were incubated for a maximum of 7 days and analyzed using the automated continuous blood culture monitoring system Bactec 9420 BC system (Becton Dickinson, Heidelberg, Germany). This approach has been recently introduced in our laboratory as a routine microbiological procedure for the etiological diagnosis of severe pyogenic infections. For dialysis effluents and ascites fluids, 5 to 10 ml was directly inoculated into each bottle, while a volume of 1 to 3 ml was inoculated into each bottle for other purulent fluids. The broth was aspirated for Gram staining when microbial growth was detected. The results were reported to the clinician in charge. The broth was then subcultured on chocolate blood agar or Schaedler agar, as appropriate. Microbial identification was achieved by

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TABLE 1. List of microorganisms detected by the SeptiFast assay

Gram-negative bacterium	Gram-positive bacterium	Fungus
Escherichia coli	*Staphylococcus aureus*	*Candida albicans*
Klebsiella pneumoniae/*Klebsiella oxytoca*	CoNS[a]	*Candida tropicalis*
Serratia marcescens	*Streptococcus pneumoniae*	*Candida parapsilosis*
Enterobacter cloacae/*Enterobacter aerogenes*	*Streptococcus* spp.[b]	*Candida krusei*
Proteus mirabilis	*Enterococcus faecium*	*Candida glabrata*
Pseudomonas aeruginosa	*Enterococcus faecalis*	*Aspergillus fumigatus*
Acinetobacter baumannii		
Stenotrophomonas maltophilia		

[a] CoNS; coagulase-negative *Staphylococcus* species, including *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. pasteuri*, *S. warneri*, *S. cohnii*, *S. lugdunensis*, *S. capitis*, *S. caprae*, *S. saprophyticus*, and *S. xylosus*.

[b] *Streptococcus* species, including *S. pyogenes*, *S. agalactiae*, *S. anginosus*, *S. bovis*, *S. constellatus*, *S. cristatus*, *S. gordonii*, *S. intermedius*, *S. milleri*, *S. mitis*, *S. mutans*, *S. oralis*, *S. parasanguinis*, *S. salivarius*, *S. sanguinis*, *S. thermophilus*, *S. vestibularis*, and viridans group streptococci.

following established and approved laboratory procedures (11).

The SF testing was performed retrospectively on clinical specimens that had been stored at −70°C. A volume of 1.5 ml of noncentrifuged clinical samples was subjected to mechanical lysis (SeptiFast lysis kit M^{grade} on the MagNA lyser instrument, Roche Diagnostics), according to the manufacturer's instructions. Total DNA extraction (from 350 μl of the lysates; eluate volume, 150 μl) was performed using the Abbott mSample preparation system DNA kit on the M2000 instrument (Abbott Diagnostics, IL), following the DNA Blood LL300/150 (version 072508) protocol. The LightCycler SeptiFast test M^{grade} assay (Roche Diagnostics GmbH, Manheim, Germany) was performed using the LightCycler 2.0 instrument (Roche Diagnostics) by following the manufacturer's instructions. A full description of this procedure has been previously reported (10). The target species detected by the SF assay are listed in Table 1.

Microbial species were recovered by conventional culturing or by using the Bactec automated culture system for 32 out of the 43 clinical specimens. A single causative microorganism was found in 19 cases, whereas a polymicrobial infection was found in 13 cases. Results from the culture and the SF assay were concordant for 23 specimens (Table 2), of which 7 tested negative by both culture and the SF assay. The remaining 16 specimens were positive according to both methods, with the organisms detected being identical. The SF assay and culture methods generated discordant results for 20 specimens (Table 3). For 5 of these specimens, one method produced a positive result, while the other method failed to detect any organisms. Of these, 4 specimens were culture negative and SF assay positive. The species detected by the SF assay were *Escherichia coli* (n = 2), *Enterococcus faecalis*, and coagulase-negative staphylococcus (CoNS). The two latter microbial species were detected in two consecutive samples from a single patient with CAPD who had been treated with vancomycin prior to sampling. In one case, *Candida albicans* was recovered by culture from an intra-abdominal abscess but was not detected by the SF assay. For the remaining 15 discordant specimens, organisms were detected by both methods, but the microbial species detected by the two methods were not identical. In 9 of these

TABLE 2. Clinical specimens yielding concordant results by culture methods and the SeptiFast assay

Specimen	Clinical condition	Species recovered by culture and detected by the SF assay[a]
Dialysis effluent	CAPD-related peritonitis	*Candida parapsilosis*
Biliary fluid	Cholangitis	*Streptococcus anginosus*/*Enterobacter cloacae*
Biliary fluid	Cholangitis	*Streptococcus anginosus*
Biliary fluid	Cholangitis	*Streptococcus constellatus*/*Enterobacter cloacae*
Biliary fluid	Cholangitis	*Escherichia coli*
Purulent fluid	Secondary peritonitis	*Escherichia coli*
Dialysis effluent	CAPD-related peritonitis	*Staphylococcus aureus*
Dialysis effluent	CAPD-related peritonitis	*Serratia marcescens*
Pleural liquid	Secondary empyema	*Staphylococcus aureus*
Biliary fluid	Cholangitis	*Enterococcus faecium*
Biliary fluid	Cholangitis	*Escherichia coli*
Biliary fluid	Cholangitis	*Escherichia coli*
Pleural liquid	Secondary empyema	*Streptococcus anginosus*/*Streptococcus agalactiae*
Pleural liquid	Primary empyema	*Streptococcus intermedius*
Intra-articular fluid	Septic arthritis	*Streptococcus pyogenes*
Pleural fluid	Primary empyema	*Streptococcus pyogenes*
Ascites fluid	SBP	None
Ascites fluid	SBP	None
Intra-articular fluid	Septic arthritis	None
Dialysis effluent	CAPD-related peritonitis	None
Dialysis effluent	CAPD-related peritonitis	None
Purulent liquid	Secondary peritonitis	None
Ascites fluid	SBP	None

[a] Eighteen specimens were simultaneously cultured by conventional methods and in Bactec bottles (aerobic and anaerobic). The SeptiFast assay does not distinguish among streptococci or between *Enterobacter cloacae* and *Enterobacter aerogenes*.

15 samples, the SF assay detected more microorganisms than those recovered from the culture methods. These microbial species were the following: *Enterobacter cloacae*/*Enterobacter aerogenes* (n = 3), *Enterococcus faecalis* (n = 3), *Candida albicans* (n = 3), *Candida parapsilosis* (n = 2), *Candida tropicalis* (n = 1), and *Pseudomonas aeruginosa* (n = 1). For the remaining 6 discordant specimens, there were organisms recovered by culture that were not detected by the SF assay. These included *Enterococcus faecalis* (n = 2), *Escherichia coli* (n = 1), *Pseudomonas aeruginosa* (n = 1), and *Morganella morganii* (n = 1); the latter bacterial species was not included in the SF test panel. In two samples, anaerobic species (*Fusobacterium mortiferum*, *Peptostreptococcus* spp., and *Clostridium perfringens*) not detectable by the SF assay were recovered by the culture methods (Table 3).

The sensitivity and the negative predictive value of the SF assay (86% and 58%, respectively) compared favorably to those of the culture methods (61% and 33%, respectively) for detecting all causative microorganisms (excluding those not listed in the master panel of the SF assay), assuming that the organisms detected by the SF assay were clinically significant (specificity and positive predictive value of 100%). The overall agreement between the culture methods and the SF assay (microorganism[s] recovered versus microorganism[s] detected) was suboptimal (κ = 0.12; 95% confidence interval [CI], 0 to 0.38). The data were analyzed with the aid of the statistical package SPSS version 17.0 (SPSS, North Chicago, IL).

Molecular methods based on species-specific PCR or broad-

TABLE 3. Clinical specimens yielding discordant results by culture methods and the SeptiFast assay

Specimen/clinical condition	Microorganism(s) recovered from culture[a]	Microorganism(s) detected by the SF assay[b]
Peritoneal fluid/secondary peritonitis	*E. coli*; *S. anginosus*	*E. coli*; *Streptococcus* spp.; *C. parapsilosis*
Biliary fluid/cholangitis	*K. pneumoniae*	*K. pneumoniae*; *E. cloacae*/*E. aerogenes*; *E. faecalis*
Pleural liquid/secondary empyema	*S. aureus*	*S. aureus*; *E. faecalis*; *P. aeruginosa*
Ascites fluid/SBP	*P. aeruginosa*; *E. faecium*	*P. aeruginosa*; *E. faecium*; *C. albicans*
Biliary liquid/cholangitis	*E. coli*	*E. coli*; *E. faecalis*
Purulent liquid/intra-abdominal abscess	*P. aeruginosa*; *E. faecium*	*P. aeruginosa*; *E. faecium*; *E. cloacae*/*E. aerogenes*
Peritoneal fluid/secondary peritonitis	*K. pneumonia*; *E. cloacae*; *C. albicans*; *S. mitis*	*K. pneumoniae*; *E. cloacae*/*E. aerogenes*; *Streptococcus* spp.
Purulent liquid/intra-abdominal abscess	*E. coli*; *P. aeruginosa*	*E. coli*
Purulent liquid/intra-abdominal abscess	*E. coli*; *E. faecalis*	*E. coli*/*C. albicans*; *C. tropicalis*
Purulent liquid/intra-abdominal abscess	*C. albicans*	*C. albicans*; *C. parapsilosis*
Pleural fluid/secondary empyema	*C. albicans*	*C. albicans*; *C. parapsilosis*
Biliary fluid/cholangitis	*E. coli*; *M. morganii*	*E. coli*; *E. cloacae*/*E. aerogenes*
Peritoneal fluid/secondary peritonitis	*E. coli*; *K. pneumoniae*; *C. krusei*; *E. faecalis*	*K. pneumoniae*; *C. krusei*
Pleural liquid/secondary empyema	*S. mitis*; *F. mortiferum*; *Peptostreptococcus* spp.	*Streptococcus* spp.
Biliary fluid/cholangitis	*E. coli*; *E. faecalis*; *C. perfringens*	*E. coli*; *E. faecalis*
Purulent liquid/intra-abdominal abscess	*C. albicans*	None
Dialysis effluent/CAPD-related peritonitis	None	*E. faecalis*
Peritoneal fluid/secondary peritonitis	None	*E. coli*
Peritoneal fluid/secondary peritonitis	None	*E. coli*
Dialysis effluent/CAPD-related peritonitis	None	CoNS

[a] Eleven specimens were simultaneously cultured by conventional methods and in Bactec bottles (aerobic and anaerobic).
[b] The SeptiFast assay does not distinguish among streptococci or between *Enterobacter cloacae* and *Enterobacter aerogenes.*

range PCR for detecting the 16S rRNA gene followed by sequencing for species identification are being increasingly used for the etiological diagnosis of purulent infections, including SBP, septic arthritis, or CAPD-related peritonitis (4, 7, 8, 12, 18, 19). These assays have been shown to improve the diagnostic efficiency of culture methods due to their higher sensitivities, especially with samples obtained from patients who have been exposed to antibiotics prior to specimen sampling. In our experience, the SF assay was found to be more sensitive than the culture methods for microbial detection in purulent fluids obtained from a single set of patients with a broad range of clinical conditions, including SBP, CAPD-related peritonitis, secondary peritonitis following abdominal surgery, acute cholangitis, intra-abdominal abscesses, and secondary pleural empyema, regardless of whether the infections were mono- or polymicrobial. Only a few microorganisms recovered from the culture methods were missed by the SF assay. In our study, most patients had been treated with broad-spectrum antibiotics prior to sampling, which may account for the relatively low diagnostic yield of the culture methods compared to the SF assay. In this context, all patients with SF-positive but culture-negative results or with samples in which the SF assay detected more microorganisms than the culture methods had been treated with antibiotics prior to sampling. From the microbiological point of view, the overall agreement between the SF assay and culture methods was rather low; yet, major discrepancies between the methods (positive results by one method and negative results by the other method) were observed only in 5 out of 20 specimens (positive by the SF assay and negative by culture methods) (Table 3). In all other samples, the SF assay and culture methods yielded concordant results with regard to the predominant microbial species present. Unfortunately, we cannot shed light on the clinical relevance of microbial species detected by one method (in most cases by the SF assay) but missed by the other. This issue is of major interest, and thus, must be addressed in further studies.

Empirical antibiotic treatment protocols for severe pyogenic infections are well established, although the early therapeutic management of such infections is often based on information obtained by DME (Gram staining) of specimens. In our study, no microorganisms were observed in the DME of 19 specimens yielding positive results by the culture methods and/or the SF assay. Moreover, early readjustment of empirical treatments is commonly made on the basis of Gram stain results of direct smears prepared from culture bottles once microbial growth is detected. The time to detection of microbial growth in the Bactec system varied widely in the current study (mean time, 8.7 h; range, 2 to 21.5 h), so that information was not available to clinicians until an average of 9 h after specimen sampling. The SF test can be completed in approximately 6 to 7 h and provides reliable information on the microbial species involved, thus allowing clinicians to prescribe the most appropriate antibiotic regimen in a timely fashion. A remarkable finding of our study was that a small volume of noncentrifuged clinical specimens was sufficient to achieve high diagnostic sensitivity. An obvious limitation of the SF assay is its inability to detect some microorganisms that are often involved in these processes, particularly anaerobic bacteria. In addition, the SF assay does not provide information about antimicrobial susceptibilities of the organisms detected. In this sense, studies addressing whether these disadvantages would have a significant impact on clinical outcome are clinically warranted. In summary, our data indicated that the SF assay is a rapid and sensitive method for the detection of microorganisms in purulent specimens from patients with severe pyogenic infections. Further studies are needed to validate the above-described findings and to determine whether or not the information

provided by the SF assay would translate into tangible clinical benefits.

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