Antimicrobial Resistance and Bacterial Identification
Utilizing a Microelectronic Chip Array

LORELEI WESTIN,1 CAROLYN MILLER,2* DANA VOLLMER,2 DAVID CANTER,3
RAY RADTKEY,3 MICHAEL NERENBERG,3† AND JAMES P. O’CONNELL1

Departments of Advanced Research,1 Assay Development,2 and
Molecular Biology,3 Nanogen, Inc., San Diego, California

Received 31 July 2000/Returned for modification 19 September 2000/Accepted 8 December 2000

Species-specific bacterial identification of clinical specimens is often limited to a few species due to the difficulty of performing multiple reactions. In addition, discrimination of amplicons is time-consuming and laborious, consisting of gel electrophoresis, probe hybridization, or sequencing technology. In order to simplify the process of bacterial identification, we combined anchored in situ amplification on a microelectronic chip array with discrimination and detection on the same platform. Here, we describe the simultaneous amplification and discrimination of six gene sequences which are representative of different bacterial identification assays: Escherichia coli gyrA, Salmonella gyrA, Campylobacter gyrA, E. coli parC, Staphylococcus mecA, and Chlamydia cryptic plasmid. The assay can detect both plasmid and transposon genes and can also discriminate strains carrying antibiotic resistance single-nucleotide polymorphism mutations. Finally, the assay is similarly capable of discriminating between bacterial species through reporter-specific discrimination and allele-specific amplification. Anchored strand displacement amplification allows multiplex amplification and complex genotype discrimination on the same platform. This assay simplifies the bacterial identification process greatly, allowing molecular biology techniques to be performed with minimal processing of samples and practical experience.

Genotype identification has been widely recognized as an effective tool in the identification and characterization of infectious disease organisms (7, 12, 16). Molecular biology-based bacterial identification assays have the potential of decreasing the time necessary for and increasing the specificity of bacterial determinations, making them efficacious alternatives to traditional biochemical and microbiological culture techniques. For example, Chlamydia trachomatis and Mycobacterium tuberculosis are traditionally very difficult organisms to culture. Molecular biology techniques circumvent the need for long culturing protocols by using amplification of either DNA (PCR, ligase chain reaction [LCR], strand displacement amplification [SDA], and nucleic acid sequence-based amplification [NASBA]) or RNA (reverse transcription-PCR, NASBA, reverse transcription-SDA, and transcription-mediated amplification [TMA]) targets. However, molecular biology techniques are also advantageous because of the amount of information that can be obtained from a single assay in a short period of time. As a result, the 1999 National Committee for Clinical Laboratory Standards (13a) guidelines have for the first time mandated the use of molecular biology methods in clinical laboratories that perform bacterial identification assays.

There are many examples of molecular biology-based assays used in the laboratory. Genotypic identification of bacterial samples is used to discriminate and identify bacteria at either the genus, species, or strain level (1, 9). Genotypic identification of antimicrobial resistance is also used as an aid in the treatment of infectious diseases (15, 19, 20, 24). Conventionnal antimicrobial susceptibility testing provides only phenotypic profiling of a potential pathogen. Low-level antimicrobial activity and heterogeneous populations of antimicrobial agent-resistant pathogens are difficult to detect with these techniques. Molecular analysis of pathogens provides a more definite means of obtaining the antimicrobial status of microorganisms by identifying organisms that possess the genetic material necessary for resistance.

As with all amplification assays, multiplex target or signal amplification is difficult. There have been numerous reports of multiplex PCR assays developed for many bacterial identification assays (15, 20). However, multiplex PCR assays, as well as all other target and signal amplification assays, are still limited in the amounts of templates that can be amplified simultaneously because of the difficulty of optimizing primer and reagent conditions. In addition, discrimination of closely matched amplicon sequences is time-consuming and laborious, consisting of either gel electrophoresis, probe hybridization, or sequencing technology.

Microchip arrays are capable of analyzing hundreds to thousands of different loci simultaneously in a relatively short period of time (3, 13). However, most microchip array systems require large amounts of template DNA, or targets from multiplex amplification, in order to detect DNA or RNA at fairly low levels (2, 13). We recently described an assay on an electronic microchip array which was capable of multiplex amplification of 10 targets simultaneously, with little decrease in amplification efficiency (22). Anchored SDA on microelectronic chips encompasses amplification on the surface of the array, requiring very small amounts of input DNA. The flexibility of the microelectronic chip array has been demonstrated repeatedly in many types of genetic discrimination assays, including single-nucleotide polymorphism (SNP) (8, 18) and...
short tandem repeat (17) discrimination assays. Here, we describe an in situ amplification assay using complex genomic DNA samples on a microelectronic chip array. We have also combined amplification with genetic discrimination assays for the identification of bacterial species and antimicrobial resistance and for SNP discrimination of fluoroquinolone resistance in Campylobacter isolates. This technology greatly simplifies molecular genotyping assays by requiring substantially less sample processing and technical expertise than most molecular biology-based assays.

MATERIALS AND METHODS

Strains, culture conditions, and isolation of genomic DNA. Bacterial strains were obtained from various sources, including the American Type Culture Collection (Manassas, Va.), the Centers for Disease Control and Prevention (Atlanta, Ga.), the National Cell and Tissue Collection, and BD Biosciences (Hunt Valley, Md.). All bacterial species were cultured as recommended by the American Type Culture Collection. Genomic DNA was isolated from liquid culture cells or plate scrapings using DNeasy genomic DNA isolation kits (Qiagen, Valencia, Calif.). All amplification systems were tested with at least three different strains.

SDA primers. Oligonucleotides (Table 1) were synthesized at Integrated DNA Technologies (Coralville, Iowa). Oligonucleotides were coupled with either BODIPY Texas red (BTR), Cy3, or Cy5 fluorophore or biotin at the 5' end. Oligonucleotides were labeled at the 5' end with a 12-mer sequence [BTR-dT(12)] that was used as a control for streptavidin integrity on the microchip were done in 50 mM histidine buffer. Biotinylated, BTR-labeled T12 oligonucleotide was used as a control for streptavidin integrity on the microchip.

Microelectronic chips, amplification primer deposition, and hybridization of templates. Microelectronic chips and the permeation layer were prepared as described previously (5, 8). In brief, the APEX chip used for these experiments consists of a 5- by 5-array of 80-m circular microelectrodes with 200-m interelectrode distance. Chips were mounted on a micromanipulator stage, and the microelectrodes were activated by a power supply and appropriately controlled relay switches. All electronic manipulations on the microchip were done in 50 mM histidine buffer. Biotinylated, BTR-labeled T12 oligonucleotide was used as a control for streptavidin integrity on the permeation layer. All SDA primers were coupled with biotin at the 5' end. After hydration of the microchip in 50 mM histidine buffer, SDA primers were deposited on the microchip using a pipette. The SDA reaction was performed in the permeation layer. All SDA primers were coupled with biotin at the 5' end. After hydration of the microchip in 50 mM histidine buffer, SDA primers were deposited on the microchip using a pipette. The SDA reaction was performed in the permeation layer. All SDA primers were coupled with biotin at the 5' end. After hydration of the microchip in 50 mM histidine buffer, SDA primers were deposited on the microchip using a pipette. The SDA reaction was performed in the permeation layer.

TABLE 1. Bacterial anchored SDA primers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Designation</th>
<th>Function</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor V</td>
<td>1933</td>
<td>Amplification</td>
<td>B-ACCGCATCGAAATGCATGTCTGGTTCGGGATATAAGGA</td>
</tr>
<tr>
<td></td>
<td>1934</td>
<td>Amplification</td>
<td>B-ACGATTCAAGCTCCAGACTTCTGGGAGAATTCTACTAAGAGG</td>
</tr>
<tr>
<td></td>
<td>1935</td>
<td>Reporter</td>
<td>ACTACAGTGACGTGGACCATC</td>
</tr>
<tr>
<td></td>
<td>1936</td>
<td>Reporter</td>
<td>TTATATACCTGGTGCTATAGG</td>
</tr>
<tr>
<td></td>
<td>1937</td>
<td>Amplification</td>
<td>B-ACCGCATCGAAATGCATGTCTGGTTCGGGATATAAGGA</td>
</tr>
<tr>
<td>Chlamydia</td>
<td>1938</td>
<td>Amplification</td>
<td>B-CGATCCAGCGACTTCTGGGAGAATTCTACTAAGAGG</td>
</tr>
<tr>
<td></td>
<td>1939</td>
<td>Reporter</td>
<td>ACTACAGTGACGTGGACCATC</td>
</tr>
<tr>
<td></td>
<td>1940</td>
<td>Reporter</td>
<td>TTATATACCTGGTGCTATAGG</td>
</tr>
<tr>
<td></td>
<td>1941</td>
<td>Bumper primer</td>
<td>BTR-GTGCAGCAAAATATG</td>
</tr>
<tr>
<td></td>
<td>1942</td>
<td>Reporter</td>
<td>BTR-CTGCATCGAAAGACGT</td>
</tr>
<tr>
<td></td>
<td>1943</td>
<td>Reporter</td>
<td>CY3-GTGGTTATATCATATG</td>
</tr>
<tr>
<td></td>
<td>1944</td>
<td>Reporter</td>
<td>CY3-TAGGATTTATATG</td>
</tr>
<tr>
<td>Campylobacter</td>
<td>1945</td>
<td>Amplification</td>
<td>B-ACGCGTAAACACAGATTGTCTGGGATAGGCG</td>
</tr>
<tr>
<td>E. coli parC</td>
<td>1946</td>
<td>Amplification</td>
<td>B-ACGTTAAACACAGATTGTCTGGGATAGGCG</td>
</tr>
<tr>
<td></td>
<td>1947</td>
<td>Reporter</td>
<td>CY3-CAGCAGTTATATG</td>
</tr>
<tr>
<td>gyrA</td>
<td>1948</td>
<td>Amplification</td>
<td>B-ACGCGTAAACACAGATTGTCTGGGATAGGCG</td>
</tr>
<tr>
<td></td>
<td>1949</td>
<td>Reporter</td>
<td>CY3-CAGCAGTTATATG</td>
</tr>
</tbody>
</table>

* B, 5' end labeled with biotin; BTR, 5' end labeled with BTR; Cy3, 5' end labeled with Cy3 fluorophore; Cy5, 5' end labeled with Cy5 fluorophore.
I. Hybridize bacterial genomic DNA: Anchored SDA amplification

II. Denature double stranded DNA; Hybridize stabilizer oligonucleotide

III. Discriminate Reporters with heat stringency

FIG. 1. Schematic representation of anchored SDA and bacterial discrimination. Biotinylated SDA amplification primer sets are first addressed to spatially distinct areas on the microchip array using electronic biasing. Templates are then electronically hybridized to the SDA primer sets, and the SDA reaction is performed in situ with the addition of bumper primers and reaction reagents to the microchip (panel I). The amplification primers contain a BsoBI restriction endonuclease site, essential for SDA. The bumper primers (not shown) are needed only to remove the initial primer extension product from the target template, allowing the primer-extended strand to bind to its complementary amplification primer. Primer extension of the complementary amplification primer and subsequent incorporation of a thiolated nucleotide (dTTP) over the BsoBI site induce nicking by BsoBI in the amplification primer region. The presence of a nick signals polymerase binding and simultaneous strand displacement-primer extension, resulting in exponential amplification of the target DNA. The SDA reaction is stopped by removal of the supernatant, double-stranded DNA products are denatured on the microchip, and internal reporters are hybridized to the amplicon products remaining on the chip (panel III). Discrimination is performed by increasing heat (thermal stringency) until only one reporter species (Cy3 or Cy5) remains (panel III). wt, wild type.

After amplification, the anchored amplicons were denatured with the addition of an alkali solution (0.5× SSC, pH 7.2) and washed five times at room temperature (0.5× SSC, pH 7.2). The amplicons were then hybridized with 1.0 μM stabilizer oligonucleotides in 4× SSC (pH 7.2) at room temperature for 5 min. After extensive washing (5 to 10 times) with 0.5× SSC, a 0.5 μM concentration of reporter oligonucleotides was hybridized for 5 min in 4× SSC at room temperature (Fig. 1, panel III). The reporters were coupled at the 5′ end with either Cy3, Cy5, or BTR fluorophore. Extensive washing (5 to 10 times) with 0.5× SSC (pH 7.2), followed by 0.2× SSC–1.0% sodium dodecyl sulfate and 0.2× SSC washes at room temperature, removed most of the unbound reporter oligonucleotides. Temperature stringency in 50 mM NaPO4 (pH 7.7) was applied to discriminate SNPs and other closely matched reporters. Temperature was ramped up in 3°C increments and maintained for at least 3 min at each step. Images were taken at every step, and the microchips were washed with 50 mM NaPO4 (pH 7.7) at every temperature interval. The entire assay from template hybridization to reporter discrimination took approximately 70 to 90 min, depending on the number of templates to be hybridized on the microchip array.

Reporter discrimination using base-stacking energy transfer. One of the advantages of anchored SDA lies in its ability to amplify and detect target DNA on the same platform. Achieving amplification of and discrimination between very similar DNA targets is difficult to accomplish with a single-platform assay. We took advantage of base-stacking energy transfer techniques to assist in reporter discrimination of SNPs (14, 17, 23). Amplification primers were designed sur-
amounts of ers to the cryptic plasmid as the target sequence (10). Figure C. trachomatis was amplified by anchored SDA using prim-ring on the microchip surface (22). Template input levels of bridized template and to ensure that amplification was occur-

ing the 5'9 reporters were designed, with either a Cy3 or a Cy5 fluorophore label coupled at labeled reporter, then the base-stacking energy of the stabilizer oligonucle-
a perfect match to the labeled reporter, then the base-stacking energies between a longer stabilizer oligonucleotide and the labeled reporter would be favorable, al-
were used such that the 5' end of the labeled reporter would base stack alongside a longer stabilizer oligonucleotide (14, 17, 23). If the amplified target presented a mismatch to lowing the shorter reporter to remain hybridized to the amplified target at ele-
if the amplified target presented a mismatch to the labeled reporter, then the base-stacking energy between the stabilizer oligonucleotide and the labeled reporter would be favorable, al-

 rounding the Thr86Ile mutation in the gyrase A region of type II DNA topo-

imersase in Campylobacter jejuni; this mutation was previously shown to confer fluoroquinolone resistance in bacteria carrying it (24). Base-stacking reporters and to ensure that amplification was occurring on the microchip surface (22). Template input levels of 1,000 copies of DNA could be routinely detected using anchored SDA in multiple experiments. Template input levels of 100 copies gave variable results (data not shown), indicating the threshold of amplification to be between 100 and 1,000 copies of plasmid DNA. No amplification could be detected when other bacterial sources were hybridized to the Chlamydia SDA primers (Table 1), indicating a high specificity of amplification. The latter is due to both the specificity of the SDA primers for amplification and the specificity of the reporter oligonucleotide for hybridization to the correct ampcion.

Antimicrobial resistance discrimination using anchored SDA. Staphylococcus aureus methicillin resistance was used as a model system for antimicrobial resistance determination with a novel gene insertion mechanism. In this experiment, genomic DNA from cultured methicillin-sensitive and methicillin-resis-
tant S. aureus was purified and hybridized to the microelec-
tron chip array. As shown in Fig. 3a, only S. aureus samples that have been characterized as methicillin resistant showed any positive signal. An anchored SDA system from the human factor V gene (22) was used as a control to show that amplification was functional in the methicillin-sensitive amplification reaction. These results demonstrate the specificity and accur-

Precision of template DNA input to determine the sensitivity of the system. Representative fluorescent images of each titration reaction are made after reporter hybridization and washing. Gray bars show SDA prim-
ers; white bars show mutated SDA primers for control of template background binding (noncleavable; bars are barely visible above the x axis).

RESULTS

Chlamydia identification using a plasmid-dependent gene. C. trachomatis was amplified by anchored SDA using prim-

erers to the cryptic plasmid as the target sequence (10). Figure 2 shows the results of a titration experiment with various amounts of Chlamydia template input. Noncleavable primers, which have a single base mutation in the recognition site for BsoBI, were used as a control for background binding of hy-

brazied template and to ensure that amplification was occurring on the microchip surface (22). Template input levels of

FIG. 2. Amplification of C. trachomatis cryptic plasmid by an-
chored SDA. Anchored SDA was performed with increasing amounts of template DNA input to determine the sensitivity of the system. Representative fluorescent images of each titration reaction are made after reporter hybridization and washing. Gray bars show SDA primers; white bars show mutated SDA primers for control of template background binding (noncleavable; bars are barely visible above the x axis).

E. coli and Salmonella discrimination using anchored SDA. Bacterial gyrase A genes have been extensively studied as a model system for molecular phylogenetic reconstruction due to the presence of highly conserved motifs interspersed with regions of divergent sequences (11). This relatively conserved sequence organization allows for the design of common amplification primers for amplification of bacterial sequences, followed by the use of specific reporters for bacterial identification discrimination assays. We used sequences from the gyrA region of type II DNA topoisomerase to amplify and discrim-

inate E. coli and Salmonella genomic DNAs in our assay (Fig. 4). The SDA primers were designed on the basis of a region that is conserved between E. coli and Salmonella, allowing both organisms to be amplified if present in a sample. Campylo-
bacter, Chlamydia, and S. aureus genomic DNAs were not amplified using this set of SDA primers (Table 1). After amplification of the samples, microchips were hybridized with
base-stacking reporters specific for either *E. coli* (Cy3) or *Salmonella* (Cy5). As shown in Fig. 4, the correct signal was dependent on the input DNA origin. Only a Cy3 signal could be seen when *E. coli* was used as the DNA template for amplification. In contrast, only a Cy5 signal could be seen when *Salmonella* was used as the DNA template. The assay was repeated with six other *Salmonella* samples and seven other *E. coli* samples, with identical results (data not shown).

Reporter discrimination is a relatively straightforward method for bacterial identification assays. However, discrimination can also be designed into amplification primers, allowing amplification of one set of sequences but not others. Allele-specific amplification has been demonstrated previously using SDA (6). We combined both reporter discrimination and allele-specific amplification in the same assay to provide a more flexible and secure means of bacterial identification. In addition to reporter discrimination of *E. coli* and *Salmonella*, Fig. 4 also demonstrates the use of allele-specific amplification of *E. coli* and *Salmonella* genomic DNA samples with amplification primers from the *parC* region of type II DNA topoisomerase. The *parC* amplification primers were designed such that *E. coli* but not *Salmonella* samples would be amplified. As shown in Fig. 4 (graph), only *E. coli* samples amplified a product when hybridized to *parC* amplification primers. No product could be detected for *Salmonella* genomic DNA samples, either on the microchip array or in solution SDA assays using polyacrylamide gels for visualizing amplicon products (data not shown).

**Assay sensitivity and cross-reactivity with other bacterial genomic DNAs.** Table 2 lists the sensitivities of the amplification systems as well as the cross-reactivity of other bacterial genomic DNAs with each primer set. Since these primer sets...
were not optimized, the levels of sensitivity differed for the systems. *Campylobacter* gyrA and *E. coli* or *Salmonella* gyrA amplification primers were the most sensitive, with detection occurring at 100 copies of template input. *Chlamydia* cryptic plasmid and *Staphylococci* mecA amplification primers were next, needing approximately 1,000 copies for successful amplification. The least sensitive system was for the *E. coli* parC gene, needing at least 10,000 copies for successful amplification. Genomic DNAs from 10 other bacterial sources were tested for cross-reactivity. None of the primer sets exhibited any level of amplification with these genomic DNA sources.

**Multiplex amplification.** Although antimicrobial resistance and strain discrimination are important in molecular diagnostic assays, it would be ideal to have a high-throughput assay that can screen a large number of genes simultaneously. In order to demonstrate this concept, the maximum numbers of amplification systems for a 25-site microchip array, with the proper controls, were amplified simultaneously. *E. coli*, *C. jejuni*, *Salmonella*, *M. tuberculosis*, *Staphylococci*, and *Chlamydia* were used to test the microchip array for cross-reaction. The *Cy3* signal was used to identify *E. coli* and *Salmonella* genomic DNA samples after amplification. Amplification primers for *parC* and *gyrA* amplifications were addressed to separate locations on the microchip array. Approximately 100 pg of *E. coli* and *Salmonella* genomic DNA was addressed to all primer sets and hybridized with reporters for both *E. coli* gyrA and parC and *Salmonella* gyrA amplicons. Reporter signals remaining after application of thermal stringency were quantified to determine the genotype of the hybridized genomic DNA sample (graph). N/C, noncleavable.

**TABLE 2. Sensitivity of anchored SDA and cross-reactivity with other bacterial genomic DNAs**

<table>
<thead>
<tr>
<th>SDA primer set</th>
<th>No. of copies needed for amplification of DNA from:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td><em>E. coli</em> or <em>Salmonella</em> gyrA</td>
<td>100</td>
</tr>
<tr>
<td><em>Campylobacter</em> gyrA</td>
<td>—</td>
</tr>
<tr>
<td><em>E. coli</em> parC</td>
<td>10,000</td>
</tr>
<tr>
<td>mecA</td>
<td>—</td>
</tr>
<tr>
<td><em>Chlamydia</em> cryptic plasmid</td>
<td>—</td>
</tr>
</tbody>
</table>

* Titration experiments were performed for each anchored SDA system, and images were quantified to determine the lower limit of amplification sensitivity. The number of copies necessary for consistent amplification of template DNA is indicated. Genomic DNA from other bacterial species (100 ng) was also tested with every system to determine the degree of cross-reactivity. Typhi, *Salmonella enterica*, serovar Typhi; MRSA, methicillin-resistant *S. aureus*. —, no amplification.
juni, methicillin-resistant S. aureus, and Chlamydia cryptic plasmid genomic DNAs were hybridized to the microchip array and amplified simultaneously. As shown in Fig. 5, all systems amplified simultaneously, giving correct discrimination patterns for the template DNA inputs used. Quantification of the signals (Fig. 5, graph) showed that the least sensitive system (parC) (Table 1) also amplified poorly in the multiplex amplification situation, whereas the most sensitive system (E. coli or Salmonella gyrA and Campylobacter gyrA) (Table 1) amplified target DNA best. Quantification of the Chlamydia cryptic plasmid amplification results could not be compared to that of the other systems, since a different fluorophore (BTR) was used as the reporter moiety. The results indicated that, independent of the numbers of amplification systems present, the different primer sets did not influence the amplification efficiency of other, neighboring primer sets. These findings confirm the previous findings (22) that anchored SDA primer sets act as discrete amplification units on the microelectronic chip array.

DISCUSSION

Anchored SDA of bacterial gene targets is an efficient method for multiplex amplification and discrimination of antimicrobial agent-resistant strains as well as for bacterial identification. The assay provides a specific and reliable means for culture confirmation and can be sensitive to an input level of approximately 100 copies of DNA. It is important to note that the primer sets described here were not optimized for either primer-primer interactions or primer amplification efficiency. Anchored SDA allows multiplex amplification to occur because, as has been shown previously (22), the amplification primer sets act as discrete units on the microelectronic chip array. In anchored SDA, amplification primer sets are spatially separated, creating distinct zones of amplification that share only enzymes and reagents. Electronic addressing of amplification primers to distinct regions on the microchip allows a reduction in primer-primer interactions while maintaining a completely open format that simplifies the amplification procedure greatly. In addition to electronic addressing, the electronic microchip format also allows electronic hybridization, which has been shown previously to be essential for anchored SDA of genomic DNA samples (22). Electronic hybridization may increase the efficiency of the reaction by both facilitating strand separation of target DNA in a low-ionic-strength environment and concentrating targets onto the array site (5). Electronic hybridization also confers advantages in time, al-
lowing hybridization reactions to be completed in minutes in- stead of hours (5, 8). These two aspects, combined with the flexibility of the assay in addressing any oligonucleotide or DNA sequence to any site on the microchip array, make the microelectronic chip a very attractive platform for molecular biology applications.

The flexibility of anchored SDA for bacterial identification makes it an ideal candidate for a task- or group-specific assay design. In this work, we have shown that five different genotypic assays (plasmid, transposon, SNP analysis, allele-specific amplification, and reporter-specific discrimination) can be accomplished simultaneously using anchored SDA and discrimination on the microelectronic chip platform. In the future, microchip arrays could include amplification primer sets, for example, for all known food-borne or respiratory pathogens on the same microchip, enabling amplification and discrimination of a whole class of bacterial pathogens on a single microchip array. In addition, anchored SDA could further discriminate, again on the same platform, possible antimicrobial resistance markers or other genetic markers that may be present in the same sample. Anchored SDA allows many different types of assays to be accommodated on the same platform, including RNA amplification (22), without adjusting for special conditions such as matching hybridization temperatures or altering stringency conditions, as in other amplification or microchip assays. This flexibility allows multiplex amplification and discrimination on the same platform, potentially streamlining the development of any nucleic-acid-based bacterial detection assay.

As demonstrated here, anchored SDA can readily discrimi- nate SNPs. This result suggests the immediate application of this assay for culture confirmation in clinical laboratories. That 100 copies of DNA are sufficient for antimicrobial resistance determination suggests that other sample sources, including blood culture bottles, could be used in conjunction with an- chored SDA. However, increases in sensitivity are necessary in order to analyze samples that have limited target possibilities, including blood, sputum, or urine samples. Studies are under way to increase the sensitivity of the assay through optimization of amplification primer design and reagent conditions, as well as increased amplification and reporter sensitivity.

Anchored SDA incorporates amplification and detection on the same platform. With the development of automated pro- tocols for anchored SDA, the process of bacterial identification could be simplified greatly by minimizing the sample processing and transfer of amplicons needed for other molecular biology-based assays. These features make anchored SDA an ideal candidate for multiplex point-of-care applications. Anchored SDA can enhance the ability of miniaturized on-site instrumentation by decreasing the complexity of the necessary molecular biology reactions and manipulations while simultane- ously allowing efficient multiplex amplification reactions and discrimination. Through integration of anchored SDA into an on-site instrument, the level of technical expertise necessary can be minimized, allowing many current clinical laboratories access to powerful molecular biology-based assays.

ACKNOWLEDGMENTS

We gratefully acknowledge the contributions of Beth Mather, Michael Heller, Richard Anderson, Bruce Wallace, and Douglas Malmi- nowski. We thank Michael Moore and Harry J. Leonhardt for critical reading of the manuscript. We are especially grateful to Halleh Aha- dian for technical assistance.

REFERENCES


