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Enhanced detection of staphylococcal genomes in positive blood cultures using a polymeric enzyme complex

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ABSTRACT

This article describes a simple and inexpensive signal amplification method, termed polymeric enzyme detection (PED), which permits rapid and sensitive detection of conserved sequences in the *tuf* gene that identify *Staphylococcus* genus, conserved sequences in the *femB* gene that specifically detect *Staphylococcus aureus* species, and the methicillin resistance gene *mecA* directly from positive blood culture bottles. Microbe-specific capture probes were immobilized onto microtiter plates or silicon chips. Target sequences and biotin-labeled, target-specific probes were hybridized to complementary capture probes to create a biotin-labeled, surface-immobilized tripartite complex. In a two-step process, signal was amplified by incubating the surface-immobilized biotin with streptavidin followed by the addition of a 500-kDa dextran polymer conjugated with approximately 80 biotins. Signal was then developed by binding of a streptavidin–horseradish peroxidase conjugate followed by incubation with the substrate tetramethylbenzidine. Use of the PED method improved the lower limit of detection 10- to 100-fold in model DNA hybridization assays with limits of detection as low as 1 fmol/L target DNA. This level of sensitivity permits detection of genomic DNA from methicillin-resistant *S. aureus* positive blood cultures within 25 to 35 min using either a thin film biosensor chip or a microtiter plate-based assay.

Nosocomial infections have become a major global health issue. In the United States, there are an estimated 2 million nosocomial infections, causing an estimated 90,000 preventable deaths, every year [1]. Methicillin-resistant *Staphylococcus aureus* (MRSA)[1] and Methicillin-resistant coagulase-negative *Staphylococcus* (MRCoNS) are the leading causes of nosocomial infection worldwide. Approximately 60% of all staphylococcal infections display antibiotic resistance [2]. Rapid diagnosis and initiation of appropriate treatment are important to reduce morbidity and mortality associated with MRSA/MRCoNS bloodstream infections. Clinical studies have shown that reducing the time to diagnose MRSA/MRCoNS decreases the length of a patient's hospital stay, has a positive impact on infection control strategies, and leads to cost savings for the hospital [3,4].

Traditional culture and biochemical identification of microorganisms that cause bloodstream infections are time-consuming, requiring 24 to 72 h after detection of microbial growth by automated, continuous-monitoring blood culture systems [5]. Polymerase chain reaction (PCR)-based tests for MRSA/MSSA (methicillin-susceptible *S. aureus*) identification have significantly reduced the time to diagnosis to between 1 and 2 h [6]. However, real-time PCR-based formats have several disadvantages in the clinical laboratory, including the requirements for expensive equipment and specialized personnel training.

Here we describe an inexpensive and simple method, termed polymeric enzyme detection (PED), to amplify signal, permitting rapid detection of MRSA or MRCoNS directly from positive blood culture specimens. Typical hybridization-based assays for detecting nucleic acid sequences use reporter probes that contain a single biotin molecule per probe; thus, only a single streptavidin (SA)/enzyme (e.g., horseradish peroxidase [HRP]) conjugate is bound per hybridization event. Although reaction of the conjugate with HRP substrates, such as tetramethylbenzidine (TMB), produces detectable signal, we reasoned that detection sensitivity could be improved considerably if multiple SA/HRP conjugates could be recruited for each hybridization event, thereby decreasing the number of target molecules required to generate a detectable signal. The PED system described here exploits the simple and straightforward approach of indirectly introducing multiple biotin

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[1] *Abbreviations used:* MRSA, methicillin-resistant *Staphylococcus aureus*; MRCoNS, methicillin-resistant coagulase-negative *Staphylococcus*; PCR, polymerase chain reaction; MSSA, methicillin-susceptible *S. aureus*; PED, polymeric enzyme detection; SA, streptavidin; HRP, horseradish peroxidase; TMB, tetramethylbenzidine; NHS, *N*-hydroxysuccinamide; PBS, phosphate-buffered saline; HABA, 4′-hydroxyazobenzene-2-carboxylic acid; EDTA, ethylenediaminetetraacetic acid; SSC, saline–sodium citrate; SDS, sodium dodecyl sulfate; pHSA, poly-HRP SA; ELISA, enzyme-linked immunosorbent assay; CFU, colony-forming units; CCD, charge-coupled device ppt, precipitated product; NC, negative control.

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molecules onto each hybridized detector probe that, in turn, can bind multiple SA/HRP conjugates for enhanced signal production. This approach does not require PCR-based nucleic acid amplification, thereby eliminating the risk of carryover contamination from previously amplified samples. This test is highly sensitive, easy to use, and rapid (25–35 min of total test time), and it requires no expensive instrumentation; thus, it should be practical for most typical hospital diagnostic laboratories.

Materials and methods

PED polymer preparation and characterization

Amino dextran (500 kDa, Molecular Probes) was reacted at 1 mg/ml with 500 μM sulfo-NHS (*N*-hydroxysuccinamide)-biotin (Pierce) in 0.1 M borate buffer (pH 8.5) at room temperature for 3 h with constant mixing. The biotinylated dextran was purified and desalted using a PD-10 column (GE Healthcare) equilibrated in 0.1 M borate (pH 8.5) following the manufacturer's instructions. The polymer solution was then diluted to 5 ml in 0.1 M borate (pH 8.5) and reacted with 100 μM sulfo-NHS-acetate at room temperature for 3 h with constant mixing. The reaction mixture was passed over another PD-10 column equilibrated with 1× phosphate-buffered saline (PBS, pH 7.0, Gibco). The biotinylated and acetylated PED polymer was eluted with 2.5 ml of 1× PBS (pH 7.0) and stored at 4 °C until further use. The degree of biotin substitution was determined by using the HABA (4′-hydroxyazobenzene-2-carboxylic acid) reagent (Pierce) following the manufacturer's instructions.

Oligonucleotide probes

The design of the capture and detector probes was performed using Vector NTI software (Invitrogen). Homology was analyzed for highly conserved sequences in the multiple species of *Staphylococcus* for the *tuf* gene to create a probe set that identifies *Staphylococcus* genus specifically. Because the *tuf* gene is present in all bacteria, multiple gram-positive organisms phenotypically similar to *Staphylococcus* were added to the alignments so that candidate sequences that react only with *Staphylococcus* sequences were designed. To create a probe set that specifically identified the species *S. aureus*, multiple strains of *S. aureus* were aligned around the *femB* gene. To specifically identify methicillin resistance within *Staphylococcus*, multiple alignments of the *mecA* gene were performed. BLAST analysis was performed on all candidate sequences to eliminate cross-reactivity. All probes were optimized for performance.

All DNA oligonucleotides were synthesized by Integrated DNA Technologies using standard phosphoramidite chemistry. To facilitate detection of genomic DNA sequences, each detector probe was terminated with a biotin-TEG phosphoramidite (Glen Research) designated **X** in the sequence. Detector probes were as follows:

mecA1: 5′-**X** TCG CAA CGT TCA ATT TAA TTT
mecA2: 5′-**X** GGT ATG TGG AAG TTA GAT TGG G
femB1: 5′-**X** GCA ACA GGA ATT GAA TGA CAA AG
femB2: 5′-**X** CGT CGT GAT CAA ATG ATG GC
tuf1: 5′-**X** CAG AAC GTG ATT CTG ACA AAC C
tuf2: 5′-**X** CAA TCA CTG GTC GTG GTA CTG.

To facilitate covalent attachment to surfaces, all capture sequences were terminated with a 5′-I linker (IDT) designated **Y** in the sequence, followed by an S-18 spacer designated **Z** in the sequence. Capture probes were as follows:

mecA: 5′-**YZ** AAA CAA ACT ACG GTA ACA TTG AT
femB: 5′-**YZ** ATT GAC CTT GAT GAA TAT GTG TTA AAG
tuf: 5′-**YZ** TGA TGC CAG TTG AGG ACG TA.

Sample preparation

To prepare MRSA-positive blood culture bottles, approximately 7 ml of human blood was injected into aerobic BD BACTEC+ blood culture bottles (Becton Dickinson). The bottles were then seeded with ATCC (American Type Culture Collection) strain 33592 (MRSA) that was initially grown on 5% sheep blood agar and placed onto a BD BACTEC automated continuous-monitoring blood culture system until microbial activity was detected.

MRSA-positive blood culture samples (0.5 ml) were mixed with 150 μl of 1 N NaOH, vortexed, and centrifuged at 5000*g* for 1 min. The supernatant was discarded, and the pellet was washed with 250 μl of 10 mM Tris (pH 7.0). After a second centrifugation, the pellet was resuspended in 100 μl of 1 U/μl achromopeptidase in Tris (pH 7.0) [7], mixed, and incubated for 5 min at room temperature. The sample was boiled for 3 min at 95 °C.

Microtiter plate detection

Capture probe immobilization on a DNA-BIND surface (Corning) was performed as described by the manufacturer. Briefly, 100 μl of 250 nM capture probes in spotting buffer (0.5 M Na_2HPO_4 and 1 mM ethylenediaminetetraacetic acid [EDTA], pH 8.5) was incubated in the plate for 30 min at room temperature. Each well was washed four times with wash A (0.1× saline–sodium citrate [SSC] with 0.1% sodium dodecyl sulfate [SDS]) followed by four times with wash B (0.1× SSC). Each well of the plate was blocked with 300 μl of biotin-free casein (Fitzgerald) at room temperature for 30 min. The wells were emptied and then used immediately. Various concentrations of target sequences and 100-nM detector probes were hybridized to the plate at 53 °C in 1× hybridization buffer (5× SSC, 0.5% alkaline-treated casein, and 0.1% Tween 20) for 30 min and then were washed four times with wash A and four times with wash B. To perform PED, 125 μl of 1 μg/ml SA (Pierce) in 1× hybridization buffer was placed in each well and incubated for 2 min at room temperature and then aspirated, followed by incubation of 125 μl of 1 μg/ml PED polymer in 1× hybridization buffer for 2 min at room temperature. The wells were aspirated and then incubated with 125 μl of 1 mg/L poly-HRP SA (pHSA, Pierce) in 1× hybridization buffer for 4 min at room temperature. For comparison with the PED method, a typical enzyme-linked immunosorbent assay (ELISA) detection method was used (standard detection). In this method, 125 μl of 1 μg/ml mouse monoclonal anti-biotin antibody HRP (Jackson/University of Manitoba) in 1× hybridization buffer was incubated for 4 min at room temperature following target hybridization and washing. Both standard detection and PED wells were washed four times with wash B and aspirated, and 100 μl of soluble Super Slow–HRP TMB (BioFX) was placed in the wells for 6 min at room temperature. To stop the reaction, 50 μl of 1 M HCl was added and the absorbance was read in a SpectraCount plate reader (Packard) at 450 nm.

Thin film biosensor detection

Thin film biosensors were purchased from Inverness Medical–BioStar. Immobilization of capture probes to the thin film biosensor surface was performed as described previously [8]. Various concentration of target and 100 nM detector probes were hybridized to the biosensor chips, arrayed in a 96-well plate, exactly as described for the microtiter plate assay except that 125 μl of precipitating TMB (BioFX) was used in place of soluble Super Slow–HRP TMB. After incubation with the precipitating TMB, the chips were washed four times each with H_2O and methanol. The chips were allowed to dry and were read visually.

Results

Signal amplification using a PED approach

Capture probes specific for the *tuf* gene present in all *Staphylococcus* species, the *femB* gene present in *S. aureus* species only, and the methicillin resistance gene *mecA* were immobilized onto plastic or silicon wafer surfaces as described in Materials and methods. Target nucleic acid sequences, obtained as aliquots from growth-positive blood culture bottles (see Materials and methods), were mixed with target complementary, biotin-labeled detector probes and hybridized to surface-immobilized capture probes to create a three-component surface-bound complex. For a standard detection approach, the surface was incubated with an anti-biotin antibody/HRP conjugate. After washing away unbound conjugate, TMB was added to generate a colorimetric signal (Fig. 1A). For the PED method, the surface-bound complex was incubated with SA, a tetrameric protein with four high-affinity biotin binding sites, to interact with the surface-immobilized, biotin-labeled reporter molecules. The SA bound to reporter probe biotin molecules served as a bridge to selectively enhance the biotin content of positive hybridization samples when they were incubated with a 500-kDa polybiotinylated dextran with approximately 80 biotins per monomer backbone (Fig. 1B). The surface-bound polybiotinylated dextran polymer was then used to recruit multiple SA/HRP conjugates from solution when samples were incubated with conjugate. Finally, after additional washes, TMB was added to generate a colorimetric signal that could be recorded either in solution, by spectrometry, or as colored precipitate on surface films.

Performance optimization of PED method

To optimize performance, we varied incubation times for each step of the PED procedure: (i) binding of SA to target-dependent, surface-immobilized biotin and (ii) binding of the polybiotinylated dextran polymer to the immobilized SA. Because the biotin–SA interaction is among the highest affinity noncovalent interactions described and has rapid association kinetics, we expected this procedure to be relatively rapid. To characterize the time required to observe maximal signal enhancement, we hybridized serial dilutions of a *mecA*-specific biotin-labeled target sequence to a complementary capture oligonucleotide immobilized onto a microtiter plate. The plate was washed and then exposed to SA at various concentrations for different incubation times. Signal increased slightly with time; however, the limit of detection did not improve after 2 min of incubation at a concentration of 1 μg/ml SA (data not shown). For the second step of the PED procedure, various concentrations of the PED polymer were incubated for various times with surface-immobilized SA. Signal was observed to reach its limit by 2 min with 1 μg/ml PED polymer (data not shown). The effect on limit of detection was also determined for the PED polymer time of incubation. No effect on limit of detection was observed from 2 to 16 min of PED incubation (data not shown). Avidity due to a high density of biotin on the PED polymer backbone must account for its rapid binding to SA, offsetting potential issues with slow diffusion of this large 500-kDa polymer.

Using the optimized incubation times and buffer conditions, the PED protocol was compared with a standard procedure for the detection of a model nucleic acid target sequence. To create a plate-based assay format, we immobilized a 5′-amino-terminal DNA capture probe that recognized conserved sequence within the *mecA* gene by reaction with a surface-immobilized NHS ester. After washing and blocking the wells, we hybridized various concentrations of biotinylated complementary target sequence to the probe for 30 min. Signal was developed as described, and the product of the HRP/TMB reaction was measured in a spectrophotometer at 450 nm. The PED protocol improved the lower limits of sensitivity 10- to 25-fold compared with the standard detection approach, allowing 120 to 300 fM target sequence to be detected (Fig. 2 and data not shown). No background was observed across a 10-fold range of PED polymer concentration.

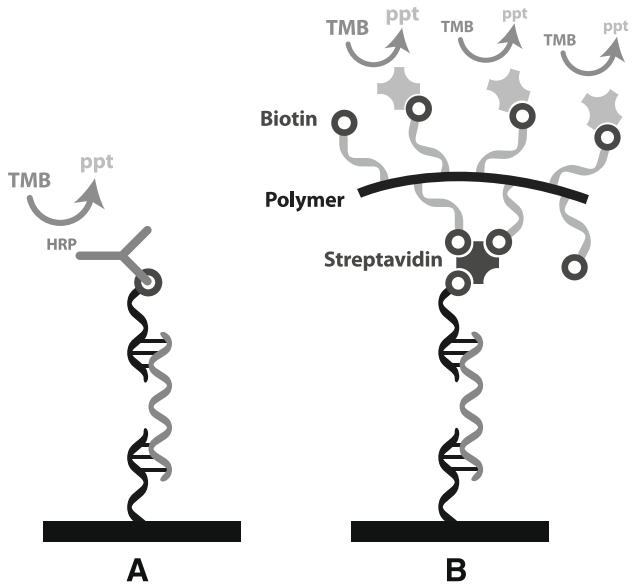

Fig. 1. Schematic representation of standard detection and PED-enhanced detection of DNA hybridization. (A) Standard detection. Surface hybridized biotin-labeled oligonucleotide is detected by anti-biotin antibody/HRP conjugate reacted with the substrate, TMB, to produce a detectable signal. (B) PED detection. Surface-hybridized, biotin-labeled oligonucleotide reacts with SA, followed by the PED polymer, to increase the number of detectable biotin molecules per hybridization event. The biotin is detected by SA/HRP conjugates reacting with the substrate, TMB, to produce a detectable signal.

Plate-based detection of positive blood culture bottles with PED

With the improvement in assay sensitivity provided by the PED method, we sought to determine whether we could detect *Staphylococcus* directly from a positive blood culture bottle. A 0.5-ml aliquot was removed from an MRSA-positive blood culture bottle, the cells were lysed as described in Materials and methods, and aliquots of the crude lysate containing MRSA genomic DNA were applied to three microtiter plate wells: one coated with a probe that recognizes conserved sequence in the *tuf* gene, a second to identify conserved sequences within the *femB* gene, and a third coated with a probe that recognizes conserved sequence within the *mecA* gene. Hybridization was allowed to proceed for various times followed by signal development. As seen in Fig. 3, significant signal above background for all three probes was observed within 8 min of hybridization time and signal saturation was observed by 15 min. Therefore, it is feasible to detect MRSA from positive blood cultures within 35 min of total assay time. For a standard detection protocol, no significant signal above background was observed after 30 min of hybridization.

Performance of PED on the thin film biosensor platform

Clinical diagnostic devices require excellent assay sensitivity and ease of use at a low cost. Thin film biosensors exploit optical principles to allow the unaided human eye to detect thickness changes of the same order as the size of intermolecular interactions of biological molecules on a chip surface [9]. These thickness

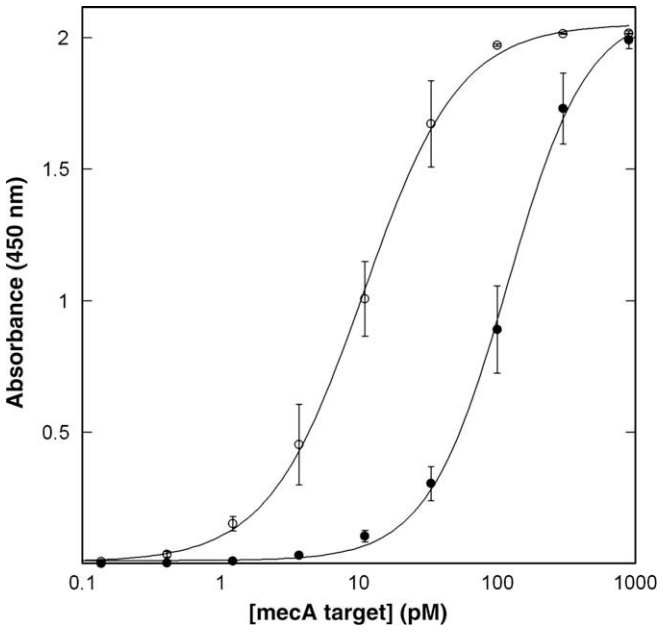

Fig. 2. Lower limit of detection of DNA hybridization using microtiter plate format. Various concentrations of a biotin-labeled, single-stranded target sequence from the *mecA* gene were hybridized to the *mecA*-specific capture probe immobilized onto microtiter wells. Signal was developed using standard detection (closed circles) or with the described PED polymer (open circles). Three experiments were performed, each in triplicate ($n = 9$), and the mean absorbance reading ± 1 SD (error bars) was plotted.

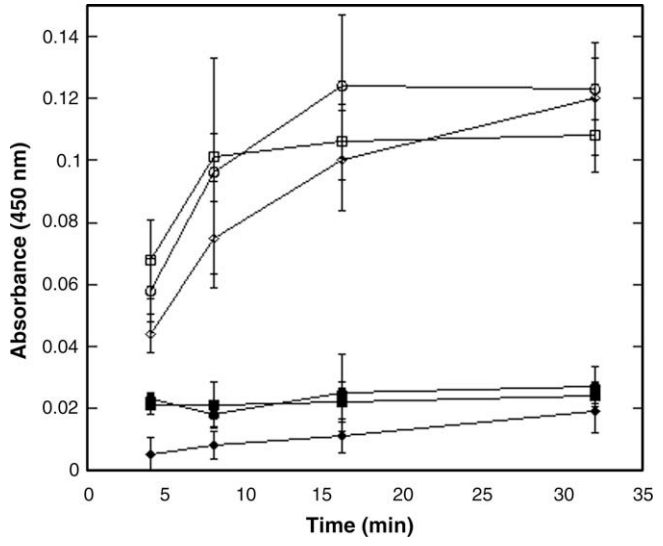

Fig. 3. Detection of MRSA-positive blood culture using microtiter plate format. MRSA-positive blood culture aliquots (0.5 ml) were processed as described in Materials and methods, hybridized to capture probes immobilized onto microtiter plate wells for various times, and detected using the PED protocol for *mecA* (open circles), *femB* (open squares), and *tuf* (open diamonds) or using standard detection for *mecA* (closed circles), *femB* (closed squares), and *tuf* (closed diamonds). Each determination was performed in triplicate in three independent experiments ($n = 9$), and the mean absorbance reading ± 1 SD was plotted.

changes are observed as a color change from gold to purple on the chip surface.

We tested the effect of the PED protocol for the detection of nucleic acid target sequences on the thin film biosensor platform. Two probes were conjugated to the chip surface using hydrazone chemistry [8]: one that recognizes conserved sequence in the *tuf* gene and the other that does so in the *femB* gene. As a model system, target DNA and biotinylated detector probes complementary to target sequences were titrated and hybridized to the chip surface for 30 min and then detected using PED or standard methods. The visual limit of detection using standard detection methods was 111 fM (Fig. 4), consistent with previously reported values [10]. The PED protocol improved the limits of detection to 4 and 1 fM for *femB* and *tuf*, respectively, or approximately 25- to 100-fold. These results verify limit of detection enhancement observed in the plate-based assays, showing that the PED approach is general to multiple detection platforms.

A rapid test for positive blood cultures using thin film biosensors

Using thin film biosensors, we sought to create a rapid clinical test for the detection of MRSA from blood culture bottles. A thin film biosensor assay that required 90 min of total assay time, 45 min of which was hybridization time, was described previously [10]. With 25- to 100-fold sensitivity improvements, assay time and complexity may be reduced by using the PED approach. As a first test, we performed a time course for the hybridization of crude genomic DNA isolated from an MRSA-positive blood culture bottle. A 0.5-ml aliquot was removed from an MRSA-positive blood culture bottle, the cells were lysed as described, and the crude lysate containing MRSA genomic DNA combined with biotinylated detector probes was applied to the thin film biosensor. Hybridization was allowed to occur for various times, and signal was developed as described. Signal from a standard detection method was not clearly apparent until 45 min and was rather weak in this study. Application of the PED protocol gave clearly visible signal within 10 min of hybridization time on both the *tuf* and *femB* probes (Fig. 5). Continuous mixing during hybridization was observed to increase signal approximately 3-fold compared with no mixing (data not shown). As a result, with the PED protocol, unambiguous detection after 5 min of hybridization occurred. Including sample preparation, this protocol creates a blood culture assay that requires only 25 to 30 min total time.

Discussion

We have described PED—a simple, inexpensive, and rapid signal amplification method based on incorporation of a polybiotinylated dextran polymer into standard detection protocols. By exploiting multivalent interactions using optimized polymer chemistry, we created a signal enhancer that is well-behaved in both plate- and chip-based assay platforms for nucleic acid detections. No nonspecific interactions were observed, and limits of detection were improved by 10- to 100-fold. This equates to a 120- to 300-fM limit of detection in a colorimetric plate-based assay and as low as 1 fM using a thin film biosensor (6×10^5 molecules/ml). This level of sensitivity permits direct detection of genomic DNA sequences from clinical specimens, creating an assay platform with faster time, lower cost (PED material cost is less than $0.01 [U.S.] per test), and reduced risk of carryover contamination compared with target amplification approaches.

The PED method was applied to two assay formats previously used for the direct detection of genomic DNA from MRSA-positive blood culture bottles. A recently described plate-based procedure required 3 h of enrichment in broth culture to raise the colony-forming units (CFU)/ml above detectable limits prior to a 3.5-h test protocol [11,12]. In contrast to PED, standard detection conditions failed to detect significant positive signal after 90 min of total assay time; these results are similar to those reported previously [11,12]. Typical positive blood culture bottles contain 10^8 to 10^9 CFU/ml for *Staphylococcus* species [10,13], equating to 0.2 to 2 pM genomic DNA. This amount of DNA is quite low relative to the limit of detec-

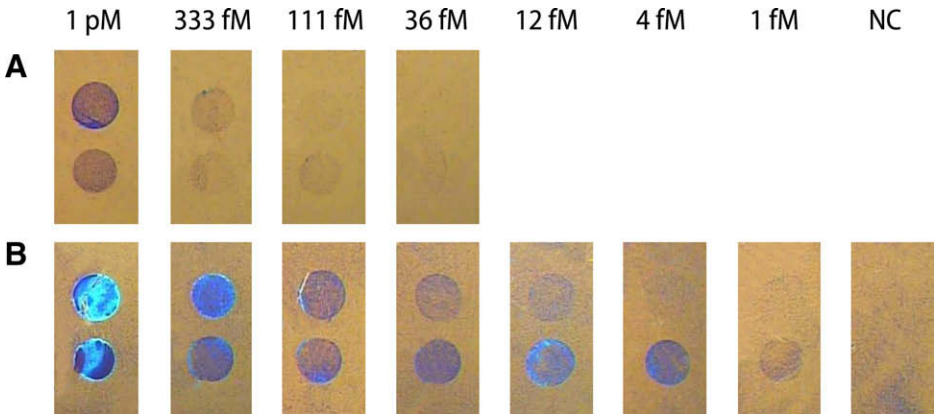

Fig. 4. Limits of detection of DNA hybridization using a thin film biosensor. Various concentrations of a biotin-labeled, single-stranded target sequences from the *tuf* (lower spot) and *femB* (upper spot) gene were hybridized to a thin film biosensor surface containing complementary capture probes. Signal was developed using either standard detection (A) or the PED protocol (B). Results are shown as color charge-coupled device (CCD) camera images. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

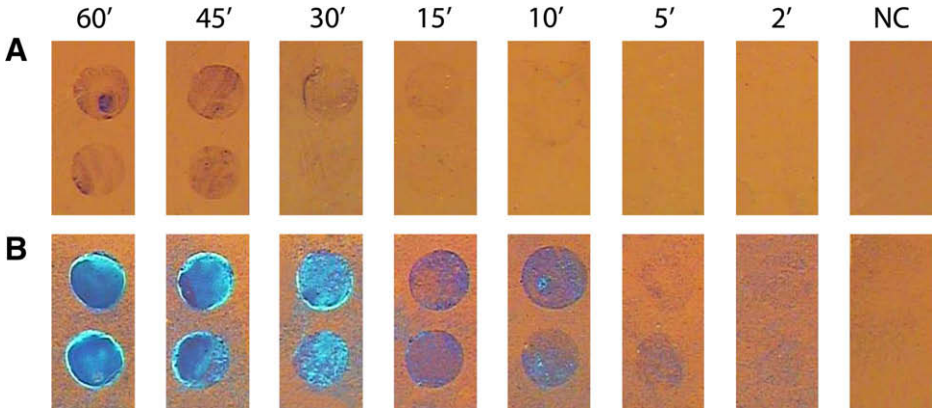

Fig. 5. Detection of MRSA-positive blood culture using a thin film biosensor. MRSA-positive blood culture aliquots (0.5 ml) were processed as described in Materials and methods, hybridized for various times (in minutes) to thin film biosensor chips coated with capture probes that recognize conserved sequences in the *femB* (upper spot) and *tuf* (lower spot) genes, and detected using either standard detection (A) or the PED protocol (B). Results are shown as color CCD camera images. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

tion for standard detection approaches (~10 pM); therefore, the lack of detection in a plate-based format is not surprising. However, the PED protocol improves the limit of detection sufficiently in plate assays to allow rapid and reliable detection of MRSA from blood culture within 35 min.

A thin film biosensor approach has been reported to detect MRSA from a positive blood culture bottle in 90 min [10]. This study reported a limit of detection of 5×10^7 CFU/ml (~0.1 pM), which is sufficient for direct detection of *Staphylococcus* from blood culture; however, relatively long incubation times were required. The PED signal enhancement protocol decreases the lower limit of detection to 0.5 to 2×10^6 CFU/ml, thereby significantly reducing the assay time to 25 min when equal quantities of sample are used. To our knowledge, this is the fastest detection of MRSA in blood cultures reported, including real-time PCR approaches that require 2 h [6].

Multivalent interactions have been reported to slow dissociation rates, resulting in improved overall binding affinity and better assay limits of detection [14–16]. We optimized each of the reagents (SA and the PED polymer) in the two-step PED procedure to maximize potential signal amplification by exploiting the multivalent binding of SA and biotin. By using unconjugated SA as a bridge between target-dependent, surface-immobilized, biotin-labeled probes and the polybiotinylated PED polymer, an additional step is required. However, all four binding sites on SA are available, and the extraordinary binding affinity for biotin is maintained such that the PED SA polymer interactions saturate within minutes. It has been demonstrated elsewhere that multiple interactions per backbone of a multiply biotin-labeled polymer significantly improve assay performance [17]. The stabilized bound state of the PED complex combined with a high-density biotin polymer capable of rapid rebinding creates a virtually undissociable interaction.

Currently, methods are being developed to eliminate the need for instrumentation in assays that use PED. As described here, all steps in the PED procedure are performed at room temperature except the hybridization step, which works equivalently well between 37 and 53 °C (data not shown). By using buffers that contain chaotropic salts, thereby depressing the melting temperature of the interactions, it has been shown that hybridization may occur at room temperature [18]. In addition, the sample preparation procedure is being simplified by eliminating the need for the two wash and centrifugation steps. Using lower amounts of blood culture, conditions that allow one-step lysis of staphylococcal cells directly in a blood culture aliquot have been developed. Furthermore, if a mixed culture of MSSA and MRCoNS were present in a positive blood culture aliquot, it could be mistaken for MRSA. We are considering the addition of probes that recognize SCCmec, which is a specific marker for MRSA, as a method to mitigate this limitation [10]. If SCCmec probes were added to the described panel, the presence of a mixed culture of MRCoNS and MSSA would

yield a positive signal on the *tuf*, *femB*, and *mecA* test probes and a negative signal on the SCCmec probe sets. These approaches will be incorporated into a chip-based format, and clinical studies will be initiated for detection and discrimination of CoNS, MRCoNS, MSSA, and MRSA in clinical specimens using the PED method.

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References

[1] Center for Disease Control and Prevention, Healthcare-associated Infections (HAIs). Available from: <www.cdc.gov/ncidod/dhqp/healthDis.html>.
[2] National Nosocomial Infections Surveillance System, National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004, Am. J. Infect. Control 32 (2004) 470–485.
[3] S.E. Beekmann, D.J. Diekema, K.C. Chapin, G.V. Doern, Effects of rapid detection of bloodstream infections on length of hospitalization and hospital charges, J. Clin. Microbiol. 41 (2003) 3119–3125.
[4] G.B. Orsi, L. Di Stefano, N. Noah, Hospital-acquired, laboratory-confirmed bloodstream infection: increased hospital stay and direct costs, Infect. Control Hosp. Epidemiol. 23 (2002) 190–197.
[5] T.L. Bannerman, *Staphylococcus*, *Micrococcus*, and other catalase-positive cocci that grow aerobically, in: P.R. Murray, E.J. Baron, M.A. Pfaller, J.H. Jorgensen, R.H. Yolken (Eds.), Manual of Clinical Microbiology, eighth ed., American Society for Microbiology, Washington, DC, 2003, pp. 384–404.
[6] P.D. Stamper, M. Cai, T. Howard, S. Speser, K.C. Carroll, Clinical validation of the molecular BD GeneOhm StaphSR assay for direct detection of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* in positive blood cultures, J. Clin. Microbiol. 45 (2007) 2191–2196.
[7] S.M. Paule, A.C. Pasquariello, D.M. Hacek, A.G. Fisher, R.B. Thomson Jr., K.L. Kaul, L.R. Peterson, Direct detection of *Staphylococcus aureus* from adult and neonate nasal swab specimens using real-time polymerase chain reaction, J. Mol. Diagn. 6 (2004) 191–196.
[8] X. Zhong, R. Reynolds, J.R. Kidd, K.K. Kidd, R. Jenison, R.A. Marlar, D.C. Ward, Single-nucleotide polymorphism genotyping on optical thin-film biosensor chips, Proc. Natl. Acad. Sci. USA 100 (2003) 11559–11564.
[9] R.D. Jenison, R. Bucala, D. Maul, D.C. Ward, Thin-film technology for direct visual detection of nucleic acid sequences: applications in clinical research, Expert Rev. Mol. Diagn. 6 (2006) 89–99.
[10] W.C. Lindsey, E.S. Woodruff, D. Weed, D.C. Ward, R.D. Jenison, Development of a rapid diagnostic assay for methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative *Staphylococcus*, Diagn. Microbiol. Infect. Dis. 61 (2008) 273–279.
[11] R.L. Skov, L.V. Pallesen, R.L. Poulson, F. Espersen, Evaluation of a new 3-h hybridization method for detecting the *mecA* gene in *Staphylococcus aureus* and comparison with existing genotypic and phenotypic susceptibility testing methods, J. Antimicrob. Chemother. 43 (1999) 467–475.
[12] K. Levi, K.J. Towner, Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in blood with the EVIGENE MRSA detection kit, J. Clin. Microbiol. 41 (2003) 3890–3892.
[13] E.M. Marlowe, L. Gibson, J. Hogan, S. Kaplan, D.A. Bruckner, Conventional and molecular methods for verification of results obtained with BacT/Alert nonvent blood culture bottles, J. Clin. Microbiol. 41 (2003) 1266–1269.
[14] J.R. Wands, R.I. Carlson, H. Schoemaker, K.J. Isselbacher, V. Zurawski, Immunodiagnosis of hepatitis B with high-affinity IgM monoclonal antibodies, Proc. Natl. Acad. Sci. USA 76 (1981) 1214–1218.
[15] N. Yoshitani, S. Takasaki, Microscale synthesis of dextran-based multivalent N-linked oligosaccharide probes, Anal. Biochem. 277 (2000) 127–134.
[16] L.S. Zuckier, E.Z. Berkowitz, R.J. Sattenberg, Q.H. Zhao, H.F. Deng, M.D. Scharff, Influence of affinity and antigen density on antibody localization in a modifiable tumor targeting model, Cancer Res. 60 (2000) 7008–7013.
[17] T. Ooya, N. Yui, Multivalent interactions between biotin–polyrotaxane conjugates and streptavidin as a model of new targeting for transporters, J. Control. Release 80 (2002) 219–228.
[18] J. Van Ness, L. Chen, The use of oligodeoxynucleotide probes in chaotrope-based hybridization solutions, Nucleic Acids Res. 19 (1991) 5143–5151.