DNA microarrays for pathogen detection and characterisation

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DNA microarrays have three main potential diagnostic uses in clinical microbiology: detection of known pathogens, pathogen typing and novel pathogen discovery. Although DNA microarray platforms offer the ability to screen for a large number of agents in parallel, sensitivity is dependent on the ability to obtain adequate amounts of pathogen nucleic acids from collected samples. In general, high levels of sensitivity require a PCR amplification step using specific primer sets, subsequently reducing the overall scope of the microarray assay. At present, relatively high costs, restricted sample throughput capabilities and validation difficulties are also major factors limiting the implementation of DNA microarray assays in diagnostic microbiology laboratories.

DNA microarrays consist of a series of DNA probes immobilised in an orderly fashion on a solid substrate such as a glass slide or a silicon chip. The probes may be DNA molecules up to several kilobases in length (from a cDNA library or synthesised by PCR) or relatively short 20-80 nt synthetic oligonucleotides. Oligonucleotide microarrays are becoming increasingly popular, mainly due to their enhanced capacity for achieving high levels of quality control. The oligonucleotides may be synthesised using conventional methods and then mechanically deposited onto the solid substrate, or they may be synthesised directly onto the substrate's surface using a range of different methods such as photolithography (e.g. Affymetrix GeneChip® Arrays), ink jet technology (e.g. Agilent SurePrint technology) or chemical synthesis onto microelectrodes (e.g. CombiMatrix Corporation's CustomArray™).

In contrast to the convention of hybridisation assays such as Southern blots, microarray terminology refers to the substrate-bound DNA molecules as probes whilst DNA (or RNA) within the sample applied to the array are referred to as the 'target' nucleic acids. Target nucleic acids are most commonly labelled with fluorescent dyes. After hybridisation on an array, unbound target is removed by a series of washing steps, after which probe-bound, labelled target nucleic acids are detected by fluorescence scanning (Figure 1).

While DNA microarrays offer the ability to represent a large range of pathogens on a single slide or chip, it must be remembered that within a diagnostic assay they represent only the final detection step. For the detection of known pathogens, although it is feasible that all known viral, bacterial, mycotic and protozoal pathogens could be represented on a single microarray, it is unlikely that genomic material from such a broad range of agents could be efficiently purified and amplified simultaneously from tissue samples. A syndrome based approach to the design of pathogen detection microarray assays is probably more realistic, making use of available clinical information to determine the most suitable strategy for target nucleic acid preparation from the sample collected.

Several syndrome based assays have been developed to date, including for the detection of viruses in cases of central nervous system infection [1, 2], for the detection of bacteria in upper respiratory tract infections [3], for the detection of enteric bacteria [4] and for the detection of potential bioterrorism agents [5]. Target nucleic amplification strategies used by these assays, including multiple species-specific PCR primer sets, multiplex PCR and universal PCR primer sets, enabled good sensitivities of detection to be achieved.

To date, Wang and colleagues [6] have described the most impressive use of random PCR amplification methods for pathogen detection on DNA microarrays. In addition to detecting a range of respiratory viruses from infected cell cultures, their approach also successfully detected rhinoviruses and parainfluenza 1 in human nasal lavage samples.

A large range of bacterial and viral typing microarray assays have been developed over the last 5 years. CombiMatrix has recently released a DNA microarray chip capable of typing Influenza A strains with haemagglutinin subtypes 1-15 and neuraminidase subtypes 1-9, using a protocol that can reportedly obtain a result in less than 4 hours (http://www.combimatrix.com/products_influenza.htm). Target viral nucleic acid is amplified using a multiplex PCR primer set designed to be pan-influenza A specific and, upon hybridisation on the chip, a further sequence of enzymatic reactions are undertaken which enable hybridisation time to be minimised. While these chips can be read by conventional fluorescence detectors, CombiMatrix has also developed a relatively small and inexpensive electrochemical detection device, with the ultimate aim of enabling the assay to be field deployable.

DNA microarray-based approaches offer exciting opportunities for rapid identification and characterisation of novel pathogens. High density microarrays incorporating many thousands of probes, combined with random nucleic acid amplification methods for the preparation of target DNA, have the potential to detect an immense range of pathogens on a single slide or chip. New pathogens can be detected as a result of hybridisation to probe sequences derived from conserved gene regions of related pathogens.

However, as discussed previously, in most cases random amplification methods are unlikely to adequately amplify novel pathogens directly from tissue samples. Therefore, successful identification most likely entails prior amplification of the unknown agent to adequate levels in a laboratory culture system.

A comprehensive microarray representing all fully sequenced viruses was one of the approaches used to identify the SARS virus as a new coronavirus [7]. Viral RNA extracted from infected Vero cell culture was amplified by a random PCR approach prior to hybridisation on the array, which subsequently revealed the presence of a coronavirus. Furthermore, physical recovery of the viral sequences hybridised to individual array probes enabled approximately 1kb of the virus genome to be characterised [8].

Suspension arrays, in which DNA probes are fixed to individually identifiable microsphere beads, offer an alternative to planar DNA microarrays. In addition to three-dimensional reaction kinetics enabling reduced hybridisation times, this format has the potential for higher sample throughput and reduced costs. To date, the Luminex LabMAP™ system, which has the ability to multiplex up to 100 different reactions in a single well, has been the most widely utilised bead-based DNA array system. Assays based on this system have been successfully developed for both the identification and genotyping of bacterial, mycotic and viral human pathogens [9-11].

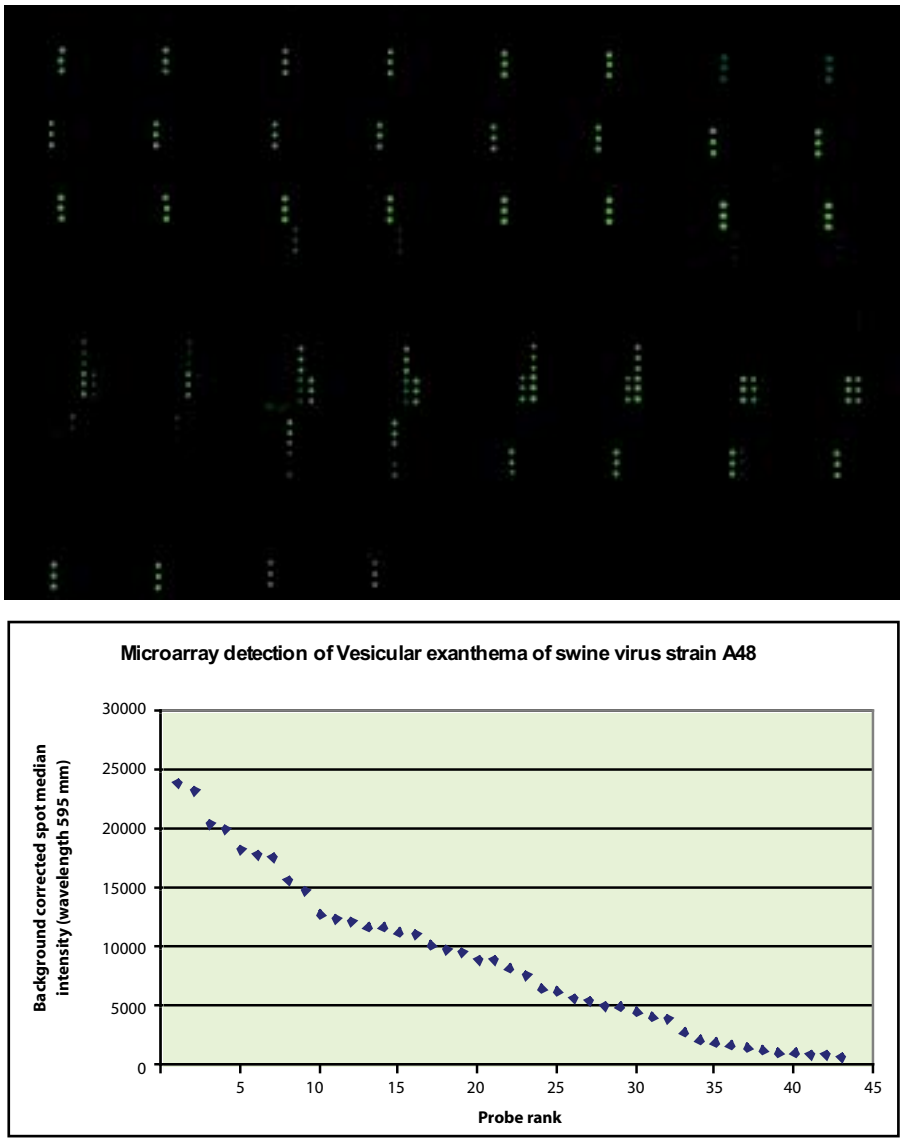

Figure 1. DNA microarray detection of Vesicular exanthema of swine virus strain A48 (VESVA48). Below: Rank order of positive probes vs background corrected spot median intensity. Top: Fluorescence scan of the hybridization array. A total of approximately 500 60-mer oligonucleotide probes, representing viruses causing vesicular or vesicular-like lesions in livestock animals, were contact printed onto Corning UltraGAPS™ glass slides using a BioRobotics MicroGrid II system. Each probe was printed in triplicate at two separate locations within the array. RNA purified from VESVA48-infected cell culture supernatant was reverse transcribed and amplified by random PCR prior to hybridising on the array. DNA bound to specific viral array probes was subsequently hybridised with UltraAmp™ Oyster ª-550 labelled 3DNA ª Dendrimer Signal Amplification Reagent (Genisphere Inc. USA). After washing, fluorescent signals were detected by an arrayWoRx[e] scanner (Applied Precision, USA) at a wavelength of 595 nm, represented here in green. Positive probes were all specific to members of the VESV species.

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