Evaluation of a commercial multiplex PCR test (SeptiFast) in the etiological diagnosis of community-onset bloodstream infections

P. Josefson, K. Strålin, A. Ohlin, T. Ennefors, B. Dragsten, L. Andersson, H. Fredlund, P. Mölling, P. Olcén

► To cite this version:

P. Josefson, K. Strålin, A. Ohlin, T. Ennefors, B. Dragsten, et al.. Evaluation of a commercial multiplex PCR test (SeptiFast) in the etiological diagnosis of community-onset bloodstream infections. *European Journal of Clinical Microbiology and Infectious Diseases*, 2011, 30 (9), pp.1127-1134. 10.1007/s10096-011-1201-6 . hal-00676224

HAL Id: hal-00676224

<https://hal.science/hal-00676224>

Submitted on 4 Mar 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24

**Evaluation of a commercial multiplex PCR (SeptiFast) in the aetiological
diagnosis of community onset bloodstream infections**

Per Josefson^{1*}, Kristoffer Strålin¹, Andreas Ohlin², Theresa Ennefors³, Birgitta Dragsten³,
Linda Andersson³, Hans Fredlund³, Paula Mölling³, Per Olcén³

1) Department of Infectious Diseases 2) Department of Paediatrics 3) Department of
Laboratory Medicine/ Clinical Microbiology, Örebro University Hospital, Örebro, Sweden

* Corresponding author. Mailing address: Department of Infectious Diseases, Örebro
University Hospital, S-701 85 Örebro, Sweden. Phone: + 46 19 6021488.
Fax + 46 19 184855. E-mail: per.josefson@orebroll.se

25 **Abstract**

26

27 *Purpose:* The commercial PCR test SeptiFast is designed to identify DNA of individual
28 bacterial and fungal pathogens in whole blood. We aimed to evaluate the usefulness of the test
29 for detection of community onset bloodstream infections.

30 *Methods:* We prospectively included adult patients who were subjected to blood culture (BC)
31 at an infectious diseases department. For the evaluation one BC/PCR set (two BC bottles and
32 one PCR tube) per patient was used. When several sets were obtained and analyzed the first
33 set with any positive result was evaluated.

34 *Results:* Among 1093 consecutively included patients, BC was positive in 138 and PCR was
35 positive in 107. Fifty positive PCR results were supported by BC in the same BC/PCR set, 10
36 were supported by other cultures, and additionally, 10 were supported by the clinical
37 presentation. Compared with BC, PCR showed specificities and negative predictive values of
38 >97% for all detectable pathogens. The following sensitivities and positive predictive values
39 (PPVs) were noted: *Staphylococcus aureus*, 67% and 43%; *Streptococcus pneumoniae*, 12%
40 and 67%; other *Streptococcus* species, 43% and 77%; *Escherichia coli*, 53% and 56%; and
41 *Klebsiella* species, 43% and 23%. If support from other cultures and the clinical presentation
42 were included in the reference standard, the PPVs for detection of these bacteria were 57%,
43 100%, 92%, 75% and 69%, respectively.

44 *Conclusions:* Although the specificities were high, the low sensitivities and suboptimal PPVs
45 noted in the present study discourage routine use of the test in its present form for detection of
46 community onset bloodstream infections.

47

48 **Keywords:** PCR, Sepsis, Bacteraemia, Diagnostic test, Evaluation

49

50 **Introduction**

51

52 Bloodstream infections are important causes of morbidity and mortality in patients
53 worldwide. Rapid etiological diagnosis and early administration of adequate antimicrobial
54 therapy soon after a critically ill patient's arrival at the hospital are important for a successful
55 outcome [1, 2]. Inadequate antimicrobial treatment has been reported to be associated with
56 increased mortality [3, 4].

57 Blood culture (BC) is at present considered the diagnostic gold standard for bloodstream
58 infections, with high specificity in species identification. However slow-growing and
59 fastidious organisms can delay diagnosis and prior antimicrobial treatment reduces the
60 sensitivity of the BC method.

61 New non-culture-based techniques are consequently being developed for the diagnosis of
62 septicaemia/bacteraemia [5, 6 , 7]. One of the approaches for potentially faster pathogen
63 identification is to use target-amplification methods, including molecular amplification with
64 polymerase chain reaction (PCR). General methods that use genes for ribosomal RNA can
65 create problems with contaminating bacteria, causing impaired analytical specificity and
66 sensitivity [8]. A new commercially available multiplex PCR (SeptiFast, Roche Diagnostics
67 GmbH) with the potential to identify 19 different bacterial species and 6 fungal species in
68 whole blood has been developed [9]. The aim of the present prospective, diagnostic study was
69 to evaluate this new PCR by comparing it with BC in a large, consecutive, non-selective
70 group of adult patients seen at a department of infectious diseases in southern Sweden. We
71 estimated that about 1000 different patients would be subjected to BC during one year, with
72 about 100 positive BCs.

73

74

75 **Materials and methods**

76

77 **Patients**

78

79 The Department of Infectious Diseases, Örebro University Hospital, Sweden, provides service
80 for a population of 275.000 inhabitants in the county of Örebro and has a ward with 30 beds
81 for adults and an outpatient clinic.

82 In the present prospective study we enrolled all adult patients who were subjected to BC at the
83 department and gave their informed consent during one full year from October 2007 to
84 September 2008. Excluded were patients with HIV and with hepatitis B and C infections for
85 local laboratory safety reasons.

86

87 **SeptiFast method**

88

89 Whole blood was collected in sterile EDTA tubes (BD Vacutainer™ K3E 15%, Becton,
90 Dickinson and Company, Plymouth, UK) through the same venepuncture as the blood
91 samples for BC were taken. The whole blood was then stored for a maximum of 4 hours at
92 room temperature, or up to three days at +4°C, or 3 months at –70°C prior to DNA
93 preparation. The DNA preparations were handled identically.

94 The internal transcribed spacer region (ITS) is used in the SeptiFast assay (Roche Diagnostics
95 GmbH, Mannheim, Germany) as the target to specifically distinguish 25 different bacterial
96 and fungal pathogens (see Table 1). As detection format, hybridization probes are used.
97 DNA was extracted from 1.5 ml of an EDTA whole-blood sample. The protocol of the
98 manufacturer was followed, including a first step of mechanical lysis using the SeptiFast Lys
99 Kit MGrade and the MagNALyser®. The DNA was prepared manually using the SeptiFast

Prep Kit MGrade (Roche Diagnostics GmbH). In the extraction step an internal control (IC) was added to each sample. The IC is a mixture of synthetic double-stranded DNA molecules with primer binding sites identical to those of the target sequences, differing in their probe binding sites. A negative control supplied by the manufacturer was included in each extraction series and the reagent controls were used as positive control of the PCR reactions. Gram-positive bacteria, Gram-negative bacteria, and fungi were amplified individually in three different mixes. The real-time PCR was performed in a LightCycler 2.0 instrument (Roche Diagnostics GmbH). The emitted fluorescence was measured in one of the four different detection channels (610, 640, 670, and 705 nm). Analysis of the melting curves were performed to strengthen the specificity of the products. The PCR amplicons from the specimens and ICs were analyzed by a pathogen identification software (SIS; SeptiFast Identification Software, Roche Diagnostics GmbH). The software automatically calculated the melting point (T_m) value and the corresponding peak height. For the Gram-positive assay the analysis was based on melting peaks (T_m and peak height) and amplification curve crossing point (C_p) values. The assays for Gram-negative and Fungi were solely based on melting peaks and C_p values were not considered. To reduce false positive results by assumed contaminants the software also includes C_p cut-off values, ex for CoNS and Streptococcus species this cut-off value is at 20 cycles. The assay was flagged as “Invalid” if the software could identify neither a specimen nor the IC. The turn-around time was 6 h. The results of the PCR assays were unknown to the clinicians until the closure of the study.

Blood culture

Blood culture was performed with the Bactec system (Becton, Dickinson and Company, Sparks, MD, USA) as a standard procedure in patients with suspected disseminated bacterial

and/or fungal infection.

For each BC a volume of 8-10 ml of venous blood was inoculated in one Bactec Plus

Aerobic/F bottle and the same volume in one Bactec Plus Anaerobic/F bottle.

After transport at room temperature the bottles were placed in a Bactec 9240 incubator, with monitoring for pH changes every 10 minutes for 6 days.

All signaling bottles were opened and an aliquot was taken for microscopy after Gram staining, culture on solid media, and further analyses for species designation according to the good laboratory practice of our accredited clinical diagnostic microbiological laboratory.

Other cultures

Cultures from normally sterile sites and from urine, wounds and respiratory secretions were performed according to standard microbiological procedures [10]. The choices of performing such cultures were based on the clinical judgement of the clinicians.

Clinical data

At inclusion clinical data were collected, including information on whether antibiotics had been taken within 3 days prior to BC. A retrospective chart review was performed by two specialists of infectious diseases (P.J., K.S.).

Analysis

The SeptiFast results were compared with the results of BC and other microbiological data. Although we routinely collected paired BC, we included for evaluation only one BC/PCR set

(two BC bottles and one PCR tube taken at the same time) per patient. If it was not negative in these analyses, the first BC/PCR set providing any positive result was included.

Definitions

A positive PCR result was considered to be fully supported when an identical microorganism was isolated in the BC of the same BC/PCR set. Other microbiological support for a positive PCR was defined as other positive BCs, cultures from normally sterile sites and from bronchial secretion, and pure culture in urine and wounds. Wound culture with mixed flora including *Staphylococcus aureus* and β -haemolytic streptococci was also regarded as other microbiological support.

In the cases of a positive PCR result without any microbiological support, we made a retrospective attempt to find clinical support for the positive PCR result. We considered a PCR result to have clinical support if the species identified by PCR generally was considered to be a common pathogen of the patient's type of infection, and if no microbiological support for any other pathogen was identified.

The regional ethical committee approved the study.

Results

During the study period the total number of patients subjected to BC at the department were 1540 and a positive BC was found in 208 of them.

Altogether 1093 patients were included in the present study, 486 females (44%) and 607 males (56%). All these patients had results from at least one BC/PCR set. Of the included patients the median age was 67 years (range 14-98 years). ICU care were received by 36 patients. The crude mortality rate within 30 days was 45/1093 (4%).

Blood culture and/or PCR was positive for any pathogen in 197 patients (18%) and in 896 patients the BC/PCR sets were negative. A positive BC was found in 138 of the patients and 107 patients had positive SeptiFast results.

The results regarding the pathogens detected by BC and/or PCR for species included in the PCR test menu are summarized in Table 2. The most commonly detected pathogens were Coagulase-negative Staphylococcus (CoNS), *Staphylococcus aureus*, *Streptococcus pneumoniae*, other Streptococcus species, *Escherichia coli*, and Klebsiella species. Results from patients who received antibiotic treatment within 3 days prior to BC are shown for each pathogen. In total there were 50 results that were positive both in PCR and BC, 64 results that were positive only with PCR and 86 results that were positive only with BC among the pathogens on the PCR test menu.

Concerning CoNS, 10 were positive only with PCR, 30 were positive only with BC and only 1 was positive with both PCR and BC. It may also be noted that for *S. pneumoniae* 14 were positive only with BC, 1 was positive only with PCR during antibiotic treatment and 2 were positive with both PCR and BC. Regarding other Streptococcus species, 13 were positive only with BC, among which 4 were positive for *Str. pyogenes* and 7 were positive for other β -haemolytic streptococci (one of which with invalid result in PCR). Three results were positive for other Streptococcus species by PCR only. PCR and BC were positive for 10 results, and of

these 2 were positive for *Str. pyogenes* and 4 were positive for other β -haemolytic streptococci according to BC.

With BC 10 different isolated pathogens that are not included in the PCR test menu, were also found in 10 patients. They were *Aerococcus viridans*, *Arcanobacterium haemolyticum*, *Pseudomonas fluorescens*, *Shigella flexneri*, Bacteroides species, Citrobacter species, Corynebacterium species, Fusobacterium species, Porphyromonas species, and Salmonella species.

Polymicrobial results were obtained in 12 patients, and are shown in Table 3.

In Table 4 the PCR-positive results are compared with results of BC and other cultures in an attempt to find other support for a positive PCR result. In the cases of positive PCR without microbiological support, clinical support is presented.

For *S. aureus* other microbiological support was in one case another positive BC and a positive joint culture, in one case another positive BC and culture from cerebrospinal fluid, and in one case a positive culture from a renal cortical cyst. For Streptococcus species other microbiological support consisted of wound culture, including β -haemolytic streptococci in 2 cases. Other microbiological support was other positive BC in 2 cases for *E. coli*, and other positive BC in 2 cases and in one case pure culture in urine for Klebsiella.

Positive PCR results without any microbiological or clinical support were obtained for example with CoNS in 10 cases, *S. aureus* in 10 cases, *E. coli* in 8 cases and *Pseudomonas aeruginosa* in 4 cases.

In total there were 114 positive PCR results from 107 patients, and 50 of them were fully supported by BC in the same BC/PCR set, and 10 by other cultures; additionally 10 results were supported by the clinical presentation. In 44 cases no microbiological or clinical support was found.

Compared with BC, the SeptiFast test showed specificities of over 98% and negative predictive values of over 97% for all detectable pathogens. In Table 5 sensitivities and positive predictive values (PPVs) are presented for major bacterial pathogens. PPVs are also shown with a combined reference standard including other microbiological support and clinical support.

Discussion

The theoretical advantages of a non-culture diagnostic method such as PCR for bacteraemia, as compared to normal BC, include a substantially shorter time for reporting the test result (both positive and negative) and detection of DNA in the blood without live microorganisms after for example antibiotic treatment.

The development of PCR tests for diagnosis of bacteraemia is hence of great clinical interest and assays have been designed and tried without a major breakthrough so far. The problems include the large number of bacterial species (and fungi) that are clinically relevant, the sometimes minute amounts of microbial DNA as compared to the huge amount of human DNA, and contaminants in connection with the blood sampling from the skin and the further

handling.

When a commercial test (SeptiFast) with a promising design was launched, the need for an evaluation was evident.

We thus chose to study an unselected group, aiming at including all adult patients seeking medical attention at our department of infectious diseases, for whom a blood culture was ordered during one full year.

In the present study there was no systematic loss of patients for inclusion. A loss was seen however in a few situations of overload of the department, and as a result of some mistakes in the sampling; also, a few single patients chose not to participate. Our handling of samples and our laboratory work strictly followed the instructions from the manufacturer of SeptiFast. In order not to create any bias, only one set of blood culture/PCR per patient was included for evaluation.

With this approach we consider the patient material to be representative of a population that, in most cases, reaches the hospital with an infection that they have developed outside the healthcare systems, named community onset blood stream infection.

In the design of the SeptiFast test the ITS region has been selected as the target region. By this approach a higher sensitivity can be achieved compared to single-copy genes, since there are often several ribosomal operons in the genomes of bacteria and fungi [9, 11]. A higher specificity can also be obtained compared to rRNA, as the ITS is more species-specific.

The selected species of bacteria and fungi that can be identified by SeptiFast was calculated to leave about 10% of our bacteraemias undiagnosed. Important pathogens are missing, such as

the anaerobes, Salmonella, Shigella, Haemophilus species, and *Neisseria meningitidis*. In our study 10 results (7%) out of 146 positive BCs, from 138 patients, included pathogens not covered by the SeptiFast.

In the evaluation, the results with the different diagnostic systems must also take into account that the blood culture system uses 8-10 ml of venous blood per bottle (one aerobic and one anaerobic bottle is taken in each culture), compared to the SeptiFast test which uses 1.5 ml in an EDTA tube with vacuum for preparation of a DNA extract for six PCR assays. One PCR assay thus contains DNA from 0.25 ml of blood or 1:32-40 of the blood culture volume used in one bottle. This may have had implications for the diagnostic sensitivity.

In the SeptiFast assay high quality PCR reagents, free of bacterial or fungal DNA contamination, are used according to the information provided by the producer. The EDTA vacuum tubes for drawing venous blood were recommended by the producer and said to be endotoxin and DNA free. The present study results, indicate, however that contamination was a problem, giving false positive *E. coli* PCR results in some cases (8 out of 32 had no support for the positive PCR result; Table 4) and *P. aeruginosa* in a few other cases (4 of 6 without support; Table 4). Whether these contaminants came from the sampling tubes or the reagents is not clear. Such problems have to be brought down to an absolute minimum by the producers.

The background of a negative result with SeptiFast, despite positive BC with a bacterium that is included in the test (false negative), is multifactorial. The reasons may be speculated on and include possibly less optimal technical steps in the PCR reaction, incorrect interpretation of amplification signals, problems with the selection of species-specific targets in the test, and the blood volume used.

In the present study the SeptiFast false negative *S. pneumoniae* bacteraemic cases are most noteworthy, since only 2 of 16 BC positive samples were detected, i.e. the sensitivity was 12%. This may be explained by the fact that the manufacturer of SeptiFast has set the detection level for *S. pneumoniae* high, in order not to have false-positive results caused by closely related alpha-haemolytic streptococci. For optimal detection of *S. pneumoniae* DNA, PCR targets specific to *S. pneumoniae* should probably be used. We recently found that PCR for *lytA* applied to plasma samples had a sensitivity of 70% and a specificity of 99% for detection of *S. pneumoniae* bacteraemia in patients with community-acquired pneumonia [12].

The same problem was encountered for other bacteraemic streptococci, mainly beta-haemolytic ones, which were negative in 57% in the SeptiFast test.

These results hamper the use of the tested version of SeptiFast for detection of bacteraemia in a general infectious disease patient population, where pneumococci and invasive haemolytic streptococci are common causes of community onset bacteraemia.

A positive result with SeptiFast when the BC is negative can be received in a number of situations. Some of these results are clinically reliable and helpful and some are misleading. A well defined contribution by the nucleic acid tests is the performance when antibiotics have been given prior to culture, and individual patients in this situation were seen in the present study (Table 2).

The presence of bacterial/fungal DNA in whole blood without live organisms has not so far been systematically explored. Biologically it is a likely situation that could focus an investigation on a search for a localised infection. The present study includes such patients, for example with *S. aureus* positivity in SeptiFast (Table 4).

Contamination of a blood sample as such in connection with the sampling of the patient involves the skin flora from which mainly different staphylococci and streptococci become positive in the diagnostic systems. The results for CoNS in the present study (Table 2) indicate that there are problems with skin contamination. Both nucleic assay tests and culture systems share these problems and results have to be repeated to be more reliable. A semiquantitative view is also useful in these situations when a late and low PCR product and a need for long-term incubation for growth favour the judgement of a contaminant [13, 14].

A number of studies, mainly with smaller study groups, using SeptiFast for the detection of microbial DNAemia, have been published with various results. Several publications have focused on compromised and highly selected patient groups, mainly from haematological units [15, 16, 17, 18, 19].

Some publications include community onset bloodstream infections. Louie et al studied 200 adult patients, sampled at emergency rooms, intensive care units and general medicine wards. They found positive PCR in 45 cases compared to 37 BC, and 69 % of the PCR results were confirmed by blood, urine, and catheter culture [20]. Tsalik et al included 306 patients with suspected sepsis. In the patients with an identified infectious aetiology, 66 were positive with BC and 46 with PCR [21]. Avolio et al compared BC with SeptiFast in patients admitted to the emergency room with suspected sepsis. Out of 144 samples, 30 identified the same organism with BC and PCR, 13 organisms were identified only with BC, and 10 were positive only with PCR [22]. West et al conducted a multicenter trial of patients with suspected bacterial or fungal septicaemia. In total 359 patients were evaluated. The rate of positivity was 17% for BC and 26% for SeptiFast. The 74 non-contaminant BC isolates were identified with PCR in 50 cases. The 174 non-contaminant microorganisms detected by SeptiFast were isolated by BC in 50 cases and additionally 67 could be confirmed as probable clinical

pathogens by culture of the same microorganism from a relevant anatomical site within the same clinical time frame. No support was found for a total of 57 samples [23].

In the present study, we found that the positivity rate of PCR was generally slightly lower than that of BC. In agreement with the previous studies on the SeptiFast test, we found that the test had high specificities and high negative predictive values for the detectable pathogens. However, the sensitivities and PPVs of the test were suboptimal, facts that became obvious with the layout of our study, with a large number of community onset consecutive patients.

Acknowledgements

The study was made possible by a research grant from the Research Committee of the County Council of Örebro.

Reagents and means for technical assistance for the study were provided by Roche Diagnostics.

There has been no other financial relationship with any other company.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, Suppes R, Feinstein D, Zanotti S, Taiberg L, Gurka D, Cheang M (2006) Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med* 34 (6):1589-1596
2. Beekmann SE, Diekema DJ, Chapin KC, Doern GV (2003) Effects of rapid detection of bloodstream infections on length of hospitalization and hospital charges. *J Clin Microbiol* 41 (7):3119-3125
3. Kollef MH, Sherman G, Ward S, Fraser VJ (1999) Inadequate antimicrobial treatment of infections: a risk factor for hospital mortality among critically ill patients. *Chest* 115 (2):462-474
4. Ibrahim EH, Sherman G, Ward S, Fraser VJ, Kollef MH (2000) The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. *Chest* 118 (1):146-155
5. Peters RP, van Agtmael MA, Danner SA, Savelkoul PH, Vandenbroucke-Grauls CM (2004) New developments in the diagnosis of bloodstream infections. *Lancet Infect Dis* 4 (12):751-760
6. Schrenzel J (2007) Clinical relevance of new diagnostic methods for bloodstream infections. *Int J Antimicrob Agents* 30 Suppl 1 S2-6
7. Mancini N, Carletti S, Ghidoli N, Cichero P, Burioni R, Clementi M (2010) The era of molecular and other non-culture-based methods in diagnosis of sepsis. *Clin Microbiol Rev* 23 (1):235-251

- 394 8. Meier A, Persing DH, Finken M, Bottger EC (1993) Elimination of contaminating
395 DNA within polymerase chain reaction reagents: implications for a general approach
396 to detection of uncultured pathogens. *J Clin Microbiol* 31 (3):646-652
- 397 9. Lehmann LE, Hunfeld KP, Emrich T, Haberhausen G, Wissing H, Hoeft A, Stuber F
398 (2008) A multiplex real-time PCR assay for rapid detection and differentiation of 25
399 bacterial and fungal pathogens from whole blood samples. *Med Microbiol Immunol*
400 197 (3):313-324
- 401 10. Murray PR, Baron EJ (2003) *Manual of clinical microbiology*. ASM Press,
402 Washington, D.C.
- 403 11. Lau A, Chen S, Sorrell T, Carter D, Malik R, Martin P, Halliday C (2007)
404 Development and clinical application of a panfungal PCR assay to detect and identify
405 fungal DNA in tissue specimens. *J Clin Microbiol* 45 (2):380-385
- 406 12. Abdeldaim G, Herrmann B, Molling P, Holmberg H, Blomberg J, Olcen P, Stralin K
407 (2010) Usefulness of real-time PCR for *lytA*, *ply*, and *Spn9802* on plasma samples for
408 the diagnosis of pneumococcal pneumonia. *Clin Microbiol Infect* 16 (8):1135-1141
- 409 13. Hall KK, Lyman JA (2006) Updated review of blood culture contamination. *Clin*
410 *Microbiol Rev* 19 (4):788-802
- 411 14. Kassis C, Rangaraj G, Jiang Y, Hachem RY, Raad I (2009) Differentiating culture
412 samples representing coagulase-negative staphylococcal bacteremia from those
413 representing contamination by use of time-to-positivity and quantitative blood culture
414 methods. *J Clin Microbiol* 47 (10):3255-3260

- 415 15. Mancini N, Clerici D, Diotti R, Perotti M, Ghidoli N, De Marco D, Pizzorno B,
416 Emrich T, Burioni R, Ciceri F, Clementi M (2008) Molecular diagnosis of sepsis in
417 neutropenic patients with haematological malignancies. *J Med Microbiol* 57 (Pt
418 5):601-604
- 419 16. von Lilienfeld-Toal M, Lehmann LE, Raadts AD, Hahn-Ast C, Orlopp KS, Marklein
420 G, Purr I, Cook G, Hoeft A, Glasmacher A, Stuber F (2009) Utility of a commercially
421 available multiplex real-time PCR assay to detect bacterial and fungal pathogens in
422 febrile neutropenia. *J Clin Microbiol* 47 (8):2405-2410
- 423 17. Varani S, Stanzani M, Paolucci M, Melchionda F, Castellani G, Nardi L, Landini MP,
424 Baccarani M, Pession A, Sambri V (2009) Diagnosis of bloodstream infections in
425 immunocompromised patients by real-time PCR. *J Infect* 58 (5):346-351
- 426 18. Casalta JP, Gourié F, Roux V, Thuny F, Habib G, Raoult D (2008) Evaluation of the
427 LightCycler(R) SeptiFast test in the rapid etiologic diagnostic of infectious
428 endocarditis. *Eur J Clin Microbiol Infect Dis* 28 569-573
- 429 19. Lamothe F, Jaton K, Prod'homme G, Senn L, Bille J, Calandra T, Marchetti O (2010)
430 Multiplex blood PCR in combination with blood cultures for improvement of
431 microbiological documentation of infection in febrile neutropenia. *J Clin Microbiol* 48
432 (10):3510-3516
- 433 20. Louie RF, Tang Z, Albertson TE, Cohen S, Tran NK, Kost GJ (2008) Multiplex
434 polymerase chain reaction detection enhancement of bacteremia and fungemia. *Crit*
435 *Care Med* 36 (5):1487-1492
- 436 21. Tsalik EL, Jones D, Nicholson B, Waring L, Liesenfeld O, Park LP, Glickman SW,
437 Caram LB, Langley RJ, van Velkinburgh JC, Cairns CB, Rivers EP, Otero RM,

438 Kingsmore SF, Lalani T, Fowler VG, Woods CW (2010) Multiplex PCR to diagnose
439 bloodstream infections in patients admitted from the emergency department with
440 sepsis. J Clin Microbiol 48 (1):26-33

441 22. Avolio M, Diamante P, Zamparo S, Modolo ML, Grosso S, Zigante P, Tosoni N, De
442 Rosa R, Stano P, Camporese A (2010) Molecular identification of bloodstream
443 pathogens in patients presenting to the emergency room with suspected sepsis. Shock
444 34 (1):27-30

445 23. Westh H, Lisby G, Breysse F, Boddinhaus B, Chomarat M, Gant V, Goglio A,
446 Raglio A, Schuster H, Stuber F, Wissing H, Hoeft A (2009) Multiplex real-time PCR
447 and blood culture for identification of bloodstream pathogens in patients with
448 suspected sepsis. Clin Microbiol Infect 15 (6):544-551

449
450
451
452
453
454
455
456
457
458
459
460
461

Table 1: Bacteria and fungi detectable by the SeptiFast assay according to the manufacturer.

Gram-negative	Gram-positive	Fungi
<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>
<i>Klebsiella pneumoniae</i>	<i>Staphylococcus epidermidis</i>	<i>Candida tropicalis</i>
<i>Klebsiella oxytoca</i>	<i>Staphylococcus haemolyticus</i>	<i>Candida parapsilosis</i>
<i>Serratia marcescens</i>	<i>Streptococcus pneumoniae</i>	<i>Candida krusei</i>
<i>Enterobacter cloacae</i>	<i>Streptococcus pyogenes</i>	<i>Candida glabrata</i>
<i>Enterobacter aerogenes</i>	<i>Streptococcus agalactiae</i>	<i>Aspergillus fumigatus</i>
<i>Proteus mirabilis</i>	<i>Streptococcus mitis</i>	
<i>Pseudomonas aeruginosa</i>	<i>Enterococcus faecium</i>	
<i>Acinetobacter baumannii</i>	<i>Enterococcus faecalis</i>	
<i>Stenotrophomonas maltophilia</i>		

Table 2: Pathogens detected by blood culture (BC) and/or PCR for species included in the PCR test menu.

	PCR + BC +	PCR+ BC –	PCR – BC +
	n ^a	n	n
Pathogens			
Coagulase-negative Staphylococcus (CoNS)	1 (0)	10 (2)	30 (8)
<i>Staphylococcus aureus</i>	10 (2)	13 (4)	5 (0)
<i>Streptococcus pneumoniae</i>	2 (0)	1 (1)	14 (0)
<i>Enterococcus faecalis</i>	3 (1)	0 (0)	2 (0)
other Streptococcus species	10 ^b (1)	3 (1)	13 ^c (0)
<i>Escherichia coli</i>	18 (3)	14 (0)	16 (2)
<i>Enterobacter cloacae/aerogenes</i>	1 (1)	4 (2)	1 (0)
<i>Klebsiella pneumoniae/oxytoca</i>	3 (1)	10 (3)	4 (0)
<i>Serratia marcescens</i>	0 (0)	1(1)	1 (1)
<i>Pseudomonas aeruginosa</i>	1 (0)	5 (1)	0 (0)
<i>Candida albicans</i>	1 (1)	1 (0)	0 (0)
<i>Aspergillus fumigatus</i>	0 (0)	2 (0)	0 (0)
Total	50 (10)	64 (15)	86 (11)

^a Number with prior antibiotic treatment is shown in brackets.

^b Blood culture was positive for *Streptococcus pyogenes* (n=2), other β -haemolytic streptococci (n=4), α -haemolytic streptococci (n=3), and *Streptococcus bovis* (n=1).

^c Blood culture was positive for *Streptococcus pyogenes* (n=4), other β -haemolytic streptococci (n=7) and α -haemolytic streptococci (n=2).

501 **Table 3:** Blood culture/PCR set with polymicrobial results (n=12): Positive results with ≥ 1
502 method for more than one pathogen.
503

Sex, Age	Diagnosis	PCR result	Blood culture aerobic bottle	Blood culture anaerobic bottle
f,68	Cholangitis	<i>Escherichia coli</i>	<i>E. coli</i> , Klebsiella species.	<i>E. coli</i> , Klebsiella species.
m,92	Cholangitis	<i>E. coli</i> , Klebsiella species. ^a	<i>E. coli</i>	<i>E. coli</i>
m,80	Cholangitis	<i>E. coli</i> , Klebsiella species	neg	neg
f,54	Infection in central venous access device	<i>Enterococcus faecalis</i> , Enterobacter species. ^b	<i>E. faecalis</i> , <i>Enterobacter cloacae</i>	<i>E. faecalis</i> , <i>E. cloacae</i>
f,80	Urinary tract infection	<i>Staphylococcus aureus</i>	neg	Coagulase-negative Staphylococcus (CoNS)
f,64	Urinary tract infection	neg	CoNS, <i>Aerococcus viridans</i>	<i>A. viridans</i>
m,80	Erysipelas	neg	Group G streptococci	Group G streptococci , CoNS
f,82	Urinary tract infection	<i>Pseudomonas aeruginosa</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
m,87	Urinary tract infection	<i>S. aureus</i> , <i>Candida albicans</i>	<i>S. aureus</i>	<i>S. aureus</i>
m,88	Suspected abdominal focus	Streptococcus species, Klebsiella species, <i>E. coli</i>	<i>Streptococcus bovis</i> , Klebsiella species, <i>E. coli</i>	<i>S. bovis</i> , Klebsiella species, <i>E. coli</i>
m,62	Diverticulitis	Streptococcus species.	neg	<i>Streptococcus anginosus</i> , Fusobacterium species.
m,48	Phlegmonous foot	Streptococcus species, <i>P. aeruginosa</i>	<i>Streptococcus pyogenes</i>	<i>S. pyogenes</i>

504 ^a *Klebsiella pneumoniae/oxytoca*

505 ^b *Enterobacter cloacae/aerogenes*

506

Table 4: Positive SeptiFast results, results of other microbiological analyses, and possible clinical support.

	PCR +	Microbiological support		No microbiological support	
		Full support by BC+ (same set) n	Other microbiological support ^a n	Clinical support n	No clinical support n
Species	n				
Coagulase-negative Staphylococcus	11	1	0	0	10
<i>Staphylococcus aureus</i>	23	10	3	0	10
Streptococcus species	13	10	2	0	1
<i>Streptococcus pneumoniae</i>	3	2	0	1 ^b	0
<i>Enterococcus faecalis</i>	3	3	0	0	0
<i>Escherichia coli</i>	32	18	2	4 ^c	8
<i>Enterobacter cloacae/aerogenes</i>	5	1	0	0	4
<i>Klebsiella pneumoniae/oxytoca</i>	13	3	3	3 ^d	4
<i>Serratia marcescens</i>	1	0	0	1 ^e	0
<i>Pseudomonas aeruginosa</i>	6	1	0	1 ^f	4
<i>Candida albicans</i>	2	1	0	0	1
<i>Aspergillus fumigatus</i>	2	0	0	0	2
Total	114	50	10	10	44

^a Other microbiological support: Other blood cultures, cultures from normally sterile sites, bronchial secretions and pure culture in urine and wounds. Wound culture with mixed flora including *Staphylococcus aureus* and β -haemolytic streptococci were also regarded as microbiological support.

^b Pneumonia.

^c Cholangitis (n=3) and cholecystitis (n=1).

^d Cholangitis (n=1) and urinary tract infection (n=2).

^e Relapsing cholangitis after liver transplantation.

^f Pneumonia after renal transplantation.

Table 5: Sensitivities and positive predictive values including a combined reference standard for major bacterial pathogens.

Species	Sensitivity ^a	Positive predictive value ^b	
		Reference standard Blood culture (BC)	Reference standard BC and/or Microbiological support and/or Clinical support
<i>Staphylococcus aureus</i>	67 (10/15)	43 (10/23)	57 (13/23)
<i>Streptococcus pneumoniae</i>	12 (2/16)	67 (2/3)	100 (3/3)
other <i>Streptococcus</i> species	43 (10/23)	77 (10/13)	92 (12/13)
<i>Escherichia coli</i>	53 (18/34)	56 (18/32)	75 (24/32)
<i>Klebsiella</i> species	43 (3/7)	23 (3/13)	69 (9/13)

^a Data are % (positive with PCR and BC/all positive with BC).

^b Data are % (positive with PCR and reference standard/all positive with PCR).