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▶ 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, Springer Verlag, 2011, 30 (9), pp.1127-1134. 10.1007/s10096-011-1201-6 . hal-00676224

## HAL Id: hal-00676224
## https://hal.archives-ouvertes.fr/hal-00676224

Submitted on 4 Mar 2012

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

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## Abstract

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

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

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

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

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

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

100 Prep Kit MGrade (Roche Diagnostics GmbH). In the extraction step an internal control (IC)
101 was added to each sample. The IC is a mixture of synthetic double-stranded DNA molecules
102 with primer binding sites identical to those of the target sequences, differing in their probe
103 binding sites. A negative control supplied by the manufacturer was included in each
104 extraction series and the reagent controls were used as positive control of the PCR reactions.
105 Gram-positive bacteria, Gram-negative bacteria, and fungi were amplified individually in
106 three different mixes. The real-time PCR was performed in a LightCycler 2.0 instrument
107 (Roche Diagnostics GmbH). The emitted fluorescence was measured in one of the four
108 different detection channels (610, 640, 670, and 705 nm). Analysis of the melting curves were
109 performed to strengthen the specificity of the products. The PCR amplicons from the
110 specimens and ICs were analyzed by a pathogen identification software (SIS; SeptiFast
111 Identification Software, Roche Diagnostics GmbH). The software automatically calculated
112 the melting point (*T*m) value and the corresponding peak height. For the Gram-positive assay
113 the analysis was based on melting peaks (*T*m and peak height) and amplification curve
114 crossing point (Cp) values. The assays for Gram-negative and Fungi were solely based on
115 melting peaks and Cp values were not considered. To reduce false positive results by assumed
116 contaminants the software also includes Cp cut-off values, ex for CoNS and Streptococcus
117 species this cut-off value is at 20 cycles. The assay was flagged as "Invalid" if the software
118 could identify neither a specimen nor the IC. The turn-around time was 6 h. The results of the
119 PCR assays were unknown to the clinicians until the closure of the study.
120
121 **Blood culture**
122
123 Blood culture was performed with the Bactec system (Becton, Dickinson and Company,
124 Sparks, MD, USA) as a standard procedure in patients with suspected disseminated bacterial

125 and/or fungal infection.

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

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

128 After transport at room temperature the bottles were placed in a Bactec 9240 incubator, with

129 monitoring for pH changes every 10 minutes for 6 days.

130 All signaling bottles were opened and an aliquot was taken for microscopy after Gram

131 staining, culture on solid media, and further analyses for species designation according to the

132 good laboratory practice of our accredited clinical diagnostic microbiological laboratory.

133

134 **Other cultures**

135

136 Cultures from normally sterile sites and from urine, wounds and respiratory secretions were

137 performed according to standard microbiological procedures [10]. The choices of performing

138 such cultures were based on the clinical judgement of the clinicians.

139

140 **Clinical data**

141

142 At inclusion clinical data were collected, including information on whether antibiotics had

143 been taken within 3 days prior to BC. A retrospective chart review was performed by two

144 specialists of infectious diseases (P.J., K.S.).

145

146 **Analysis**

147

148 The SeptiFast results were compared with the results of BC and other microbiological data.

149 Although we routinely collected paired BC, we included for evaluation only one BC/PCR set

150 (two BC bottles and one PCR tube taken at the same time) per patient. If it was not negative
151 in these analyses, the first BC/PCR set providing any positive result was included.
152
153 **Definitions**
154
155 A positive PCR result was considered to be fully supported when an identical microorganism
156 was isolated in the BC of the same BC/PCR set. Other microbiological support for a positive
157 PCR was defined as other positive BCs, cultures from normally sterile sites and from
158 bronchial secretion, and pure culture in urine and wounds. Wound culture with mixed flora
159 including *Staphylococcus aureus* and β-haemolytic streptococci was also regarded as other
160 microbiological support.
161
162 In the cases of a positive PCR result without any microbiological support, we made a
163 retrospective attempt to find clinical support for the positive PCR result. We considered a
164 PCR result to have clinical support if the species identified by PCR generally was considered
165 to be a common pathogen of the patient's type of infection, and if no microbiological support
166 for any other pathogen was identified.
167
168 The regional ethical committee approved the study.
169
170 ## Results
171
172 During the study period the total number of patients subjected to BC at the department were
173 1540 and a positive BC was found in 208 of them.
174

175 Altogether 1093 patients were included in the present study, 486 females (44%) and 607
176 males (56%). All these patients had results from at least one BC/PCR set. Of the included
177 patients the median age was 67 years (range 14-98 years). ICU care were received by 36
178 patients. The crude mortality rate within 30 days was 45/1093 (4%).
179
180 Blood culture and/or PCR was positive for any pathogen in 197 patients (18%) and in 896
181 patients the BC/PCR sets were negative. A positive BC was found in 138 of the patients and
182 107 patients had positive SeptiFast results.
183
184 The results regarding the pathogens detected by BC and/or PCR for species included in the
185 PCR test menu are summarized in Table 2. The most commonly detected pathogens were
186 Coagulase-negative Staphylococcus (CoNS), *Staphylococcus aureus*, *Streptococcus*
187 *pneumoniae*, other Streptococcus species, *Escherichia coli*, and Klebsiella species. Results
188 from patients who received antibiotic treatment within 3 days prior to BC are shown for each
189 pathogen. In total there were 50 results that were positive both in PCR and BC, 64 results that
190 were positive only with PCR and 86 results that were positive only with BC among the
191 pathogens on the PCR test menu.
192
193 Concerning CoNS, 10 were positive only with PCR, 30 were positive only with BC and only
194 1 was positive with both PCR and BC. It may also be noted that for *S. pneumoniae* 14 were
195 positive only with BC, 1 was positive only with PCR during antibiotic treatment and 2 were
196 positive with both PCR and BC. Regarding other Streptococcus species, 13 were positive only
197 with BC, among which 4 were positive for *Str. pyogenes* and 7 were positive for other β-
198 haemolytic streptococci (one of which with invalid result in PCR). Three results were positive
199 for other Streptococcus species by PCR only. PCR and BC were positive for 10 results, and of

200 these 2 were positive for *Str. pyogenes* and 4 were positive for other β-haemolytic
201 streptococci according to BC.
202
203 With BC 10 different isolated pathogens that are not included in the PCR test menu, were also
204 found in 10 patients. They were *Aerococcus viridans*, *Arcanobacterium haemolyticum*,
205 *Pseudomonas fluorescens*, *Shigella flexneri*, Bacteroides species, Citrobacter species,
206 Corynebacterium species, Fusobacterium species, Porphyromonas species, and Salmonella
207 species.
208
209 Polymicrobial results were obtained in 12 patients, and are shown in Table 3.
210
211 In Table 4 the PCR-positive results are compared with results of BC and other cultures in an
212 attempt to find other support for a positive PCR result. In the cases of positive PCR without
213 microbiological support, clinical support is presented.
214
215 For *S. aureus* other microbiological support was in one case another positive BC and a
216 positive joint culture, in one case another positive BC and culture from cerebrospinal fluid,
217 and in one case a positive culture from a renal cortical cyst. For Streptococcus species other
218 microbiological support consisted of wound culture, including β-haemolytic streptococci in 2
219 cases. Other microbiological support was other positive BC in 2 cases for *E. coli,* and other
220 positive BC in 2 cases and in one case pure culture in urine for Klebsiella.
221
222 Positive PCR results without any microbiological or clinical support were obtained for
223 example with CoNS in 10 cases, *S. aureus* in 10 cases, *E. coli* in 8 cases and *Pseudomonas*
224 *aeruginosa* in 4 cases.

225

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

230

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

236

## 237 Discussion

238

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

243

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

249 handling.

250

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

252 evaluation was evident.

253 We thus chose to study an unselected group, aiming at including all adult patients seeking

254 medical attention at our department of infectious diseases, for whom a blood culture was

255 ordered during one full year.

256

257 In the present study there was no systematic loss of patients for inclusion. A loss was seen

258 however in a few situations of overload of the department, and as a result of some mistakes in

259 the sampling; also, a few single patients chose not to participate. Our handling of samples and

260 our laboratory work strictly followed the instructions from the manufacturer of SeptiFast. In

261 order not to create any bias, only one set of blood culture/PCR per patient was included for

262 evaluation.

263 With this approach we consider the patient material to be representative of a population that,

264 in most cases, reaches the hospital with an infection that they have developed outside the

265 healthcare systems, named community onset blood stream infection.

266

267 In the design of the SeptiFast test the ITS region has been selected as the target region. By

268 this approach a higher sensitivity can be achieved compared to single-copy genes, since there

269 are often several ribosomal operons in the genomes of bacteria and fungi [9, 11]. A higher

270 specificity can also be obtained compared to rRNA, as the ITS is more species-specific.

271

272 The selected species of bacteria and fungi that can be identified by SeptiFast was calculated to

273 leave about 10% of our bacteraemias undiagnosed. Important pathogens are missing, such as

274 the anaerobes, Salmonella, Shigella, Haemophilus species, and *Neisseria meningitidis*. In our
275 study 10 results (7%) out of 146 positive BCs, from 138 patients, included pathogens not
276 covered by the SeptiFast.
277 In the evaluation, the results with the different diagnostic systems must also take into account
278 that the blood culture system uses 8-10 ml of venous blood per bottle (one aerobic and one
279 anaerobic bottle is taken in each culture), compared to the SeptiFast test which uses 1.5 ml in
280 an EDTA tube with vacuum for preparation of a DNA extract for six PCR assays. One PCR
281 assay thus contains DNA from 0.25 ml of blood or 1:32-40 of the blood culture volume used
282 in one bottle. This may have had implications for the diagnostic sensitivity.
283
284 In the SeptiFast assay high quality PCR reagents, free of bacterial or fungal DNA
285 contamination, are used according to the information provided by the producer. The EDTA
286 vacuum tubes for drawing venous blood were recommended by the producer and said to be
287 endotoxin and DNA free. The present study results, indicate, however that contamination was
288 a problem, giving false positive *E. coli* PCR results in some cases (8 out of 32 had no support
289 for the positive PCR result; Table 4) and *P. aeruginosa* in a few other cases (4 of 6 without
290 support; Table 4). Whether these contaminants came from the sampling tubes or the reagents
291 is not clear. Such problems have to be brought down to an absolute minimum by the
292 producers.
293
294 The background of a negative result with SeptiFast, despite positive BC with a bacterium that
295 is included in the test (false negative), is multifactorial. The reasons may be speculated on and
296 include possibly less optimal technical steps in the PCR reaction, incorrect interpretation of
297 amplification signals, problems with the selection of species-specific targets in the test, and
298 the blood volume used.

299 In the present study the SeptiFast false negative *S. pneumoniae* bacteraemic cases are most
300 noteworthy, since only 2 of 16 BC positive samples were detected, i.e. the sensitivity was
301 12%. This may be explained by the fact that the manufacturer of SeptiFast has set the
302 detection level for *S. pneumoniae* high, in order not to have false-positive results caused by
303 closely related alpha-haemolytic streptococci. For optimal detection of *S. pneumoniae* DNA,
304 PCR targets specific to *S. pneumoniae* should probably be used. We recently found that PCR
305 for *lytA* applied to plasma samples had a sensitivity of 70% and a specificity of 99% for
306 detection of *S. pneumoniae* bacteraemia in patients with community-acquired pneumonia
307 [12].
308 The same problem was encountered for other bacteraemic streptococci, mainly beta-
309 haemolytic ones, which were negative in 57% in the SeptiFast test.
310 These results hamper the use of the tested version of SeptiFast for detection of bacteraemia in
311 a general infectious disease patient population, where pneumococci and invasive haemolytic
312 streptococci are common causes of community onset bacteraemia.
313
314 A positive result with SeptiFast when the BC is negative can be received in a number of
315 situations. Some of these results are clinically reliable and helpful and some are misleading.
316 A well defined contribution by the nucleic assay tests is the performance when antibiotics
317 have been given prior to culture, and individual patients in this situation were seen in the
318 present study (Table 2).
319 The presence of bacterial/fungal DNA in whole blood without live organisms has not so far
320 been systematically explored. Biologically it is a likely situation that could focus an
321 investigation on a search for a localised infection. The present study includes such patients,
322 for example with *S. aureus* positivity in SeptiFast (Table 4).

323 Contamination of a blood sample as such in connection with the sampling of the patient
324 involves the skin flora from which mainly different staphylococci and streptococci become
325 positive in the diagnostic systems. The results for CoNS in the present study (Table 2)
326 indicate that there are problems with skin contamination. Both nucleic assay tests and culture
327 systems share these problems and results have to be repeated to be more reliable. A
328 semiquantitative view is also useful in these situations when a late and low PCR product and a
329 need for long-term incubation for growth favour the judgement of a contaminant [13, 14].
330
331 A number of studies, mainly with smaller study groups, using SeptiFast for the detection of
332 microbial DNAemia, have been published with various results. Several publications have
333 focused on compromised and highly selected patient groups, mainly from haematological
334 units [15, 16, 17, 18, 19].
335 Some publications include community onset bloodstream infections. Louie et al studied 200
336 adult patients, sampled at emergency rooms, intensive care units and general medicine wards.
337 They found positive PCR in 45 cases compared to 37 BC, and 69 % of the PCR results were
338 confirmed by blood, urine, and catheter culture [20]. Tsalik et al included 306 patients with
339 suspected sepsis. In the patients with an identified infectious aetiology, 66 were positive with
340 BC and 46 with PCR [21]. Avolio et al compared BC with SeptiFast in patients admitted to
341 the emergency room with suspected sepsis. Out of 144 samples, 30 identified the same
342 organism with BC and PCR, 13 organisms were identified only with BC, and 10 were positive
343 only with PCR [22]. West et al conducted a multicenter trial of patients with suspected
344 bacterial or fungal septicaemia. In total 359 patients were evaluated. The rate of positivity was
345 17% for BC and 26% for SeptiFast. The 74 non-contaminant BC isolates were identified with
346 PCR in 50 cases. The 174 non-contaminant microorganisms detected by SeptiFast were
347 isolated by BC in 50 cases and additionally 67 could be confirmed as probable clinical

348 pathogens by culture of the same microorganism from a relevant anatomical site within the
349 same clinical time frame. No support was found for a total of 57 samples [23].
350
351 In the present study, we found that the positivity rate of PCR was generally slightly lower
352 than that of BC. In agreement with the previous studies on the SeptiFast test, we found that
353 the test had high specificities and high negative predictive values for the detectable pathogens.
354 However, the sensitivities and PPVs of the test were suboptimal, facts that became obvious
355 with the layout of our study, with a large number of community onset consecutive patients.
356
357

## 358 Acknowledgements

359

360 The study was made possible by a research grant from the Research Committee of the County
361 Council of Örebro.
362
363 Reagents and means for technical assistance for the study were provided by Roche
364 Diagnostics.
365
366 There has been no other financial relationship with any other company.
367

## 368 Conflict of interest

369

370 The authors declare that they have no conflict of interest.
371

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447 and blood culture for identification of bloodstream pathogens in patients with
448 suspected sepsis. Clin Microbiol Infect 15 (6):544-551
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461

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

463

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

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485 **Table 2**: Pathogens detected by blood culture (BC) and/or PCR for species included in the
486 PCR test menu.
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| | PCR + BC + n [a] | PCR+ BC – n | PCR – BC + n |
|---|---|---|---|
| **Pathogens** | | | |
| Coagulase-negative Staphylococcus (CoNS) | 1 (0) | 10 (2) | 30 (8) |
| *Staphylococcus aureus* | 10 (2) | 13 (4) | 5 (0) |
| *Streptococcus pneumoniae* | 2 (0) | 1 (1) | 14 (0) |
| *Enterococcus faecalis* | 3 (1) | 0 (0) | 2 (0) |
| *other* Streptococcus species | 10 [b] (1) | 3 (1) | 13 [c] (0) |
| *Escherichia coli* | 18 (3) | 14 (0) | 16 (2) |
| *Enterobacter cloacae/aerogenes* | 1 (1) | 4 (2) | 1 (0) |
| *Klebsiella pneumoniae/oxytoca* | 3 (1) | 10 (3) | 4 (0) |
| *Serratia marcescens* | 0 (0) | 1(1) | 1 (1) |
| *Pseudomonas aeruginosa* | 1 (0) | 5 (1) | 0 (0) |
| *Candida albicans* | 1 (1) | 1 (0) | 0 (0) |
| *Aspergillus fumigatus* | 0 (0) | 2 (0) | 0 (0) |
| | | | |
| **Total** | 50 (10) | 64 (15) | 86 (11) |

488 [a] Number with prior antibiotic treatment is shown in brackets.
489 [b] Blood culture was positive for *Streptococcus pyogenes* (n=2), other β-haemolytic streptococci (n=4), α-
490 haemolytic streptococci (n=3), and *Streptococcus bovis* (n=1).
491 [c] Blood culture was positive for *Streptococcus pyogenes* (n=4), other β-haemolytic streptococci (n=7) and α-
492 haemolytic streptococci (n=2).
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501 **Table 3:** Blood culture/PCR set with polymicrobial results (n=12): Positive results with ≥ 1
502 method for more than one pathogen.
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| Sex, Age | Diagnosis | PCR result | Blood culture aerobic bottle | Blood culture anaerobic bottle |
|---|---|---|---|---|
| f,68 | Cholangitis | *Escherichia coli* | *E. coli*, Klebsiella species. | *E. coli*, Klebsiella species. |
| m,92 | Cholangitis | *E. coli*, Klebsiella species. [a] | *E. coli* | *E. coli* |
| m,80 | Cholangitis | *E. coli*, Klebsiella species | neg | neg |
| f,54 | Infection in central venous access device | *Enterococcus faecalis*, Enterobacter species. [b] | *E. faecalis, Enterobacter cloacae* | *E. faecalis, E. cloacae* |
| f,80 | Urinary tract infection | *Staphylococcus aureus* | neg | Coagulase-negative Staphylococcus (CoNS) |
| f,64 | Urinary tract infection | neg | CoNS, *Aerococcus viridans* | *A. viridans* |
| m,80 | Erysipelas | neg | Group G streptococci | Group G streptococci , CoNS |
| f,82 | Urinary tract infection | *Pseudomonas aeruginosa* | *P. aeruginosa* | *E. coli* |
| m,87 | Urinary tract infection | *S. aureus, Candida albicans* | *S. aureus* | *S. aureus* |
| m,88 | Suspected abdominal focus | Streptococcus species, Klebsiella species, *E. coli* | *Streptococcus bovis*, Klebsiella species, *E. coli* | *S. bovis*, Klebsiella species, *E. coli* |
| m,62 | Diverticulitis | Streptococcus species. | neg | *Streptococcus anginosus*, Fusobacterium species. |
| m,48 | Phlegmonous foot | Streptococcus species, *P. aeruginosa* | *Streptococcus pyogenes* | *S. pyogenes* |

504 [a] *Klebsiella pneumoniae/oxytoca*
505 [b] *Enterobacter cloacae/aerogenes*
506

507 **Table 4:** Positive SeptiFast results, results of other microbiological analyses, and possible
508 clinical support.
509

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

510 [a] Other microbiological support: Other blood cultures, cultures from normally sterile sites, bronchial secretions
511 and pure culture in urine and wounds. Wound culture with mixed flora including *Staphylococcus aureus* and β-
512 haemolytic streptococci were also regarded as microbiological support.
513 [b] Pneumonia.
514 [c] Cholangitis (n=3) and cholecystitis (n=1).
515 [d] Cholangitis (n=1) and urinary tract infection (n=2).
516 [e] Relapsing cholangitis after liver transplantation.
517 [f] Pneumonia after renal transplantation.
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520 **Table 5:** Sensitivities and positive predictive values including a combined reference standard

521 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** |
| *Staphylococcus aureus* | 67 (10/15) | 43 (10/23) | 57 (13/23) |
| *Streptococcus pneumoniae* | 12 (2/16) | 67 (2/3) | 100 (3/3) |
| other Streptococcus species | 43 (10/23) | 77 (10/13) | 92 (12/13) |
| *Escherichia coli* | 53 (18/34) | 56 (18/32) | 75 (24/32) |
| Klebsiella species | 43 (3/7) | 23 (3/13) | 69 (9/13) |

522 [a] Data are % (positive with PCR and BC/all positive with BC).

523 [b] Data are % (positive with PCR and reference standard/all positive with PCR).