INTRODUCTION

Molecular detection techniques continue to increase in utility in clinical microbiology laboratories. The implementation of in vitro nucleic acid amplification techniques, led by real-time PCR, in diagnostic laboratories has transformed viral detection and select bacterial detection. Although not likely to completely replace culture techniques in the near future, molecular applications in the diagnosis of infectious diseases have become commonplace in academic medical centers and tertiary-care facilities and are becoming more tangible in community-based settings as more FDA-cleared products are available. The further advancement of molecular infectious disease diagnostics is dependent on the ability of multiplexing technologies, or the ability to detect and identify more than one pathogen simultaneously from the same specimen, to be implemented in clinical microbiology laboratories with ease and accuracy. One approach to multiplex detection and characterization is microarray analysis.

Simply defined, a microarray is a collection of microscopic features (most commonly DNA) which can be probed with target molecules to produce either quantitative (gene expression) or qualitative (diagnostic) data. Although other types of microarrays exist, such as protein microarrays (122, 125), this review will focus on DNA microarrays. The initial production of arrays in the research arena included radiolabeled macroarrays such as Southern blots and dot blots (91, 177). Scientific ingenuity in research laboratories in the 1990s led to the development of modern two-dimensional hybridization microarrays (167, 172). Largely due to advances in fabrication, robotics, and bioinformatics, microarray technology has continued to improve in terms of efficiency, discriminatory power, reproducibility, sensitivity, and specificity (135). In addition, microarray platforms have expanded to include three-dimensional arrays or suspension bead arrays. These improvements have allowed the transition of microarrays from strictly research settings to clinical diagnostic applications. The number of articles on microarrays and articles describing their use in microbiology and infectious diseases has rapidly increased over the past 9 years (Fig. 1). Although many of these articles can still be attributed to clinical microbiology or infectious disease “research,” research in the diagnostic realm has led to the optimization of the diagnostic potential of microarrays and has led to the development of commercially available qualitative detection platforms. Thus, we have now entered a new era in molecular diagnostics where the use of microarray technology in clinical microbiology is a reality.

BASIC CONCEPTS OF MICROARRAYS

Microarrays can be distinguished based upon characteristics such as the nature of the probe, the solid-surface support used, and the specific method used for probe addressing and/or target detection (135). Below, we review the methodologies of printed and in situ-synthesized microarrays, high-density bead arrays, and electronic and suspension bead microarrays. In all
of these approaches, the probe refers to the DNA sequence bound to the solid-surface support in the microarray, whereas the target is the “unknown” sequence of interest. In general terms, probes are synthesized and immobilized as discrete features, or spots. Each feature contains millions of identical probes. The target is fluorescently labeled and then hybridized to the probe microarray. A successful hybridization event between the labeled target and the immobilized probe will result in an increase of fluorescence intensity over a background level, which can be measured using a fluorescent scanner (135). The fluorescence data can then be analyzed by a variety of methods. Experimental details including probe length and synthesis, number of possible features (i.e., density of the microarray), and the solid surface used vary depending on the type of microarray employed and are discussed below and summarized in Table 1. The goals of the manuscript are to review the concepts behind each of these microarray technologies, highlighting their benefits and disadvantages, as well as provide a detailed review of the applications of these techniques in clinical microbiology.

**Printed Microarrays**

Printed arrays were the first microarrays utilized in research laboratories and are so called because of the “printing” or spotting of the probes onto the microarray surface, which is most commonly a glass microscope slide. Glass slides are an attractive medium for microarrays because they are economical; are stable throughout high temperatures and stringent washes; are nonporous, allowing for efficient kinetics during hybridization; and have minimal background fluorescence (25). The probe spots, or features, can be applied by either noncontact or contact printing. A noncontact printer uses the same technology as computer printers (i.e., bubble jet or inkjet) to expel small droplets of probe solution onto the glass slide. In contact printing, each print pin directly applies the probe solution onto the microarray surface. The result in both cases is the application of a few nanoliters of probe solution per spot to create an array of 100- to 150-μm features. During the printing process it is imperative to control for cross-contamination and printing consistency to preserve the integrity of the microarray and subsequent hybridization data. Due to the relatively large size of the features, printed microarrays are of lower density (~10,000 to 30,000 features) than in situ-synthesized microarrays and high-density bead arrays (discussed below) but offer considerably more features than either electronic microarrays or suspension bead arrays. The general workflow for the processing of printed microarrays is depicted in Fig. 2.

Printed arrays can be further classified based upon the nature of the probes: double-stranded DNA (dsDNA) or oligonucleotide microarrays. For dsDNA microarrays, the probes consist of amplification products (amplicons) obtained by PCR using primers designed from a known genomic sequence, shotgun library clones, or cDNA (74, 167, 194). The double-stranded amplicons are denatured, either in print buffer or after immobilization, which allows the probes to be available for hybridization. Amplicons can be attached to the glass slide surface by the electrostatic interaction of the negative charge of the phosphate backbone of the DNA with a positively charged coating of the slide surface (51) or by UV-cross-linked covalent bonds between the thymidine bases in the DNA and amine groups on treated slides (25). Typically, each 200- to 800-bp dsDNA probe represents a different gene. Ideally, PCR amplicons for microarrays should have high specificity and yield but no contamination, including nonspecific amplification and contaminants that affect attachment to the microarray surface or that autofluoresce (19). Unfortunately, dsDNA probes generally have a high sensitivity but suffer in specificity. For example, in a report by Hager, 21 to 34% of probes did not match the intended target and/or were contaminated (72). The ultimate assessment of probe specificity is sequencing of the products. However, due to financial constraints, most laboratories test the purity and quantity of the amplified product by agarose gel electrophoresis. The specificity of hybridization

![FIG. 1. Microarray publications. The number of primary manuscripts published using microarray technology (bars) and the number of microarray publications that have infectious disease and/or microbiology applications (line) are depicted.](http://cmr.asm.org/)

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<table>
<thead>
<tr>
<th>Microarray Principle(s)</th>
<th>Format(s)</th>
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<tr>
<td>Printed DNA probes on glass slides</td>
<td>For dsDNA, PCR amplicons (200–800 bp) from known genomic sequence, shotgun library clones, or DNA are used; for oligonucleotides, 25-80-bp probes are synthesized</td>
<td>Moderate (10,000–30,000)</td>
<td>$$$</td>
<td></td>
<td>No commercially available applications; pathogen detection and identification, antimicrobial resistance detection, viral discovery, molecular surveillance</td>
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<tr>
<td>In situ synthesized oligonucleotides</td>
<td>Oligonucleotides are synthesized directly on the surface of a quartz wafer using photochemistry; multiple probe sets (one perfect-match probe and one mismatch probe) are included per target</td>
<td>High (Affymetrix, 10^6; NimbleGen and Agilent, 15,000–10^6)</td>
<td>$$$</td>
<td></td>
<td>No commercially available applications; pathogen detection and identification, antimicrobial resistance detection, viral discovery, molecular surveillance, strain typing</td>
</tr>
<tr>
<td>High-density bead arrays</td>
<td>Sequence-tagged beads are randomly assorted onto fiber-optic bundles or silicon slides</td>
<td>High (50,000–10^6)</td>
<td>$$$</td>
<td></td>
<td>No commercially available applications; potential use in microbiology but no studies published to date</td>
</tr>
<tr>
<td>Electronic</td>
<td>Electric fields are used to promote active hybridization of nucleic acids on a microelectronic device; streptavidin-biotin bonds immobilize the probes on the array surface</td>
<td>Low (400 max)</td>
<td>$$</td>
<td></td>
<td>Commercially available products discontinued; pathogen detection and identification</td>
</tr>
<tr>
<td>Liquid-bead suspension</td>
<td>Spectrally unique microspheres provide solid support for application of probes or universal sequence tags; bead hybridization with fluorescently labeled target DNA is measured using flow cytometry</td>
<td>Low (100 max)</td>
<td>$$$</td>
<td></td>
<td>FDA-cleared xTAG RVP assay; pathogen detection and identification, antimicrobial resistance detection, strain typing</td>
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*Data from reference 135. $$, low-moderate cost; $$$, moderate cost; $$$$, high cost.
data can be improved by incorporating redundancy by the inclusion of multiple gene segments among the probes. Although decreased specificity can be beneficial when analyzing a genomic sequence rich in natural polymorphisms, it is disadvantageous when trying to discriminate among highly similar target sequences and unacceptable for clinical diagnostic applications (105).

The spotted probes of oligonucleotide microarrays consist of short, chemically synthesized sequences. In contrast to the relatively large size of dsDNA probes discussed above, the length of probes typically used in printed oligonucleotide microarrays ranges from 25 to 80 bp but may be as long as 150 bp for gene expression microarrays (30). The use of shorter probe lengths allows fewer errors to be introduced during probe synthesis and facilitates the interrogation of small genomic regions, including polymorphisms. While the decreased probe length may adversely affect sensitivity compared to dsDNA probes, specificity is often greater when short, specific genomic regions are interrogated. Longer probes have higher melting temperatures and greater mismatch tolerance, leading to decreased specificity. Generally, the strength of the hybridization signal and the sensitivity increase with an increasing length of the probe. The addition of spacers coupled to very short oligonucleotides or the application of a higher concentration of probe during printing can improve the hybridization signal strength (30). Ramdas et al. reported an eightfold increase in sensitivity with 70-mer oligonucleotides relative to the sensitivity of 30-mer probes, especially for low-expression genes (159). Since, as with dsDNA probes, very long oligonucleotide probes may decrease specificity due to random hybridization to nontarget sequences, it is critical to cautiously determine the optimal probe length for each microarray design. Although easier to manufacture than dsDNA probes, oligonucleotide probes require careful design so that all probes have comparable melting temperatures (within 5°C) and lack palindromic sequences. Preferably, each probe should be tested experimentally to guarantee nonbiased hybridization data, although this may not always be financially possible (30). Oligonucleotide probes are attached to glass slides by covalent linkage because, due to their small size, a significant quantity of probes would be lost during wash steps after noncovalent electrostatic immobilization and cross-linking. The probes are coupled to the microarray surface via modified 5′ or 3′ ends (most commonly a 5′ amino group) on coated slides that provide aldehyde or epoxy functional groups.

Compared to the in situ-synthesized microarrays discussed below, printed microarrays are relatively simple and inexpensive. However, the initial setup of microarray facilities is costly and requires dedicated space in which environmental variables such as dust, humidity, and temperature are well controlled. Dedicated microarray core facilities are available at many universities, making these challenges minimal for individual investigators. A major advantage of printed microarrays is flexibility. The ability to quickly adjust spotted probes based upon updated annotations or the discovery of new, emerging pathogens or resistance mechanisms makes printed microarrays attractive for use in clinical microbiology. However, the implementation of a printed microarray in a clinical diagnostic laboratory is complicated by the arduous and expensive tasks of monitoring production reproducibility, performing clinical validation studies, and continuously assessing the quality of downstream data. A major drawback in the manufacturing of printed dsDNA microarrays is the enormous scale of amplicon production and the associated difficulties of quality control, information management, efficiency, and accuracy. Likewise, the design of oligonucleotide probes is labor-intensive, and errors introduced from probe synthesis are a problem. Although printed microarrays are conducive to "homebrew" or user-defined testing, their use in diagnostic microbiology remains limited to specific research applications.
microarrays are also crucial to the study of organisms that have not been fully sequenced. The lack of commercially available printed microarrays for use in clinical infectious disease diagnostics makes it unlikely that printed microarrays will soon transition to clinical microbiology laboratories. There are, however, commercially available whole-genome microarrays for select organisms that are useful for research endeavors.

In Situ-Synthesized Oligonucleotide Microarrays

In situ-synthesized arrays are extremely-high-density microarrays that use oligonucleotide probes, of which GeneChips (Affymetrix, Santa Clara, CA) are the most widely known. Unlike the printed oligonucleotide arrays described above, the oligonucleotide probes are synthesized directly on the surface of the microarray, which is typically a 1.2-cm² quartz wafer. Because in situ-synthesized probes are typically short (20 to 25 bp), multiple probes per target are included to improve sensitivity, specificity, and statistical accuracy. Classically, 11 probes are used per 600 bases being examined (38). The use of probe sets further increases the specificity. A probe set includes one perfect-match probe and one mismatch probe that contains a 1-bp difference in the middle position of the probe (i.e., position 13 of a 25-bp probe). Each member of the probe set is located in a separate feature, which allows the mismatch probe to act as a negative control to identify possible nonspecific cross-hybridization events. Recent advances in GeneChips include the use of longer probes, the design of arrays that interrogate across entire genes or exons, and the implementation of multiple independent and nonoverlapping perfect-match probes in lieu of classic probe sets.

Affymetrix GeneChips typically have \(10^6\) features per microarray depending on the interface distance (38, 59). GeneChip oligonucleotide probes are synthesized using semiconductor-based chemical synthesis. On the quartz surface, synthesis linkers modified with light-sensitive protecting groups (59). Thus, the microarray surface is chemically protected from a nucleotide addition until deprotected by light. When the array surface is exposed to UV light, reactive nucleotides modified with a photolabile protecting group can be added to growing oligonucleotide chains. To target specific nucleotides to exact probe sites, photolithographic masks are used. Each photolithographic mask has a defined pattern of windows, which acts as a filter to either transmit or block UV light from specific features on the chemically protected microarray surface. Areas of the microarray surface in which UV light has been blocked will remain protected from the addition of nucleotides, whereas areas exposed to light will be deprotected, and specific nucleotides can be added. The pattern of windows in each mask directs the order of nucleotide addition. In situ probe synthesis is therefore accomplished through the cycling of masking, light exposure, and the addition of either A, C, T, or G bases to the growing oligonucleotide (Fig. 3) (38, 51).

Other high-density oligonucleotide microarrays include those manufactured by Roche NimbleGen (Madison, WI) and Agilent Technologies (Palo Alto, CA). Both platforms use longer oligonucleotide probes (60 to 100 bp), but NimbleGen uses maskless photo-mediated synthesis, and Agilent employs inkjet technology for the in situ manufacturing of the probes. While experiments performed with GeneChips are limited to one label, the NimbleGen and Agilent platforms allow multicolor hybridizations. As mentioned above for printed microarrays, the use of longer oligonucleotides increases sensitivity. The Roche NimbleGen approach to in situ synthesis is similar to that of the GeneChips described above, but photolithographic masks are replaced by “virtual” or digital masks in Roche NimbleGen’s maskless array synthesizer technology. Maskless array synthesizer technology uses an array of programmable micromirrors to create digital masks that reflect the desired pattern of UV light to deprotect the features where the next nucleotide will be coupled (Fig. 4). Each NimbleGen microarray can contain \(10^6\) features and can be purchased in the following formats per slide: \(1 \times 2.1\) million features, \(3 \times 720,000\) features, \(1 \times 385,000\) features, \(4 \times 72,000\) features, and \(12 \times 135,000\) features. In contrast to the quartz wafers used for the above-described technologies, Agilent microarrays use glass slides and inkjet printing, which eliminates the need for either lithographic or digital masks (Fig. 5). The in situ synthesis of 60-mer oligonucleotides is achieved using five-“ink” (4 bases plus catalyst) printing of nucleotide precursors combined with coupling and deprotection steps (83). Agilent microarrays are available in the following formats: \(1 \times 244,000\) features, \(2 \times 105,000\) features, \(4 \times 44,000\) features, and \(8 \times 15,000\) features.

Due to the complex nature of chemical synthesis and the expense involved in production, synthesized microarrays are dependent on commercial manufacturing and are therefore not conducive to user-defined development. There is a growing number of microbial genome microarrays available commercially for gene expression studies. Also, several resequencing microarrays have been developed by TessArae (Potomac Falls, VA) on the Affymetrix GeneChip platform to simultaneously detect and differentiate large numbers of microbial pathogens (115, 116). Currently, a synthesized oligonucleotide array appropriate for use in a diagnostic microbiology laboratory would need to be ordered as a custom microarray. The expense of a custom Affymetrix microarray and the inherent inflexibility of its custom mask make the use of an Affymetrix-synthesized array impractical for the clinical laboratory. In contrast, other in situ-synthesized platforms (Nimblegen and Agilent) can be easily customized with unique oligonucleotide sequence content. Furthermore, a Web-based tool provided by Agilent (eArray) allows users to custom design microarrays with no minimum manufacturing batch size, making Agilent microarrays a primary choice for homebrew and pilot applications. Although it is relatively expensive to obtain a custom oligonucleotide array, many universities already have one or more of these platforms on campus for hybridization and analysis, which may offset the upfront costs. The major advantages to these systems are the reproducibility of the manufacturing process and the standardization of reagents, instrumentmation, and data analysis, all of which are critical for methodologies to transition to the clinical laboratory (38). Further advantages that make this approach attractive for clinical diagnostics include controls, such as reference probes for intensity normalization; internal standards of known concentrations; and probes arranged in a checkerboard pattern that are homologous to an internal control included in the hybridization mix.

Whether printed or synthesized, oligonucleotide microar-
rays generally allow much cleaner downstream hybridization data than do amplicon-based microarrays. With oligonucleotide arrays, the ability to standardize probe concentrations and hybridization temperatures while avoiding or controlling for significant nonspecific hybridization has resulted in considerable improvements in the accuracy and reproducibility of microarray data (105). Although in situ-synthesized oligonucleotide microarrays are very robust systems and have significant control measures included, there are currently none with direct diagnostic infectious disease applications that are commercially available.

**High-Density Bead Arrays**

Similar to the printed and in situ-hybridized microarrays discussed above, BeadArrays (Illumina, San Diego, CA) provide a patterned substrate for the high-density detection of target nucleic acids. However, instead of glass slides or silicon wafers as direct substrates, BeadArrays rely on 3-μm silica beads that randomly self-assemble onto one of two available substrates: the Sentrix Array Matrix (SAM) or the Sentrix BeadChip (Fig. 6) (52, 146). The SAM contains 96 1.4-mm fiber-optic bundles. Each bundle is an individual array consisting of 50,000 5-μm light-conducting fibers, each of which is chemically etched to create a microwell for a single bead (52). In the universal BeadArray, up to 1,536 bead types (each with a unique capture sequence) assemble onto each fiber bundle, resulting in ~30 beads of each type in the array (146). Each SAM allows the analysis of 96 independent samples. The BeadChip can be used to assay 1 to 16 samples at a time on a silicon slide that has been processed by microelectromechanical systems technology to provide microwells for individual beads (53). BeadChips are more appropriate for very-high-density applications such as whole-genome genotyping, which requires 10^5 to 10^6 features for determining genome-wide single nucleotide polymorphisms (SNP) (Infinium assay; Illumina) (70).

Unlike the known locations of printed and in situ-hybridized microarray features, the beads in BeadArrays randomly assort to their final location on the array. Thus, the bead location must be mapped, which is accomplished by a decoding process (71). This “decoding” process is in contrast to the use of internal dyes for “encoding” the Luminex microspheres discussed in “Suspension Bead Arrays” below. Each bead has ~700,000 copies of a unique capture oligonucleotide covalently attached to it, which serves as the bead’s identifier (107). In the universal arrays, the capture sequences specifically avoid homology with human and mouse nucleic acid sequences and are referred to as IllumiCodes. The mapping of
the Illumina beads is accomplished by a series of hybridization and rinse steps, allowing fluorescently labeled complementary oligonucleotides to bind to their specific bead sequence (IllumiCode) and thus track the location of the bead type (52). An additional advantage to the decoding process is the quality control provided for each feature of the microarray (53).

The SAM can be processed using a standard microtiter plate, which makes it amenable to standard automation and high-throughput processing. The distance between individual arrays on the 16-sample BeadChip is identical to that of a standard multichannel pipettor, thereby facilitating ease of use. BeadArrays can support up to 10^5 to 10^6 features and have built-in redundancy. This redundancy is a crucial experimental control for intermicroarray comparative data since each manufactured microarray will not be identical. An additional advantage to the uniqueness of each microarray is that altering the bead pattern provides a means to identify spatial bias.

Although the analysis tools available for BeadArray-specific data analysis, background correction, and spatial artifact recognition have been lagging behind those provided by other microarray manufacturers, independent researchers have begun to fill the gaps (20, 47, 49, 50, 182, 217). BeadArrays have been successfully applied to DNA methylation studies (12, 13), gene expression profiling (14, 54, 107), and SNP genotyping, including the International HapMap Project (www.hapmap.org) (23, 53, 70).

**Electronic Microarrays**

The printed and in situ-synthesized microarrays and BeadArrays described above rely on passive transport for the hybridization of nucleic acids. In contrast, electronic microarrays utilize active hybridization via electric fields to control nucleic acid transport. Microelectronic cartridges (NanoChip 400; Nanogen, San Diego, CA) use complementary metal oxide semiconductor technology for the electronic addressing of nucleic acids (175). Each NanoChip cartridge has 12 connectors that control 400 individual test sites. Negatively charged nucleic acids are transported to specific sites, or features, when a positive current is applied to one or more test sites on the cartridge.
The surface of the microarray contains streptavidin, which allows for the formation of streptavidin-biotin bonds once electronically addressed biotinylated probes reach their targeted location. The positive current is then removed from the active features, and new test sites can be activated by the targeted application of a positive current. Once the probes have been hybridized at discrete features, the microarray is ready for the application of fluorescently labeled target DNA. Typically, target DNA passively hybridizes with the immobilized probes on the microarray but can also be concentrated electronically (Fig. 7). Although addressing the capture probe down first is the most commonly used format, amplicon-down and sandwich assays have also been utilized. Regardless of the addressing format used, if hybridization occurs between the probe and the target DNA, fluorescent reporters will be present at the positive test, which will be detected when the electronic microarray is scanned and analyzed.

Electronic microarrays offer several advantages. For example, multiplex detection can be accomplished at an individual test site since multiple probes, each with a distinct fluorophore, can be sequentially addressed to the same feature. The flexibility of this platform allows nucleic acids from a single sample to be hybridized to multiple (but not necessarily all) test sites for the detection of multiple targets, or nucleic acids from multiple samples can be analyzed on the same microarray cartridge, minimizing waste. Furthermore, the NanoChip is a universal blank chip, and the content of the microarray is specified directly by the user, which allows more flexibility in assay design and decreases costs associated with microarray manufacturing. Although the density of electronic microarrays is currently limited to 400 spots, this is sufficient for the majority of diagnostic microbiology applications. In 2007, NanoGen announced the termination of its microarray business. Nonetheless, this technology demonstrates the evolution of microarray technology to a platform that is more practical for diagnostic applications.

**Suspension Bead Arrays**

In contrast to the two-dimensional, or planar, arrays discussed above, suspension bead arrays are essentially three-dimensional arrays based on the use of microscopic polystyrene spheres (microspheres or beads) as the solid support and flow cytometry for bead and target detection. Furthermore, they are distinct from the high-density Illumina BeadArrays discussed above, in which the beads are immobilized on fiber-optic strands or silicon slides. Suspension-bead-based assays were initially described in 1977 and focused on the detection of antigens and antibodies (78). Multiplexing was first achieved by using different-sized microsphere sets for the simultaneous detection of multiple antibodies (130, 169). Currently, more-robust multiplexing is accomplished using different microsphere sets based on color. Red (658-nm emission) and infrared (712-nm emission) fluorochromes are used at various
concentrations to fill 5.6-μm microspheres. Each bead of the 100-microsphere set has a distinct red-to-infrared ratio, and therefore, each bead has a unique spectral address (Fig. 8A). Microspheres with a specific spectral address coupled to a specific probe are equivalent to a feature in a planar microarray. Once multiple individual microspheres have been coupled to separate specific probes, a mixture of microspheres (in theory, up to 100) can be used to interrogate extracted and amplified nucleic acids (Fig. 8B). The subsequent detection of a fluorescent reporter that indicates probe-target DNA hybridization is accomplished using a bench-top flow cytometer. A single-file microsphere suspension passes by two lasers. A 635-nm laser excites the red and infrared fluorochromes impregnated in the microspheres, which allows the classification of the bead and therefore the identity of the probe-target being analyzed. A 532-nm laser excites reporter fluorochromes such as R-phycoerythrin and Alexa 532 to quantify any hybridization that occurs on the microsphere (Fig. 8C).

Several chemistries have been developed for nucleic acid detection by suspension bead arrays, including direct DNA hybridization, competitive DNA hybridization, and solution-based chemistries with microsphere capture (48). In direct DNA hybridization, PCR amplicons hybridize directly to probe capture sequences immobilized on the microspheres (Fig. 8B) (8, 179). Generally, a biotinylated primer is used during amplification, which allows streptavidin–R-phycoerythrin to bind and label hybridized microspheres. Competitive DNA hybridization utilizes unlabeled PCR amplicons and biotinylated competitor oligonucleotides. In contrast to the direct hybridization method, competitive DNA hybridization yields high fluorescence in the absence of target DNA. When target DNA is present, it binds the labeled competitor DNA, which, in turn, is not available to hybridize to the microsphere, yielding low fluorescence. Allele-specific primer extension (ASPE) or target-specific primer extension (TSPE), oligonucleotide ligation assay (OLA), and single-base-chain extension (SBCE) are solution-based chemistries coupled with subsequent microsphere capture. By exploiting the natural properties of DNA polymers and ligases, these chemistries incorporate a capture sequence during the solution-based reaction (48). Both ASPE or TSPE and OLA use a capture primer, which contains a unique 5′ sequence followed by a target-specific sequence. In ASPE and/or TSPE, the primer can be extended by DNA polymerase only if target DNA is present to supply the complementary base for the 3′ nucleotide. The label in ASPE and/or TSPE is provided by a biotinylated deoxynucleotide triphosphate. The OLA reaction is ligase dependent. In addition to the capture primer, a biotinylated probe homologous to target DNA is present during an OLA. The capture primer and reporter probes can be ligated only if target DNA is present in the sample. Used for multiplex SNP detection, SBCE requires independent reactions for each nucleotide query. For every SNP being interrogated, one probe with a unique capture sequence is used to assay the possible alleles in separate wells containing a different dideoxynucleoside triphosphate per well (24). When the capture and target sequences are homologous, a biotinylated dideoxynucleoside triphosphate is incorporated, thereby terminating further extension.

The solution-based chemistries described above all take advantage of universal microspheres with nonspecific capture
sequences. The first universal sequences used to tag microspheres were ZipCode/cZipCode capture sequences originally used with SBCE in SNP genotyping assays (24, 84, 192, 219). The 25-bp ZipCode sequences are based on random genomic sequences from *Mycobacterium tuberculosis* (24). A unique ZipCode sequence is included in the 5′/H11032 end of the capture probe used in the chemistries described above, while microspheres are tagged with the complementary sequence (cZipCode). Additional sets of universal capture sequences have been developed, including those by Tm Biosciences (xTAG; Luminex Molecular Diagnostics, Inc., Toronto, Canada) and EraGen (Madison, WI) (EraCode) (90). The sequences of the xTAG (also Tag-It and FlexMAP) system consist of 3 of the 4 nucleotides, thereby decreasing the likelihood of nonspecific hybridization to naturally occurring sequences. Since all of the xTAG sequences are thermodynamically matched, variability in hybridization efficiency is not an issue. The xTAG universal bead technology is used for all commercial assays available through Luminex. Based on the expanded genetic alphabet of MultiCode technology, EraCode sequences incorporate synthesized isoguanosine and 5-Me-isocytosine bases. EraCode sequences are highly specific since the isoguanosine and 5-Me-isocytosine bases will pair with each other but not naturally occurring bases.

Although the feature density of suspension bead arrays is the lowest of all the platforms reviewed, advantages abound that make this platform the most practical for clinical microbiology applications. The availability of universal bead sets and their inherent flexibility make the development of user-defined applications feasible and relatively inexpensive. Although users must carefully validate the positive fluorescent threshold for each analyte in the multiplex, user-defined bead-based assays provide experienced users a multitude of clinically relevant applications (see below). Importantly, in 2008, Luminex obtained FDA clearance for the first infectious-disease suspension bead array (xTAG RVP), which detects 12 respiratory viruses and subtypes (106, 132). Although analyte-specific reagents (ASRs) also exist, the availability of FDA-cleared products is a critical step in getting this technology into less-experienced diagnostic microbiology laboratories. However, many established clinical molecular microbiology laboratories rely heavily on real-time PCR, which has minimal contamination
risks. In contrast, the opening of postamplification tubes and the subsequent pipetting steps in the workflow of suspension arrays increase the risk for intra- and interrun contamination. Careful consideration should be paid to contamination control measures and the reestablishment of postamplification laboratory space in the era of real-time PCR. Nonetheless, the relative simplicity, powerful multiplexing capabilities, and affordability of suspension bead arrays make this platform the most attractive for high-throughput nucleic acid detection in clinical infectious disease diagnostics.

**POTENTIAL APPLICATIONS IN CLINICAL MICROBIOLOGY**

Microarray technology has been used for over a decade to investigate the differential gene expression of pathogens. Although gene expression analyses have contributed significantly to our understanding of pathogenic mechanisms, pathogen responses to environmental stimuli, and host-pathogen interactions, one could argue that the data from these investigations have little direct impact on diagnostic microbiology. However, as outlined below, microarray technology has been applied to the detection and identification of various pathogens, pathogen discovery, antimicrobial resistance monitoring, and strain typing. In addition, the monitoring of host responses to infection and therapy represents a burgeoning field that, when coupled with pathogen-specific detection and monitoring, will be the ultimate diagnostic platform for infectious diseases.

**Microbial Detection and Identification**

Perhaps the most promising area in applying microarray technology in clinical microbiology is the use of low- or middle-density microarrays for the simultaneous assessment of large numbers of microbial genetic targets (64, 183). Specific microbial gene amplification by either a broad-range or a multiplex PCR prior to microarray analysis enhances test sensitivity. The amplification of universal microorganism targets by broad-range PCR followed by sequencing analysis has been considered a standard procedure (190); however, microarrays have
emerged as potential tools for bacterial detection and identification given their high parallelism in screening for the presence of a wide diversity of genes. The most commonly used gene targets have been the 16S bacterial and 28S fungal and intergenic transcribed spacers (ITSs) in rRNA genes, and microarray technology has been incorporated to compensate for the time-consuming sequencing identification procedure (190). An oligonucleotide microarray targeting the 16S rRNA gene was developed for the detection of a panel of 40 predominant human intestinal bacterial pathogens in human fecal samples (208). Assays using broad-range PCR incorporated with microarrays have been shown to allow rapid bacterial detection and identification with positive blood cultures (5, 128). A similar procedure was developed and used for the rapid diagnosis of bloodstream infections caused by common bacterial pathogens in the pediatric and general populations (32, 173). PCR amplification, in combination with an oligonucleotide microarray, was used to identify Bacillus anthracis based on the rRNA ITS region (144). Several studies reported the use of microarrays to identify pathogenic yeasts and molds by targeting the ITS regions in fungal rRNA genes (80, 81, 110). Recently, a DNA microarray was established to detect and identify 14 commonly encountered fungal pathogens in clinical specimens collected from neutropenic patients (178).

The key for broad-range PCR amplification followed by microarray identification to work is to target the right gene. It is critical to use a gene “broad” enough so that most related microorganisms can be covered in one amplification reaction. On the other hand, the targeted gene should possess enough polymorphic information to supply sufficient discriminatory power to differentiate and characterize related microorganisms. Degenerate primer sets can be designed to increase the coverage of relatively variable genes. Other universal bacterial genes have been used to detect and identify organisms using microarrays. For mycobacterial detection and identification, the gyrB, rpoB, and katG genes have been targeted by using microarrays (61, 197). Microarrays targeting the 23S rRNA and gyrB genes for bacterial detection and identification using clinical specimens have been described (92, 102, 136). In addition to bacterial and mycobacterial organisms, microarrays following broad-range PCR amplification have been used to detect and identify fungal, parasitic, and viral pathogens (43, 101, 210).

Microarrays have also been incorporated with multiplex PCR amplification for the simultaneous detection and identification of a panel of microbial pathogens in a single reaction. Khodakov et al. described a novel microarray-based approach for the simultaneous identification and quantification of human immunodeficiency virus type 1 (HIV-1) and hepatitis B and C viruses in donor plasma specimens (96). A microarray technique for the detection and identification of enteropathogenic bacteria at the species and subspecies levels was developed, covering pathogenic Escherichia coli, Vibrio cholerae, Vibrio parahaemolyticus, Salmonella enterica, Campylobacter jejuni, Shigella spp., Yersinia enterocolitica, and Listeria monocytogenes (220). A microarray-based multiplexed assay was developed to detect foot-and-mouth disease virus with rule-out assays for two other foreign animal diseases and four domestic animal diseases that cause vesicular or ulcerative lesions that are indistinguishable from those of foot-and-mouth disease virus infection of cattle, sheep, and swine (111). Boving et al. reported the development of a novel multiplex PCR with product detection by the Luminesx suspension array system covering a panel of bacterial and viral pathogens causing meningitis. This system detected and identified nine microorganisms including Neisseria meningitidis, Streptococcus pneumoniae, E. coli, Staphylococcus aureus, L. monocytogenes, Streptococcus agalactiae, herpes simplex virus types 1 and 2, and varicella zoster virus directly from cerebrospinal fluid (15). The ResPlex I system, manufactured by Qiagen (Valencia, CA), was used to detect a panel of bacterial pathogens related to community-acquired pneumonia from tracheal aspirates collected from hospitalized antibiotic-treated children. The data indicated that the ResPlex I system significantly enhanced the pathogen-specific diagnosis of community-acquired pneumonia in children (39). This gene-specific, multiplex amplification followed by a microarray identification system provides a great example for additional clinical diagnostic applications such as the detection and differentiation of respiratory viral pathogens, which is described in detail below.

**Respiratory Viral Pathogen Detection in Connection with Multiplex PCR Amplification**

Respiratory infections caused by a panel of bacterial, viral, and fungal pathogens usually present with similar signs and symptoms that are nearly indistinguishable by clinical diagnosis. Simultaneous testing for all possible pathogens is an efficient means to obtain a conclusive result. In addition, assaying for all potential pathogens may yield information regarding possible coinfections or induced secondary infections. The first promising respiratory microarray system was described in 2002, which incorporated 1,600 unique 70-mer-long oligonucleotide probes covering approximately 140 viral genome sequences (206, 207). This ViroChip system was used to identify the severe acute respiratory syndrome virus as a coronavirus (163), for the discovery of a human parainfluenza virus type 4 infection associated with respiratory failure (27) and human coronavirus and rhinovirus in nonasthmatic patients (98), and for the diagnosis of a human metapneumovirus causing critical respiratory illness (26). A resequencing microarray based on the Affymetrix GeneChip platform that used short oligonucleotides to simultaneously provide both species-level and strain-level identification of respiratory pathogens was developed (127, 209). The system was able to detect and identify 26 respiratory pathogens including the novel influenza virus subtypes H5N1 and H1N1 (115, 116). Another comprehensive and panmicrobial microarray, the GreeneChipResp system, was developed and later used for the detection of respiratory viruses and the subtype identification of influenza A viruses (150, 157). In addition to the detection and identification of respiratory pathogens, several formats of microarrays that detect the whole coronavirus genus (42) and detect and type influenza viruses (113, 131, 170, 227) have been described.

Several commercial products are available for the detection of a panel of respiratory viruses, which incorporate microarrays as the identification method (18, 106, 109, 112, 126, 132, 143, 148, 160, 185). These products include the Infiniti RVP from AutoGenomics, Inc. (Carlsbad, CA); the MultiCode-PLx RVP from EraGen Biosciences (Madison, WI); the ResPlex II assay...
from Qiagen (Valencia, CA); the Ngen respiratory virus ASR assay from Nanogen (San Diego, CA); and the xTAG RVP from Luminox Molecular Diagnostics (Toronto, Canada). Table 2 contrasts these commercially available kits. Among them, the EraGen, Qiagen, and Luminox molecular diagnostics systems incorporate multianalyte profiling by a liquid-bead microarray system developed by Luminox (discussed above) (48). Specific applications of this technology for nucleic acid detection include SNP genotyping, genetic disease screening, gene expression profiling, and microbial detection and typing. Although suspension bead arrays are amenable to high-throughput nucleic acid detection, the efficiency of the front-end multiplex PCR amplification limits the number of pathogens that can be included in one reaction. With the implementation of novel multiplex amplification procedures, numbers of targets included in one reaction can be significantly increased without a significant loss of sensitivity (112, 166).

**Simultaneous Detection and Typing of Human Papillomaviruses**

Persistent infection with known high-risk human papillomavirus (HPV) types is a significant risk factor for cervical cancer and is increasingly being recognized as playing a role in other cancers. Recently, HPV vaccines have demonstrated effectiveness in preventing type-specific persistent infection and disease. To monitor the impact of vaccine implementation strategies, determine type-specific persistence, and evaluate the clinical significance of coinfection with multiple genotypes, HPV testing will require type-specific results. A high-throughput, sensitive, specific, and reproducible HPV detection and typing assay is therefore highly desirable. Most established HPV typing assays are based on consensus PCR to amplify the relatively conserved L1 gene region with hybridization, restriction enzyme digestion, or sequencing of the amplicon to determine type(s). Recently, several studies were aimed at evaluating the usefulness of microarray technology for the simultaneous detection and typing of HPV in routine clinical specimens. A user-developed HPV DNA microarray for high-risk HPV genotyping was evaluated by using a panel of malignant and nonmalignant cervical smears. This approach provides the potential to improve the clinical management of patients with cervical cytological abnormalities (3). Several systems that combine multiplex PCR amplification and microarray identification have been reported to provide rapid and reliable diagnostic tools for HPV detection and typing that are amenable to automation (73, 119, 140, 145). Additional studies that incorporated microarrays to detect and characterize high-risk mucosal HPV types (66), betapapillomavirus types (65), and the frequencies of 23 HPV types in women with and without cytological anomalies (193) have been reported.

### Table 2. Comparison of commercially available, microarray-based kits for detection and identification of respiratory viruses

<table>
<thead>
<tr>
<th>Product</th>
<th>Company</th>
<th>Viruses and/or genotypes detected</th>
<th>Amplification platform(s)</th>
<th>Microarray platform</th>
<th>Characteristic(s)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infiniti RVP</td>
<td>AutoGenomics, Inc. (Carlsbad, CA)</td>
<td>Flu-A, Flu-B, PIV-1, PIV-2, PIV-3, PIV-4, RSV-A, RSV-B, hMPV-A, hMPV-B, RhV-B, EnV, CoV, and Adv</td>
<td>Multiplex PCR and RT-PCR</td>
<td>Infiniti analyzer (solid chip)</td>
<td>The detection step by the Infiniti analyzer is completely automatic</td>
<td>160</td>
</tr>
<tr>
<td>ResPlex II assay</td>
<td>Qiagen (Valencia, CA)</td>
<td>Flu-A, Flu-B, PIV-1, PIV-2, PIV-3, PIV-4, RSV-A, RSV-B, hMPV, RhV, EnV, and severe acute respiratory CoV</td>
<td>Multiplex RT-PCR (Tem-PCR)</td>
<td>Luminex (liquid chip)</td>
<td>A unique Tem-PCR allows large numbers of targets included in one reaction without significant loss of sensitivity</td>
<td>18, 112</td>
</tr>
<tr>
<td>NGEN respiratory virus ASR</td>
<td>Nanogen (San Diego, CA)</td>
<td>Flu-A, Flu-B, PIV-1, PIV-2, PIV-3, and RSV</td>
<td>Multiplex RT-PCR</td>
<td>NanoChip (solid chip)</td>
<td>Probe labeling, target capture, and detection are accomplished using electronic microarray technology</td>
<td>112, 185</td>
</tr>
<tr>
<td>xTAG RVP</td>
<td>Luminox Molecular Diagnostics (Toronto, Ontario, Canada)</td>
<td>Flu-A, Flu-B, PIV-1, PIV-2, PIV-3, PIV-4, RSV-A, RSV-B, hMPV, AdV, EnV, CoV, and RhV</td>
<td>Multiplex PCR and RT-PCR</td>
<td>Luminex (liquid chip)</td>
<td>TSPE is used in combination with universal detection beads</td>
<td>126, 132, 148</td>
</tr>
</tbody>
</table>

**Abbreviations:** Tem, target-enriched multiplex; Flu, influenza virus; PIV, parainfluenza virus; RSV, respiratory syncytial virus; hMPV, human metapneumovirus; RhV, rhinoviruses; EnV, enteroviruses; CoV, coronavirus; RT, reverse transcription.
novel DNA detection assay incorporating the Luminex suspension array was reported and was applied to the genotyping of HPV in cervical samples (141). The molecular inversion probe microarray assay, originally applied to large-scale human SNP detection, has been used for HPV detection and typing to demonstrate the potential of the method for the detection and characterization of any microbe (1).

Rapid Detection and Characterization of Methicillin-Resistant Staphylococcus aureus

*Staphylococcus aureus*, including methicillin-resistant *S. aureus* (MRSA), is an important pathogen in hospitals and, increasingly, in communities around the world. Advanced laboratory techniques, including diagnostic microarray analysis, have been sought to rapidly identify staphylococcal isolates and determine antimicrobial susceptibility patterns. DNA microarray analyses of large samples of clinically characterized community-acquired MRSA strains have been reported, which provide broad insights into evolution, pathogenesis, and disease emergence (57, 99, 168). DNA microarrays based on the Array-Tube platform (ClonDiag Chip Technologies, Jena, Germany) have been used for characterizing and genotyping staphylococcal DNA, including their relevant resistance determinants and virulence factors (137–139). Microarrays provide a valuable epidemiological tool for the detailed characterization of MRSA isolates and comparison of strains at a global level (137). In addition, several techniques incorporating peptide and/or nucleic acid probes and conventional and real-time PCR have been used to take advantage of the rapid enrichment of automated blood culture instruments to rapidly identify MRSA from flagged blood cultures when gram-positive cocci in clusters are observed. The combination of novel multiplex PCR amplification and suspension bead array detection (StaphPlex) for the rapid detection and characterization of staphylococci directly from positive blood culture bottles was described (191). The StaphPlex system provides simultaneous staphylococcal identification, antibiotic resistance determinant detection, detection of Panton-Valentine leukocidin, and determination of staphylococcal cassette chromosome mec types I to IV within 5 h. This approach potentially impacts antibiotic treatment of MRSA isolates and comparison of strains at a global level (137). In addition, several techniques incorporating peptide and/or nucleic acid probes and conventional and real-time PCR have been used to take advantage of the rapid enrichment of automated blood culture instruments to rapidly identify MRSA from flagged blood cultures when gram-positive cocci in clusters are observed. The combination of novel multiplex PCR amplification and suspension bead array detection (StaphPlex) for the rapid detection and characterization of staphylococci directly from positive blood culture bottles was described (191). The StaphPlex system provides simultaneous staphylococcal identification, antibiotic resistance determinant detection, detection of Panton-Valentine leukocidin, and determination of staphylococcal cassette chromosome mec types I to IV within 5 h. This approach potentially impacts antibiotic usage when gram-positive cocci in clusters are detected by reducing the unnecessary use of vancomycin, which is often used empirically to treat patients until susceptibility results are available (191). A similar system (MVPllex) was developed and was used to screen for MRSA in nasal swabs (154). Notably, the MVPllex system detects 13 different molecular targets including vancomycin-resistant *Enterococcus*.

Determination of Antimicrobial Drug Resistance

Another successful application of microarray techniques in clinical microbiology is the determination of antimicrobial resistance by simultaneously detecting a panel of drug resistance-related mutations in microbial genomes (21, 36, 72, 153, 196, 224, 225). The emergence of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis and time-consuming phenotypic antimycobacterial susceptibility procedures have stimulated the pursuit of microarray platforms in antimycobacterial drug resistance determinations. High-density DNA oligonucleotide arrays have been used for parallel species identification and rifampin resistance-related mutations in mycobacteria (197) and, more specifically, for the detection of *M. tuberculosis* strains that are resistant to rifampin (40, 176, 222) or isoniazid, kanamycin, streptomycin, pyrazinamide, and ethambutol (41, 189, 204). Oligonucleotide microarrays were developed to analyze and identify drug-resistant *M. tuberculosis* strains, and it was found that the results were comparable with those of standard antimicrobial susceptibility testing (69, 134, 184). A low-cost and -density DNA microarray was designed to detect mutations that confer isoniazid and rifampin resistance in *M. tuberculosis* isolates. The low-cost and -density array protocol takes 45 min after PCR amplification, with only minimal laboratory equipment required (7). Antonova and colleagues developed a method for the detection and identification of mutations in the *M. tuberculosis* genome determining resistance to fluoroquinolones by hybridization on biological microchips (6). A recently developed QIAplex system combines a novel multiplex PCR amplification and suspension bead array identification for the simultaneous detection of 24 *M. tuberculosis* gene mutations responsible for resistance to isoniazid, rifampin, streptomycin, and ethambutol (63). Several studies that detected antibiotic resistance-related mutations in bacterial genomes have been reported (2, 68, 153, 202, 221, 225).

Microarray-based techniques face several application challenges to determine antimicrobial resistance in the clinical setting. First, genomes of some pathogens continue to mutate under natural and therapeutic selective pressures, which is well demonstrated by HIV-1. An Affymetrix microarray was developed to provide HIV-1 antiretroviral-drug-resistant profiles (104, 198, 213). The product was discontinued due to rapidly emerging HIV-1 genome mutations. The company now has a comprehensive, high-density microarray available to identify every mutation in resistance-related HIV-1 genomes. Second, molecular mechanisms for many antimicrobial drug resistances remain to be discovered while novel resistance genes and mutations continue to emerge. It takes considerable time and effort to decipher all of the resistance-related mutations and transfer the basic science findings to clinical applications. For *M. tuberculosis*, until such knowledge is available, the currently used phenotypic methods for identifying resistance will continue to play an invaluable role in optimizing the therapy of persons with tuberculosis.

Microbial Typing

Numerous studies that use microarrays for microorganism typing by taking advantage of its simultaneous detection of a variety of genomes have been reported. The accurate identification and prompt typing of pathogens causing diarrheal diseases are critical for directing clinical intervention, including appropriate antibiotic administration, and facilitating epidemiological investigations. Microarray-based approaches along with other genetic approaches that can be used to support or replace the classical serotyping method for several conventional diarrheal bacterial pathogens have already been offered. The use of microarrays has included *Salmonella*, *Helicobacter*, and *Campylobacter* species (46, 56, 155, 164, 201, 212). PCR followed by a microarray hybridization step has been used for
the detection and typing of *E. coli* virulence genes (28, 199). A serotype-specific DNA microarray for the identification of clinically encountered *Shigella* and pathogenic *E. coli* strains was recently described (114). Diagnostic microarrays based on the ArrayTube format were devised for virulence determinant detection as well as for protein-based serotyping of *E. coli* (4, 100). A novel ArrayTube assay, which incorporates oligonucleotide DNA probes representing 24 of the most epidemiologically relevant O antigens and 47 H antigens, has been described for fast DNA serotyping of *E. coli* (9). Microarrays have also been used to characterize and type other gastroenteritis-causing viral pathogens including rotavirus, norovirus, and astrovirus (29, 77, 86, 103, 123). Beyond diarrheal illnesses, Pas et al. reported the comparison of reverse hybridization, microarray, and sequence analysis for hepatitis B virus (HBV) genotyping, suggesting that the InnoLipa HBV genotyping strip assay, a microarray-based system, detected dual infections and was an easy and quick tool for HBV genotyping (152).

**Microbial Gene Expression Profiling**

The quantification of multiple microorganisms simultaneously using microarray techniques has rarely been reported, probably due to technical difficulties. Instead, the detection and monitoring of the gene expressions of individual microorganism genomes during infection have begun to generate meaningful data (16, 62). Whole-genome microarrays for *M. tuberculosis* were first described using the amplicon arrays developed at Stanford to define gene expression responses to isoniazid and ethambutol (214). Subsequently, microarrays have been used to monitor *M. tuberculosis* gene expression responses to a variety of environmental conditions and exposure to antibiotics (11, 93, 161, 180, 203). *M. tuberculosis* gene expression patterns associated with resistance and susceptibility and mycobacterial survival during infection have been investigated by use of oligonucleotide microarrays (60, 94, 165). The transcriptional profile of *M. tuberculosis* from human lung samples has been studied; during pulmonary tuberculosis, *M. tuberculosis* actively transcribes a number of genes involved in active fortification and evasion from host defense systems (158). Microbial candidate genes have been studied by differential-expression microarrays for discrimination between infection and disease caused by *M. tuberculosis* (87).

Gene expression profiles of other bacterial and fungal infections have also been studied by microarrays (17, 37, 44, 45, 67, 85, 121, 124, 162, 181, 218). A concordance of the gene expression data between intracellular *Shigella* and *Salmonella* has been noted, although they colonize different niches inside the cell (124). So far, most studies of microbial gene expression profiling have been limited in research. Techniques used in these studies need to be validated to ensure that sufficient amounts of mRNA are extracted such that gene expression data are not compromised. The host-pathogen interactions that define a disease are clearly complex, and other genotypic and phenotypic data need to be integrated to clarify the intricate cross talk from host to pathogen and the environmental cues that lead to the expression of bacterial virulence factors in vivo. Nevertheless, microbial gene expression profiles reveal a complete picture of the metabolic state of bacteria under a particular condition, thereby providing a potential tool for the diagnosis and monitoring of microbial infection and disease.

**Host Gene Expression Profiling during Microbial Infections**

Pathogen-induced phenotypic changes in a host are often accompanied by marked changes in host gene expression. Genome-wide expression profiling of the hosts, in addition to the pathogens, has become increasingly important for studying host-pathogen interactions (88). The advent of microarray technology has greatly expanded our ability to monitor changes in host gene expression. The cellular transcriptional response to human cytomegalovirus was globally monitored with an oligonucleotide array in 1998 (223). Subsequently, oligonucleotide microarrays have been generated to measure host gene expression profiles in response to *E. coli, Candida albicans, L. monocytogenes*, influenza virus, and respiratory syncytial virus infections (34, 58, 82). A microarray was generated to incorporate a serial of host response genes including those involved in inflammation and chemotaxis as well as those involved in the synthesis of prostaglandins, Toll-like receptors, and T-cell regulation (97). Such a microarray system has been used to determine immune responses in normal human monocytes after fungal pathogen infections and antifungal drug inoculation (35, 97, 174). Microarrays have been implemented to generate gene expression profiles for viral hepatitis infections, which provide enormous diagnostic and therapeutic potential (75, 76, 147, 171, 211).

Several studies that used microarray-based techniques to detect and characterize host gene expression profiles for sepsis have been reported. Microarray technology was first used to analyze tissue-specific changes in gene expression induced by sepsis in animal models (33). Subsequently, numerous studies that used host gene expression profiling toward sepsis diagnostics, pathogen type differentiation, and clinical outcome prediction have been described (Table 3) (55). The rapid determination of a host sepsis transcriptome provides an early differential diagnosis and clinical outcome prediction. Current microarray-based techniques using host gene expression profiles are limited due to the background variation among and within the individuals studied and poor quality control built into the microarrays. Therefore, the gene expression differences notified by the microarrays have to be verified by gene-specific quantitative real-time PCR assays (95).

**Host Genomic Polymorphism Determination**

When infections, especially chronic infections, are viewed as horizontally acquired genetic diseases, it makes sense to view the pathogen and host as an integrated system. Host genetic polymorphisms that influence the host immune response to infectious agents, thereby determining susceptibility to certain diseases and pathological conditions, which has been well explored in sepsis, have been described (118). SNP analysis is a powerful tool for the mapping and diagnosing disease-related alleles. While sequencing remains the “gold standard” to determine host genetic variabilities, microarray-based techniques may become a simple, rapid, automatic, and user-friendly format for screening and detecting a large panel of related SNPs simultaneously. An Affymetrix HuSNP assay was used to study the role of human
genomic SNPs in the pathogenesis of human parvovirus B19 infection, and relevant SNPs revealed by the microarray study were further confirmed by allele-specific real-time PCR assays (95). A microarray was developed for the simultaneous genotyping of four host SNPs associated with the therapeutic effect of interferon in hepatitis C virus patients (186). These preliminary data suggest that a genetic predisposition is associated with the pathogenesis and development of microbial infections.

**CONCLUDING REMARKS**

Microarrays have the unprecedented potential to simultaneously detect and identify thousands of microbial genes, which provides another evolutionary technical advance in the field of clinical microbiology. Although, historically, microarrays have been used largely for gene expression studies, microarrays have gradually been applied in the detection and classification of microbial pathogens. The development of microarrays has revolutionized the field of clinical microbiology by providing a powerful new tool for rapid and sensitive detection of pathogens and the simultaneous analysis of thousands of genes.

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### TABLE 3. Selected sepsis studies using microarray-based host gene expression

<table>
<thead>
<tr>
<th>Chip used</th>
<th>Subject(s)</th>
<th>Main findings and conclusions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlas array, Clontech Laboratories (Mountain View, CA)</td>
<td>Mouse</td>
<td>Microarray technology provides a powerful new tool for rapidly analyzing tissue-specific changes in gene expression induced by sepsis</td>
<td>33</td>
</tr>
<tr>
<td>Hu95aVer2 GeneChip, Affymetrix (Santa Clara, CA)</td>
<td>Adult patients</td>
<td>The host inflammatory responses to gram-negative and gram-positive stimuli share some common response elements but also exhibit distinct patterns of cytokine appearance and leukocyte gene expression</td>
<td>55</td>
</tr>
<tr>
<td>Image consortium libraries, Livermore National Laboratory (Livermore, CA)*</td>
<td>Mouse</td>
<td>Both gram-positive sepsis and gram-negative sepsis share a final common pathway involved in the pathogenesis of sepsis, but certain genes are differentially expressed under distinct regulation</td>
<td>221</td>
</tr>
<tr>
<td>Arraytor human 500-1 cDNA, SIRS-Laboratory (Jena, Germany)</td>
<td>Adult patients</td>
<td>Microarrays can identify typical gene expression profiles for blood samples from patients with severe sepsis</td>
<td>156</td>
</tr>
<tr>
<td>Hu 133A and 133B GeneChip, Affymetrix</td>
<td>Healthy adult blood leukocytes receiving bacterial endotoxin stimulus</td>
<td>Human blood leukocyte response to acute systemic inflammation includes the transient dysregulation of leukocyte bioenergetics and modulation of translational machinery; these findings provide insight into the regulation of global leukocyte activities as they relate to innate immune system tolerance and increased susceptibility to infection in humans</td>
<td>22</td>
</tr>
<tr>
<td>MGU74Av2 GeneChip, Affymetrix</td>
<td>Mouse</td>
<td>A(2A)R blockade may be useful for treatment of infection and sepsis</td>
<td>142</td>
</tr>
<tr>
<td>HG-U133A GeneChip, Affymetrix</td>
<td>Adult patients</td>
<td>Blood transcriptional profiling is a valuable approach not only for patient stratification but also to identity new genes possibly involved in sepsis pathophysiology</td>
<td>149</td>
</tr>
<tr>
<td>Mouse 430 2.0 GeneChip, Affymetrix</td>
<td>Mouse</td>
<td>T-cell receptor signaling and mitogen-activated protein kinase signaling were significantly altered by sepsis</td>
<td>129</td>
</tr>
<tr>
<td>U74Av2 GeneChip, Affymetrix</td>
<td>Mouse</td>
<td>Sepsis induces common inflammatory response gene changes in mouse leukocyte gene expression that can be used to diagnose sepsis</td>
<td>31</td>
</tr>
<tr>
<td>Adelaide Microarray, Compugen, San Jose, CA</td>
<td>Adult patients</td>
<td>The signature genes reflect suppression of neutrophils’ immune and inflammatory function by sepsis; gene expression profiling therefore provides a novel approach to advance our understanding of the host response to sepsis</td>
<td>188</td>
</tr>
<tr>
<td>430A GeneChip, Affymetrix</td>
<td>Mouse</td>
<td>Sepsis induces alterations in balance of pro- and antiapoptotic transcriptional networks, and bcl-2 overexpression improves survival in sepsis</td>
<td>205</td>
</tr>
<tr>
<td>U133 Plus 2.0 GeneChip, Affymetrix</td>
<td>Pediatric patients</td>
<td>Genome-level alterations of zinc homeostasis may be prevalent in clinical pediatric septic shock</td>
<td>216</td>
</tr>
<tr>
<td>U133 Plus 2.0 GeneChip, Affymetrix</td>
<td>Adult patients</td>
<td>Sepsis has a unique gene expression profile that is different from that for uninfected inflammation and becomes apparent prior to expression of the clinical sepsis phenotype</td>
<td>89</td>
</tr>
<tr>
<td>U133 Plus 2.0 GeneChip, Affymetrix</td>
<td>Adult patients</td>
<td>Toll-like receptors and downstream signaling genes are differentially expressed in critically ill patients developing sepsis compared with those with sterile inflammation; these expression differences occur before phenotype-based diagnosis of clinical sepsis</td>
<td>120</td>
</tr>
<tr>
<td>U133 Plus 2.0 GeneChip, Affymetrix</td>
<td>Adult patients</td>
<td>There was evidence of sepsis-related immunosuppression and reduced inflammatory response in mononuclear cells on a transcriptome level; these characteristic transcriptional changes can be used to aid the diagnosis of sepsis</td>
<td>187</td>
</tr>
</tbody>
</table>

* Distributed by ResGen Invitrogen (Huntsville, AL).
characterization of microbial pathogens, determination of antimicrobial resistance, typing of microbial pathogens, and monitoring of microbial infections by investigating host genomic expression and polymorphism profiles. Even with these major advances, the potential power behind microarray applications in clinical microbiology has yet to be fully realized. The ability to detect multiple pathogens and/or monitor the variability of normal microbial populations in a disease process could transform our current understanding of infectious diseases. In addition, massively parallel sequencing performed by microarray analysis offers the opportunity of sequencing directly from complex clinical specimens. This metagenomics approach will allow a comprehensive analysis of every nucleic acid in the specimen. For these robust applications, high-density microarray platforms must be able to transition from translational research laboratories to the clinical laboratory. It is unlikely that traditional, planar microarrays will soon appear in clinical microbiology laboratories due to their high cost, relative lack of flexibility, and limited throughput. The ideal microarray platform for the diagnostic laboratory is a low- to medium-density array that offers limited, reliable, and straightforward results without the need for sophisticated equipment and data management (133). Indeed, platforms that have begun to meet these criteria have been developed, such as electronic microarrays and suspension bead arrays.

With the potential power of microarray analysis comes abundant challenges, particularly in relation to the diagnostic laboratory. Several critical issues need to be resolved before microarray-based techniques can be widely implemented in clinical microbiology services. Due to the potential variability in multiple steps included in the microarray analysis, it is difficult to compare quantitative data between, and even within, microarray experiments. Substantial obstacles still exist along the entire spectrum of preanalytical-to-postanalytical analysis. Heterogeneous clinical specimens present unique challenges with respect to sensitivity, specificity, quantification, and data analysis of microarrays that are not encountered during the analysis of pure cultures. In addition, optimization of extraction, labeling, and hybridization; incorporation of appropriate quality controls, design, and implementation of clinical validation studies; and management and interpretation of data remain challenges in a clinical setting. Moreover, laboratories must account for microarray reproducibility in production and analysis, cost of implementation, acquisition of appropriately skilled laboratorians, as well as intellectual property and reimbursement issues. Compared to real-time PCR, microarray analysis requires additional manipulations including hybridization and washing, which increase the contamination risk and the amount of hands-on time needed, both steps backwards in diagnostic molecular microbiology.

Although improvements are still needed to make the majority of microarray applications amenable to clinical microbiology laboratories, the future role of these robust technologies in diagnostic microbiology is indisputable. Microarray-based analyses will revolutionize infectious disease diagnostics through the detection and identification of previously unknown or unsuspected pathogens, by transforming our current view of multiplexed laboratory testing, and by expanding pathogen detection to include bacterial population-based analyses and host-specific responses (135). As more pathogen genomes and targeted genes are sequenced, costs associated with microarray production decrease, and FDA-cleared products become available, diagnostic applications of microarray-based analyses will continue to expand. As PCR has done in the last 25 years, and more recently real-time PCR, microarray technology will undoubtedly transform the diagnostic capabilities of clinical laboratories, ushering us into a new molecular revolution.

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