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Evaluation of the Hyplex BloodScreen Multiplex PCR–Enzyme-Linked Immunosorbent Assay System for Direct Identification of Gram-Positive Cocci and Gram-Negative Bacilli from Positive Blood Cultures

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We evaluated the Hyplex BloodScreen PCR–enzyme-linked immunosorbent assay (ELISA) system (BAG, Lich, Germany), a new diagnostic test for the direct identification of gram-negative bacilli and gram-positive cocci from positive blood cultures, with 482 positive BACTEC 9240 blood culture bottles. The test involves amplification of the bacterial DNA by multiplex PCR and subsequent hybridization of the PCR product to specific oligonucleotide probes in an ELISA-based format. The available probes allow the separate detection of *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Klebsiella* spp., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*/*Enterococcus faecium*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae* and the staphylococcal *mecA* gene. The Hyplex BloodScreen test showed an overall sensitivity of 100% for the identification of gram-negative bacilli and 96.6 to 100% for the identification of gram-positive cocci (*S. aureus*, 100%; *S. epidermidis*, 97.2%; *Enterococcus faecalis*/*Enterococcus faecium*, 96.6%; and *Streptococcus pneumoniae*, 100%). The specificities of the test modules ranged from 92.5 to 100% for gram-negative bacilli and 97.7 to 100% for gram-positive cocci (*Escherichia coli*, 92.5%; *Pseudomonas aeruginosa*, 98.5%; *Klebsiella* spp., 100%; *Enterobacter aerogenes*, 100%; *S. aureus*, 100%, *S. epidermidis*, 97.7%; *Enterococcus faecalis*/*Enterococcus faecium*, 99.6%; *Streptococcus pyogenes*, 100%; and *Streptococcus pneumoniae*, 99.3%). The result of the *mecA* gene detection module correlated with the result of the phenotypic oxacillin resistance testing in all 38 isolates of *Staphylococcus aureus* investigated. In conclusion, the Hyplex BloodScreen PCR-ELISA system is well suited for the direct and specific identification of the most common pathogenic bacteria and the direct detection of the *mecA* gene of *Staphylococcus aureus* in positive blood cultures.

Bloodstream infections are potentially life-threatening conditions that require rapid identification of the causative agent in order to facilitate a specific antimicrobial therapy. Rapid bacterial identification and susceptibility testing not only improves patient therapy and outcome but can also reduce costs and may prevent the development of bacterial resistance, for instance by allowing a shorter duration of antimicrobial therapy or an early switch from empirically administered broad-spectrum antibiotics to narrow-spectrum substances (8, 15, 18).

Several methods for direct detection and susceptibility testing of bacteria in positive blood cultures have been described, including PCR methods, as well as DNA and RNA probes and restriction fragment length polymorphism profile analysis (4). The target sequences of the primers and probes were the eubacterial 16S rRNA gene and family-, genus-, or species-specific genes for identification of the bacteria, as well as specific resistance genes, such as the staphylococcal *mecA* gene, for the determination of antimicrobial susceptibility (2, 5, 9–12). The application of fluorescence-based real-time PCR even allowed specific detection of pathogenic bacteria in blood cultures within a few hours (17). However, there is no commercial kit for molecular diagnostics of blood cultures yet available and standardization of the assays is lacking. In addition to molecular methods, the Vitek 2 system has recently been evaluated for the identification and susceptibility testing of pathogenic bacteria by direct inoculation from positive BACTEC blood culture bottles and showed promising results (13). However, thus far these evaluations have exclusively used gram-negative bacilli, and problems with the identification of gram-positive cocci have been described (1).

Recently, a commercially available test kit, the Hyplex BloodScreen multiplex PCR-enzyme-linked immunosorbent assay (ELISA) system (BAG, Lich, Germany), has been developed that facilitates direct identification of pathogenic bacteria from positive blood culture bottles within few hours. Independently of the result of the initial Gram stain, a PCR assay for either gram-positive or gram-negative bacteria is applied. For subsequent hybridization in an ELISA-based format, the assay is made up of a panel of test modules (microtiter plate cavities coated with a specific probe) for the identification of *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Klebsiella* spp., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *Enterococcus faecalis*/*Enterococcus faecium*. In addition, the staphylococcal *mecA* gene can be detected with a specific test.

In the present study, we first evaluated the Hyplex BloodScreen multiplex PCR-ELISA system for the direct identification of pathogenic bacteria and the detection of the staphylo-

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coccal *mecA* gene on 482 positive aerobic and anaerobic BACTEC 9240 blood culture bottles. The results of the Hyplex BloodScreen test were compared to the results of culture and biochemical identification as "gold standard."

MATERIALS AND METHODS

Sample collection and bacterial strains. Samples consisted of 482 positive BACTEC 9240 blood culture bottles, including 376 aerobic and 106 anaerobic bottles from patients of all departments of the hospital of Ulm University. Samples were collected between July 2001 and August 2002 after automatic detection of the bottles in the BACTEC 9240 (Becton Dickinson) blood culture system and processed in parallel to routine diagnostics. On each day of collection, all blood cultures positive with gram-positive cocci, gram-negative cocci, or gram-negative rods in the initial Gram stain, including mixtures of different bacteria, were used, and no preselection of samples was done. In cases where both the aerobic and the anaerobic bottle were detected to be positive, only the aerobic bottle was included in the study.

Cultural identification of bacteria. Identification and differentiation of bacteria grown in BACTEC 9240 bottles was performed according to the results of the Gram stain. Identification of all bacteria apart from most staphylococci was done by Api (Api 20 Strep, Api Rapid ID 32 Strep, Api NH, Api 20E, and Api 20NE [all from BioMerieux]). For staphylococci, diagnosis was based on typical morphology (color, hemolysis, etc.), a positive catalase reaction, positive clumping factor (Slidex; BioMerieux), positive aurease detection (BioMerieux), and mannitol-salt-agar detection. If differentiation was ambiguous, an Api Staph analysis was performed.

Susceptibility testing. Methicillin resistance in *S. aureus* was detected by determination of *PBP2a* by a latex agglutination test (MRSA Screen; Innogenetics, Ghent, Belgium) and phenotypically by growth on Mueller-Hinton agar supplemented with 6 μg of oxacillin and 4% NaCl (Heipha)/ml. In coagulase-negative staphylococci (CNS), oxacillin resistance was determined phenotypically by agar diffusion test according to the NCCLS guideline M100-S11, with a 1-μg oxacillin disk on Muller-Hinton agar supplemented with 2% NaCl. An inhibition zone of ≤17 mm indicated resistance to oxacillin. In addition, oxacillin MIC was determined by microbouillon (broth) dilution on the Merlin Micronaut System (Merlin) by using a range of 0.25 to 32 μg of oxacillin of 2% NaCl in the assay. In all strains, the results of both methods were consistent.

DNA preparation. Total DNA from positive blood culture bottles was prepared by an alkali wash lysis method, according to the protocol of Millar et al. (14). Briefly, 0.5 ml of inoculated blood culture medium was mixed with 1.0 ml of alkali wash solution (0.5 M NaOH, 0.05 M sodium citrate) and then further mixed on a rotator for 10 min at room temperature. The suspension was centrifuged at 13,000 × *g* for 5 min, the pellet was washed twice in 0.5 ml of 0.5 M Tris-HCl (pH 8.0) and centrifuged as described above, and the resulting pellet was resuspended in 0.1 ml of Tris-EDTA (10 mM Tris-HCl [pH 8.0], 1 mM EDTA). The suspension was transferred in a screw-cap reaction tube, incubated at 95°C for 10 min, and subjected to two cycles of 2 min of freezing in liquid nitrogen and 2 min of boiling in a boiling water bath (freezing at −20°C for 5 to 10 min each was also effective). After a centrifugation step at 13,000 × *g* for 15 min, the supernatant was stored at −20°C until use in the Hyplex BloodScreen test.

Hyplex BloodScreen multiplex PCR-ELISA system. The Hyplex BloodScreen multiplex PCR-ELISA system (version 1; BAG) involves an initial amplification of the bacterial DNA by multiplex PCR and a subsequent hybridization of the PCR product to specific oligonucleotide probes in an ELISA-based format with color-coded wells. The test takes ca. 4.5 to 6 h, including DNA isolation, PCR amplification, and detection by hybridization. Positive samples are identified by measurement of the optical densities (ODs) in the ELISA plate wells. An OD of <0.2 has been defined as negative, an OD between 0.2 and 0.4 has been defined as borderline, and an OD of >0.4 has been defined as positive by the manufacturer. All samples with borderline results (<2% of all samples) were repeated three times (extending the turnaround time of the assay for ~2.5 h), and the means of the measurements were considered. The system includes a gram-positive PCR kit for the amplification of the DNA of gram-positive bacteria and test modules (microtiter plate cavities coated with a specific probe) for the detection of *S. aureus*, *S. epidermidis*, *E. faecalis*/*E. faecium*, *S. pyogenes*, *S. pneumoniae*, and the staphylococcal *mecA* gene, as well as a gram-negative PCR kit for the amplification of the DNA of gram-negative bacteria and test modules for the detection of *E. coli*, *P. aeruginosa*, *E. aerogenes*, and *Klebsiella* spp. Target genes of the assay are species-specific housekeeping genes. The test was performed as indicated by the manufacturer except for the use of 0.5-ml reaction tubes (instead of 0.2-ml tubes) for the multiplex PCR on a thermal cycler (Thermal Cycler Touch Down; Hybaid, Ashford, United Kingdom) and the following modification of the PCR protocol: a denaturation time of 1 min, an annealing time of 1 min, and an elongation time of 90 s.

Nucleotide sequence analysis of PCR products. The nucleotide sequences of amplicons were determined in eight samples in which the biochemical identification of certain bacterial isolates was found to be ambiguous or divergent with respect to the PCR result. Briefly, the primers 16sfor (AGAGTTTGATCCTG GCTCAG) and 16srev (GTTTACCTTGTTACGACTT) were used as sequencing primers; these primers span a region from positions 8 to 1509 of the eubacterial 16S rRNA gene (7). Amplification products were purified by using the HighPure PCR product purification kit (Roche Diagnostics), and cycle sequencing reactions of the eubacterial 16S ribosomal DNA (rDNA) sequence were performed as described in the dye terminator cycle sequencing ready reaction kit (ABI Prism Biosystems). The fluorescence-labeled reaction products were analyzed with a ABI Prism 310 genetic analyzer. Obtained sequences were compared to the GenBank/EMBL databank for identification of isolates. Identification was defined as a sequence homology of ≥98.5% with the respective strain in the database.

LightCycler PCR assays. For confirmation of the identification of *S. epidermidis* and the *mecA* gene in selected samples, LightCycler PCR assays with hybridization probes were performed. For the identification of *S. epidermidis*, primer DG74 and RW01, amplifying a 386-bp fragment of the 16S rRNA gene, and the probes EpiFL and EpiLC were used according to the protocol published by Wellinghausen et al. (17). For identification of the *mecA* gene, primers Mec-F2 and Mec-R2 and probes Mec-HP-1 and Mec-HP-2 were used strictly according to the protocol published by Reischl et al. (16).

RESULTS

Identification of samples with gram-negative microscopy. In 140 blood cultures, exclusively gram-negative bacteria were visible in the initial Gram stain and grown in pure culture. In all samples, the PCR assay for gram-negative bacteria and the hybridization modules for *E. coli*, *P. aeruginosa*, *Klebsiella* spp., and *E. aerogenes* were applied. Compared to culture and subsequent biochemical identification, the diagnostic sensitivity of the assays resulted in 100% (Table 1). For the *E. aerogenes* assay, the sensitivity was not determined since no isolate of this species was included in the panel of samples tested. Regarding diagnostic specificity, the *Klebsiella* spp. and the *E. aerogenes* assay also had a specificity of 100%. The *P. aeruginosa* assay showed a specificity of 98.5% since cross-reactions with *Citrobacter koseri* and *Enterobacter cloacae* were observed. However, these false-positive results had much lower OD values than the true positive isolates and only reached the cutoff OD value of 0.4, which discriminates borderline and positive samples, as stated by the manufacturer (Table 1). The *E. coli* assay showed a specificity of only 92.5% since cross-reactions with *Bacteroides fragilis*, *E. cloacae*, and *Morganella morganii* were observed. Although OD values of the false-positive samples that contained *B. fragilis* and *M. morganii* clearly differed from the OD values from the true positive samples (median OD of <0.6 versus 2.5, $P < 0.001$ by Mann-Whitney U test; see also Table 1), the false-positive *E. cloacae* isolate had an OD value similar to that of *E. coli* (Table 1). Identification of this *E. cloacae* isolate was further confirmed by 16S rDNA sequencing (99.6% homology to the respective strain in the database).

Identification of samples with gram-positive microscopy. In 309 blood cultures, exclusively gram-positive cocci were visible in the initial Gram stain and grew in pure culture. In all samples, the PCR assay for gram-positive bacteria and the hybridization modules for *S. aureus*, *S. epidermidis*, *E. faecium*/*E. faecalis*, *S. pyogenes*, and *S. pneumoniae*, as well as the *mecA* gene, were applied. A sensitivity of 100% was observed for the *S. aureus* and *S. pneumoniae* modules. The *E. faecalis*/*E. fae-*

TABLE 1. PCR and culture results of blood cultures positive for a single species of gram-negative bacteria

Species identified by culture	No. of blood cultures (n = 140)	No. of positive PCR results[a] with module for detection of:			
		E. coli	*P. aeruginosa*	*Klebsiella* spp.	*E. aerogenes*
Acinetobacter baumannii	5	0	0	0	0
Acinetobacter junii/johnsonii	2	0	0	0	0
Acinetobacter spp.	3	0	0	0	0
Agrobacterium radiobacter	1	0	0	0	0
Bacteroides caccae	1	0	0	0	0
Bacteroides fragilis	4	4[b] (0.38)	0	0	0
Bacteroides vulgatus	1	0	0	0	0
Chryseomonas luteola	1	0	0	0	0
Citrobacter koseri	1	0	1 (0.39)	0	0
Citrobacter spp.	1	0	0	0	0
Escherichia coli	58	**58 (2.52)**	0	0	0
Enterobacter cloacae	20	1[c] (2.50)	3[d] (0.41)	0	0
Enterobacter sakazakii	3	0	0	0	0
Fusobacterium spp.	1	0	0	0	0
Haemophilus influenzae	1	0	0	0	0
Klebsiella oxytoca	4	0	0	**4 (1.94)**	0
Klebsiella pneumoniae	18	0	0	**18 (2.10)**	0
Morganella morganii	1	1 (0.59)	0	0	0
Neisseria meningitidis	1	0	0	0	0
Pseudomonas aeruginosa	6	0	**6 (2.37)**	0	0
Proteus mirabilis	1	0	0	0	0
Salmonella typhimurium	1	0	0	0	0
Serratia marcescens	1	0	0	0	0
Serratia spp.	1	0	0	0	0
Stenotrophomonas maltophilia	3	0	0	0	0

[a] Median ODs of the measurements are shown in parentheses. In the case of false-positive results, the OD value represents the median of at least three repeated measurements of each individual sample. The diagnostic sensitivities and specificities for *E. coli*, *P. aeruginosa*, *Klebsiella* spp., and *E. aerogenes* were 100 and 92.5%, 100 and 98.5%, 100 and 100%, and not determined and 100%, respectively. True-positive results are in boldface.

[b] From anaerobic blood culture bottles (not recommended by the manufacturer).

[c] 16S rDNA sequencing confirmed the biochemical identification result (*Enterobacter* spp., 99.6% homology in 1,455 bases sequenced).

[d] Three isolates from three different blood cultures of the same patient representing only one bacterial strain.

cium module repeatedly revealed borderline positive OD values (median OD of 0.23) in one sample of *E. faecium*, most probably due to low amount of DNA in the sample. The diagnostic sensitivity of the *S. pyogenes* module could not be calculated since no isolate of this species was included in the panel of samples tested.

All assays had a diagnostic specificity exceeding 97.5%. The *S. epidermidis* module showed false-positive results with three samples of CNS, four samples of *E. faecalis* and one sample of *Streptococcus mitis* (Table 2). In these eight samples, an *S. epidermidis*-specific LightCycler PCR was also performed that resulted in a positive signal in six of the eight samples, suggesting the simultaneous presence of *S. epidermidis* DNA, possibly due to contamination of the sample during preparation of the blood culture. Both samples of CNS, which were false positive in the Hyplex BloodScreen test but negative in the *S. epidermidis* LightCycler PCR, were identified as *S. hominis* by 16S rDNA sequencing and, thus, must be regarded as "true" false positives. The *E. faecalis*/*E. faecium* module was positive in one isolate of *Enterococcus durans* that was identified by Api ID 32 Strep (code 32115701351). 16S rDNA sequencing of this isolate revealed the highest similarity (99.8%) to the 16S rDNA of *E. faecium*, which might explain the positive test result. The *S. pneumoniae* module reacted positively in one isolate of *E. faecalis* and in one isolate of *S. mitis*. The false-positive result of the *S. mitis* can be explained since this isolate harbors the PCR target gene (unpublished data). The *S. aureus* and the *S. pyogenes* module had specificities of even 100% (Table 2).

Identification of the *mecA* gene of staphylococci in pure culture. The Hyplex BloodScreen test for the detection of the *mecA* gene was applied in all samples showing gram-positive bacteria in the Gram stain. Apart from staphylococci, no other isolates reacted positive in the *mecA* test. In *S. aureus*, the *mecA* gene was detected in all methicillin-resistant *S. aureus* strains (n = 3) and in none of the phenotypically confirmed methicillin-susceptible *S. aureus* isolates (n = 35).

Concerning *S. epidermidis*, 95.8% of the phenotypically resistant isolates (136 of 142) and 94.4% of the phenotypically susceptible isolates (34 of 36) were correctly identified in the *mecA* assay (Table 3). In these isolates, which reacted as false negative (n = 6) or false positive (n = 2) in the Hyplex BloodScreen assay compared to the phenotypic result, an additional *mecA* gene LightCycler PCR was performed. This PCR confirmed the presence of the *mecA* gene in all false-negative isolates. Both "false-positive" isolates were also confirmed to carry the *mecA* gene and therefore have to be considered as true positives.

Concerning CNS except *S. epidermidis*, the Hyplex BloodScreen *mecA* test yielded false-negative results in 12 of 20 phenotypically resistant isolates, and the additionally performed *mecA* gene LightCycler PCR confirmed the presence of the *mecA* gene in all isolates. Interestingly, 9 of 12 isolates were identified as *S. haemolyticus*. All phenotypically susceptible CNS were correctly identified by the *mecA* test (Table 3).

Identification of samples showing a mixture of gram-negative and gram-positive bacteria. A mixed culture of different species of bacteria was grown in 33 samples. In 22 samples a

TABLE 2. PCR and culture results of blood cultures positive for a single species of gram- positive bacteria

Species identified by culture	No. of blood cultures (n = 309)	No. of positive PCR results[a] with module for detection of:				
		S. aureus	*S. epidermidis*	*E. faecium*/*E. faecalis*	*S. pyogenes*	*S. pneumoniae*
Staphylococcus aureus MSSA[h]	35	**35 (2.53)**	0	0	0	0
Staphylococcus aureus MRSA[i]	3	**3 (2.6)**	0	0	0	0
Staphylococcus epidermidis[b]	179	0	**174 (2.48)**	0	0	0
CNS (except *Staphylococcus epidermidis*)[b]	30	0	3[g] (0.55)	0	0	0
Micrococcus luteus	2	0	0	0	0	0
Stomatococcus mucilagenosus	1	0	0	0	0	0
Enterococcus faecalis	18	0	4[d] (0.85)	**18 (1.17)**	0	1 (1.77)
Enterococcus faecium	11	0	0	**10**[e] **(2.2)**	0	0
Enterococcus durans	1	0	0	1[f] (2.5)	0	0
Streptococcus agalactiae	2	0	0	0	0	0
Streptococcus anginosus	6	0	0	0	0	0
Streptococcus mitis	7	0	1[d] (1.34)	0	0	1[c] (2.5)
Streptococcus oralis	1	0	0	0	0	0
Streptococcus pneumoniae	10	0	0	0	0	**10 (2.23)**
Streptococcus salivarius	2	0	0	0	0	0
Streptococcus spp. (alpha-hemolytic)	1	0	0	0	0	0

[a] Median ODs of the measurements are shown in parentheses. In the case of false-positive results, the OD value represents the median of at least three repeated measurements of each individual sample. The diagnostic sensitivities and specificites for *S. aureus*, *S. epidermidis*, *E. faecium*/*E. faecalis*, *S. pyogenes*, and *S. pneumoniae* were 100 and 100%, 97.2 and 97.7%, 96.6 and 99.6%, not determined and 100%, and 100 and 99.3%, respectively.
[b] Identification obtained by 16S rDNA sequencing in four cases of *S. epidermidis* and two cases of CNS (*S. hominis* and *S. haemolyticus*) in an earlier study (17).
[c] Atypical *S. mitis* harboring the PCR target gene.
[d] Also positive with specific *S. epidermidis* PCR, suggesting the coexistence of *S. epidermidis* DNA in the sample.
[e] The remaining sample with culture positive for *E. faecium* was repeatedly tested with borderline results (OD = 0.23), most probably due to low amount of DNA in the sample.
[f] 16S rDNA sequence of isolate with highest similarity to sequence of *E. faecium* (99.8% homology with 1,319 bases compared).
[g] One isolate of *S. haemolyticus* was also positive with the specific *S. epidermidis* PCR, suggesting the coexistence of *S. epidermidis* DNA in the sample; two isolates of *S. hominis* were negative in the *S. epidermidis* PCR.
[h] MSSA, methicillin-susceptible *S. aureus*.
[i] MRSA, methicillin-resistant *S. aureus*.

pure microscopy resulted in a mixed culture, while in 11 samples the mixture was already seen on the initial Gram stain. In 29 of 33 samples all species that could be identified with a specific hybridization module of the Hyplex BloodScreen kit were successfully identified (Table 4). False-negative results were obtained for *S. epidermidis* in four samples containing mixtures of different gram-positive cocci. Interestingly, in all four samples the additionally performed *S. epidermidis* PCR was negative, suggesting either low amounts of DNA in the sample or contamination of the culture plates. In four samples, bacterial species were identified by the Hyplex BloodScreen test that were not grown in culture, including *Klebsiella* spp. in two samples and both *S. aureus* and *S. epidermidis* in another two samples (Table 4). Since the *S. epidermidis* PCR was also positive in both samples growing *S. epidermidis*, the Hyplex BloodScreen test result might be regarded as true positive. The additional detection of *Klebsiella* spp. in two samples appears plausible since both samples were obtained from the same patient treated in the MICU for sepsis and large-bowel necrosis. The two samples positive for *S. aureus* were from two patients with abdominal sepsis and urosepsis, respectively.

Of the 15 isolates of *S. epidermidis* grown in a mixed culture, 13 were phenotypically resistant to oxacillin. In 5 of these 13 isolates *mecA* gene detection was false negative, including all four samples that were false negative for *S. epidermidis*. False-positive results were not seen with the *mecA* gene detection test.

DISCUSSION

In the present study, the Hyplex BloodScreen PCR-ELISA system was evaluated for the first time for direct identification of pathogenic bacteria in a large panel of positive BACTEC 9240 blood culture bottles. This test system allows identification of the bacteria within ca. 4.5 to 6 h, including DNA isolation, PCR amplification, and detection by reverse hybridization. Therefore, in contrast to conventional culture and biochemical identification techniques, which usually take 1 to 2 days, the Hyplex BloodScreen PCR-ELISA system generates results much quicker.

Concerning pure cultures of bacteria, the Hyplex Blood-

TABLE 3. Identification of the *mecA* gene in staphylococci grown in pure culture

Organism and parameter	n	No. of strains identified by the Hyplex BloodScreen *mecA* gene module	
		No. positive	No. negative
S. aureus			
Oxacillin resistance (phenotypic and PRP2a latex test)	3	3	0
Oxacillin susceptibility (phenotypic)	35	0	35
S. epidermidis			
Oxacillin resistance (phenotypic)	142	136	6[a]
Oxacillin susceptibility (phenotypic)	36	2[a]	34
Phenotypic susceptibility testing result not available	1	1	0
Coagulase-negative staphylococci except *S. epidermidis*			
Oxacillin resistance (phenotypic)	20	8	12[a]
Oxacillin susceptibility (phenotypic)	10	0	10

[a] All isolates were positive in the additionally performed *mecA* gene LightCycler PCR.

TABLE 4. Results of mixed bacterial cultures

Gram stain	Cultured species identifiable by Hyplex BloodScreen test[a]	Cultured species not identifiable by Hyplex BloodScreen test	False-positive results by Hyplex BloodScreen test
Gram-negative rods	***K. pneumoniae***	*P. mirabilis*	
	P. aeruginosa	*E. cloacae*	
		E. cloacae, *C. freundii*	
	P. aeruginosa*, *K. pneumoniae		
		A. junii/johnsonii, *A. lwoffii*	
	E. coli	*E. cloacae*	***Klebsiella* spp.**
	S. maltophilia	*A. baumannii*	
Gram-positive diplococci	***E. faecalis***	CNS	***S. aureus*, *S. epidermidis*[b]**
	E. faecalis*, *E. coli		
	E. faecalis*, *S. epidermidis		
Gram-positive cocci in clusters	***S. aureus*, *S. epidermidis***		
	***S. aureus*, *S. epidermidis*[c]**		
	***S. aureus*, *S. epidermidis*[c]**		
	***S. aureus*, *S. epidermidis*[c]**		
	S. epidermidis	*Acinetobacter* spp.	
	S. epidermidis*, *P. aeruginosa	Alpha-hemolytic streptococci	
	E. faecalis*, *S. epidermidis		
Gram-positive cocci in chains	***E. faecalis*, *E. faecium***		
	***E. faecalis*, *S. epidermidis*[c]**		
	E. faecalis*, *S. aureus		
		S. mitis, *S. salivarius*	
	S. epidermidis	*S. mitis*	
Gram-negative rods, gram-positive cocci in chains		*Acinetobacter* spp., *P. fluorescens*, *C. indologenes*	
	P. aeruginosa*, *E. coli	*S. agalactiae*	***S. aureus*, *S. epidermidis*[b]**
Gram-negative rods, gram-positive diplococci	***E. coli***	*S. oralis*	
		P. mirabilis, *S. mitis*	
Gram-negative rods, gram-positive cocci in clusters	***E. coli*, *S. epidermidis***		
	***P. aeruginosa*, S. epidermidis**		
	P. aeruginosa*, *S. epidermidis		
	E. faecium	*E. cloacae*	***Klebsiella* spp.**
Gram-positive diplococci and cocci in clusters	***E. faecalis***	CNS	
Gram-positive cocci in chains and in clusters	***S. epidermidis***	Alpha-hemolytic streptococci	
	S. epidermidis	*S. oralis*	

[a] Species printed in boldface were identified with the specific module of the Hyplex BloodScreen test.
[b] *S. epidermidis*-specific LightCycler PCR also positive.
[c] *S. epidermidis*-specific LightCycler PCR negative.

Screen PCR-ELISA system had a very high sensitivity, ranging from 96.6 to 100% for the various test modules (Table 1 and 2). The specificities of the different modules were also high and exceeded 97.5% in all assays but one. The test module for the detection of *E. coli* cross-reacted with *B. fragilis*, *M. morganii*, and one isolate of *E. cloacae* and therefore had a specificity of only 92.5%. Nevertheless, all isolates of *Bacteroides* spp. were grown exclusively from anaerobic blood culture bottles which should not be applied in the test as stated by the manufacturer. Interestingly, borderline OD values (OD = 0.2 to 0.4) were exclusively observed in false-positive samples, whereas most (330 of 336) true-positive samples had an OD of >1.0. Therefore, it may be applicable to regard borderline results as negative without a loss in sensitivity. An OD between 0.4 and 0.6 was observed in one true-positive sample, an OD between 0.6 and 0.8 was observed in three samples, and an OD between 0.8 and 1.0 was observed in two samples. Altogether, the Hyplex BloodScreen PCR-ELISA system detected 74.8% (336 of 449) of all bacteria, i.e., 61.4% of gram-negative bacilli and 80.9% of gram-positive cocci, directly in the positive blood culture bottle.

Concerning mixed infections with at least two different species of gram-positive and/or gram-negative bacteria, the Hyplex BloodScreen PCR-ELISA system also showed a high sensitivity, identifying all detectable species in 29 of 33 samples. False-positive results compared to the culture results were obtained in 4 of 33 samples.

The Hyplex BloodScreen PCR-ELISA system not only allows identification of the most important pathogenic bacteria causing bloodstream infections in humans but also includes a test module for the detection of the *mecA* gene in staphylococci that codes for methicillin susceptibility. In our study, the detection of the *mecA* gene in *S. aureus* proved 100% sensitive and specific. Phenotypic detection of methicillin resistance in CNS is difficult due to the heterogeneous expression of the

mecA gene, whereas detection of the *mecA* gene by PCR is reported to be the gold standard for the determination of methicillin resistance (3, 6, 19). In our study, the correlation of a negative Hyplex BloodScreen *mecA* gene PCR result with a phenotypic oxacillin susceptibility in CNS was high. The only two isolates of *S. epidermidis* that reacted positive in the *mecA* gene PCR but were phenotypically susceptible to oxacillin were confirmed to harbor the *mecA* gene by an additional LightCycler PCR. However, in *S. epidermidis* and other CNS, especially *S. haemolyticus*, the sensitivity of the *mecA* gene detection in phenotypically oxacillin-resistant isolates was lower (95.8% in pure cultures of *S. epidermidis*, 61.5% in mixed cultures containing *S. epidermidis*, and only 40.0% in CNS other than *S. epidermidis*). Thus, the results of the *mecA* gene PCR of the Hyplex BloodScreen system must be interpreted carefully in the absence of *S. aureus* and *S. epidermidis*.

Due to the minimal technical prerequisites that are needed by the test, including only a thermal cycler, an incubator for the hybridization, and a standard ELISA reader or an automated ELISA processor, the test is suited for both large laboratories and smaller laboratory units, e.g., in teaching or district hospitals. The costs of the kit and reagents are moderate and amount to ca. $4.50 (U.S. dollars) per sample and test.

Although it is stated by the manufacturer that the test should only be used on aerobic blood culture bottles, we decided, since all of the species covered by the test represent aerobically growing bacteria, to include both aerobic and anaerobic blood culture bottles since, in our experience, aerobically growing bacteria, especially staphylococci and *Enterobacteriaceae*, are sometimes found in anaerobic blood culture bottles. For instance, in our study 29 of 133 aerobic or facultative anaerobic gram-negative rods, including 16 of 58 isolates of *E. coli*, were detected in the anaerobic blood culture bottle.

In conclusion, the Hyplex BloodScreen PCR-ELISA system is well suited for the direct and specific identification of the most common pathogenic bacteria in positive blood cultures. It allows earlier identification of pathogenic bacteria compared to routine cultures and may contribute to a timely and cost-effective pathogen-adapted antimicrobial therapy even before availability of phenotypic antimicrobial susceptibility testing results. In addition, it also allows early sensitive and specific detection of the *mecA* gene in *S. aureus*.

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