

Recent Application of DNA Microarray Techniques to Diagnose Infectious Disease

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Abstract: Infectious diseases are considered a major cause of death globally, accounting for one-third of all deaths. Traditionally, microbiological approaches to diagnose infectious diseases have been labor-intensive and time-consuming; molecular methods significantly improve on these constraints. DNA microarray is a novel and advanced diagnostic test for infectious disease that is designed for specific identification of a wide variety of organisms simultaneously. Microarray has various advanced applications in infectious disease diagnostics, including the 2019 pandemic COVID 19, detection of food-borne infectious agents, and detection of *Neisseria meningitidis*. The aim of this review is to provide updated application of DNA microarray techniques for the diagnosis of infectious diseases and to explore the development of new DNA microarray methods. In conclusion, DNA microarray techniques are the future rapid and accurate diagnostic tool for infectious disease diagnosis and detection of antimicrobial resistance.

Keywords: DNA microarray, infectious disease diagnostics, molecular techniques

Introduction

Infectious diseases are illnesses caused by pathogenic microbes such as bacteria, viruses, parasites, or fungus, which can be transmitted directly or indirectly from one person to another.¹ Throughout human history, infectious illnesses have been a major cause of morbidity and mortality. One-third of all death worldwide is caused by an infectious illness, with respiratory infections, diarrheal infections, HIV/AIDS, and malaria being the most prevalent causes of infectious disease mortality.²

The most successful way to preventing or controlling microbial infections or illnesses is early detection. To make a good diagnosis and, as a result, to accomplish an effective therapy, the infecting pathogens, such as bacteria, fungi, viruses, and parasites, must be identified and characterized accurately and quickly.³ Microbiological techniques, like microorganism isolation, have traditionally been considered as the best method for identifying infections. However, culture is laborious and time-consuming, and the presence of non-cultivable bacteria makes speedy diagnosis impossible.^{4,5} Molecular technologies that have significantly enhanced microbial diagnostics are now routinely utilized in clinical laboratories and are the primary approaches for the diagnosis of infectious illnesses.⁴

Polymerase Chain Reaction (PCR), Deoxyribonucleic Acid (DNA) microarrays, Reverse Transcriptase PCR (RT-PCR), and others are among the common molecular methods used to diagnose infectious diseases.^{3,6} Molecular methods are used to identify pathogens by selecting housekeeping genes that are universally conserved or by screening random regions of the organism's genome.³

Particularly for the identification and characterization of isolates as well as the diagnosis of diseases caused by virulent, slow-growing, highly contagious agents, non-viable, or uncultivable organisms that cannot be identified by traditional culture techniques, molecular techniques have been increasingly incorporated in laboratories.^{3,6} In the clinical diagnostic laboratory, molecular diagnostics has gained prominence and demonstrated benefits for the routine identification and epidemiological analysis of microorganisms.⁷ When compared to culture, molecular approaches are highly sensitive, offer the benefits of a quick turnaround time and are highly specific. However, they are costly and some methods may miss discovering new species.^{3,6}

DNA microarray is one of the most recent and advanced molecular diagnostic test for the comprehensive analysis of etiologic agent of disease.^{8,9} Microarray diagnosis works through the principle of hybridization of nucleotides from patients sample to oligonucleotide probes.^{10,11} Microarrays are now used for pathogen genotyping and identification, diagnosis of cancer, detection of antibiotic resistance, detection of gene mutations and diagnosis of the recent pandemic COVID-19.^{10,12} Microarrays following used to detect and identify different microorganisms by targeting the Intergenic transcribed spacers (ITSs), 16S bacterial and 28S fungal ribosomal subunits and others.¹³

The advantage of microarray techniques is it have a high sensitivity and specificity and able to detect polymicrobial pathogens from a single specimen simultaneously but it can only be used to identify detect pathogens which are previously sequenced and have designed probes, those novel species without previous known genome will not be detected.^{14,15} Now a day many DNA microarray techniques are used for the diagnosis of infectious disease. The aim of this review is to provide updated and comprehensive information on the latest DNA microarray techniques and to explore the development of new DNA microarray methods.

DNA Microarray

Microarray technology is one of the most advanced molecular techniques that enables comprehensive genetic analysis of an organism or sample for the diagnosis of various bacterial, viral, fungal, and parasitic diseases that have already been standardized at the genus and species levels.^{9,10} The detection threshold for pathogenic DNA in Gene Chip is as low as 10 femto grams, much below the thresholds of currently available technologies.⁹ As compared to whole-genome sequencing DNA, microarray detects multiple pathogens simultaneously which is better to diagnose infections caused by polymicrobial pathogens.¹⁶

Microarrays can be spotted arrays or in situ-synthesized arrays. Spotted microarray is performed after printing the probes onto the microarray surface, where as in situ-synthesized arrays works through the probes synthesized directly on the microarray surface.¹⁷

Principle of DNA Microarrays

Nucleic acids are extracted from the clinical sample, amplified by PCR using primers for possible pathogens which able to cause the infection, and then labeled with a fluorescent dye and labeled nucleic acids are hybridized on a DNA microarray, finally scanned to detect the sample's particular probe/DNA interactions by fluorescence.⁵ Direct fluorescence method or enzymatic detection can be used to identify the hybridization, producing a semi-quantitative result.¹⁸

Recent Application of DNA Microarray in Infectious Disease Diagnosis

Recently, there have been new methods for detection, identification of pathogens and determine antimicrobial resistances including the detection of the 2019 pandemic COVID-19 (Table 1).⁸

Pathogen Detection and Species Identification

To identify microorganisms, DNA content, gene-expression patterns, and host gene products, such as the expression of genes encoding for cytokines, apoptosis-causing agents, growth factors, and other stress-related and signaling responses can be analyzed.^{25,26} Because of the excellent specificity of the binding of probes to pathogen gene sequences, it is possible to detect a wide range of organisms with high discriminatory power.²⁵ The most often used gene targets in microarrays are 16S bacterial ribosomal subunits, 28S fungal ribosomal subunits, and ITSs.¹³

In a number of comparative bacteria genome hybridization investigations, microarrays were utilized to identify and discriminate distinct strains of *M. tuberculosis*, as well as detection of *Neisseria meningitidis*, Enteropathogenic bacteria such as *E. coli*, *Vibrio*, *Salmonella*, *Campylobacter*, *Shigella*, *Yersinia*, and *Listeria* species.^{13,27}

A colorimetric vertical-flow DNA microarray for the detection of *Neisseria meningitidis* was recently developed. This method takes advantage of isothermal amplification with recombinase polymerase amplification as well as the screening abilities of DNA microarrays in a paper format.¹⁹ Ma et al also developed a DNA microarray assay for the simultaneous identification of fifteen bacterial species from respiratory tract in pneumonia patients.²⁰ The target for this assay was 16S rRNA genes and other specific genes of each pathogen and have detection limit was 10³ copies/μL.²⁰

Table I Recent Applications of DNA Microarray in Microbial Pathogens

Type of DNA Microarray	Pathogen		Target Gene	References
Paper DNA VFM with RPA	<i>N. meningitidis</i>		<i>ctrA</i> gene	[19]
Spotted microarray	Polymicrobial that cause pneumonia		16s rRNA and specific genes for each pathogen	[20]
Magnetic nanoparticles enhanced DNA microarray	Food borne Pathogens	<i>E. coli</i> <i>S. enterica</i> <i>C. jejuni</i> <i>V. cholerae</i>	<i>rfbE</i> <i>envA</i> <i>ceuE</i> <i>ompW</i>	[21]
In situ synthesized chip	Food borne Pathogens	<i>S. typhimurium</i> <i>S. aureus</i> <i>E. coli O157:H7</i> <i>L. monocytogenes</i> <i>V. parahemolyticus</i>	<i>invA</i> <i>sasH</i> <i>wzy</i> <i>hlyA</i> <i>comEC/ rec2</i>	[22]
Covidarray	SARS CoV-2		Nucleocapsid(N1&N2)	[23]
SMAvirusChip	Arboviruses		Structural and non-structural proteins	[24]

Microarrays are now being used to identify food-borne diseases.¹⁴ A recently developed magnetic nanoparticles augmented oligonucleotide microarray approach has significant potential for the identification of food-borne pathogens such as *S. enterica*, *V. cholerae*, *C. jejuni*, *E. coli*, and others.^{21,28} This technique uses PCR to amplify double-stranded DNA that has been biotin and Cy3 labeled, using streptavidin-modified magnetic nanoparticles (SA-MNPs) to collect the biotinylated PCR products. The PCR strand labeled with Cy3 can be collected and concentrated for down-stream hybridization following denaturation and magnetic separation.²¹ The hybridization signal was increased by up to 15 times using this magnetic nanoparticles-based technology as compared to the traditional single-stranded target preparation techniques.²¹ Hu et al developed an in situ-synthesized gene chip to detect foodborne pathogens such as Salmonella Typhimurium, *V. parahemolyticus*, *S. aureus*, *L. monocytogenes*, and *E. coli* on fresh fruits and vegetables.²²

The first application of microarray in diagnostic virology was to identify HIV mutations associated with antiretroviral treatment resistance. Since then, microarrays have been designed to identify a wide range of viruses, including those that cause central nervous system infection, hepatitis C virus, and respiratory viruses.²⁹ Microarray approaches in virology can be grouped into four viral infection groups: respiratory diseases (Influenza and Coronaviruses), hemorrhagic fever (*Arenaviridae*, *Bunyaviridae*, *Flaviviridae*, and *Filoviridae*), neurotropic infection (Herpes simplex Virus and Epstein Barr Virus), and HIV and associated viruses.²⁷

Together with P16 immunohistochemistry, DNA microarray is the approved Human Papilloma Virus (HPV) test.³⁰ P16 immunohistochemistry is a widely used and approved diagnostic method for HPV-driven head and neck cancer by the American Joint Committee on Cancer (AJCC). This test is extremely sensitive but only moderately specific when used as a single test to diagnose HPV-positive oropharyngeal cancer.³¹ The DNA microarray approach is accurate enough to identify all known HPV subtypes at the same time. Kwon et al analyzed the eighth edition AJCC and recommended integrating at least two HPV testing methodologies, such as the HPV DNA microarray methodology.³⁰

The causal agent of COVID-19 (SARS-CoV 2) is also being identified using microarray by taking into account SARS-CoV rapid mutation, to find 24 Single Nucleotide Polymorphism (SNP) mutations among SARS-CoV spike (S) gene.³² Microarray methods identify coronaviruses by producing cDNA from viral RNA via reverse transcription and then labeling it with particular probes.¹² Damin et al have developed CovidArray, a microarray-based oligonucleotide microarray-based test to detect SARS CoV-2 in nasopharyngeal swabs targeting two areas (N1 and N2) of nucleocapsid. The process is based on fluorescently labelled amplicons being solid-phase hybridized.²³

A DNA microarray for the detection of Enteroviruses was developed using 1256 70-mer microarray probes from conserved regions of more than 100 viral species associated with the gastrointestinal tract, with at least 6 probes designed

for each viral species and detection limits of 10^3 virus particles of Human adenovirus C, Human astrovirus, and group A Rotavirus.³³ Khan et al created a DNA microarray method (SMAVirusChip) that enables for the screening of over 400 arboviruses difficult to identify with immunoassays due to cross reactivity such as Chikungunya, Dengue, and Zika with only one biological sample.²⁴

The FDA has approved many diagnostic tests for detecting fungal pathogens such as supramagnetic nanoparticles coated with designated candida capture molecules that are used in a suspension array-based diagnostic test that detects the five most relevant *Candida* spp.³⁴ DNA microarrays are used to identify pathogenic yeasts and moulds by targeting the Internal transcribed spacer regions of fungal rRNA genes.²⁷ A DNA microarray was designed to detect 14 fungal pathogens including *Aspergillus*, *Candida*, *Fusarium* and *zygomycetes* from samples from high-risk patients.³⁵ This technique combines multiplex PCR with successive DNA microarray hybridization by targeting sequences from the fungal rRNA genes' 18S, 5.8S, and ITS.³⁵

Microbial Typing

Microarrays have been widely used as SNP genotyping platforms.¹⁵ Genomic hybridization of a whole genome array enables genome-wide comparison of their genetic contents by identifying the presence or absence of homologous DNA sequences in microorganisms. Differences in the genomes of *Mycobacterium* species, *S. pneumoniae*, *H. pylori* and *C. trachomatis* have been studied using DNA microarrays.^{13,36} Through the use of DNA microarray-based analysis, *Staphylococcus aureus* can be genotyped, and gene profiling is available to investigate their virulence potential and resistance mechanisms.⁵

Determination of Virulence Factors and Pathogenicity

Microarray technology enables a global analysis of the genetic determinants that contribute to pathogenicity by investigating molecular mechanisms of host invasion, immune evasion, and survival strategies, as well as determining the genes expressing bacterial toxins, adhesins, and other putative virulence factors promoting colonization or tissue damage.²⁵ Comparative genomics might aid in discovering putative virulence factors by investigating genome-wide gene expression patterns under relevant circumstances, such as physiological changes during interaction with the host, for example the whole-genome microarray of *H. pylori* has shown to be an effective technique for finding differences in the gene content of two strains of the parasite that generate distinct pathogenic consequences.¹³

Antibiotic Resistance

Traditional drug resistance testing on *M. tuberculosis* clinical samples takes a long time, causing therapy to be delayed. The microarray can determine *M. tuberculosis* antimicrobial resistance pattern by characterizing mutations in the *rpoB* and *katG* genes that give rifampin and isoniazid resistance.²⁷ Microarray technology may also detect Extended spectrum beta lactamase and carbapenemase-producing Enterobacteriaceae,^{37,38} and identification of resistance in *P. falciparum* due to SNPs.²⁷ For the detection of resistance genes in both gram-negative and gram-positive bacteria for different antibiotics AMR Direct Flow Chip Kit DNA microarray is a better and rapid assay.³⁹

Conclusion

As infectious diseases are the major cause of illness and death globally accurate and fast diagnostic techniques are required for effective management of it. DNA microarray techniques are the future promising tools for the simultaneous detection of polymicrobial pathogens responsible for infectious disease. In addition to the diagnosis of infectious disease DNA microarray techniques can simultaneously detect antimicrobial resistance among microorganisms. Even if this paper is concerned only on DNA microarray, there are different microarray techniques such as protein, peptide, glycan, antibody, and aptamer microarrays which can be used for screening vaccine candidate, and study posttranslational modifications.

Abbreviations

AIDS, Acquired Immunodeficiency Syndrome; AJCC, American Joint Committee on Cancer; DNA, Deoxyribonucleic Acid; HIV, Human Immunodeficiency Virus; HPV, Human Papilloma Virus; ITS, Intergenic Transcribed Spacers; PCR,

Polymerase Chain Reaction; RNA, Ribonucleic Acid; SARS, Severe Acute Respiratory Syndrome; SNP, Single Nucleotide Polymorphism.

Ethical Consideration

Ethical consideration is not applicable for this review.

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