Review

A role for arrays in clinical virology: fact or fiction?

Jonathan P. Clewley*

Sexually Transmitted and Blood Borne Virus Laboratory, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT, UK

Abstract

Microarrays of DNA probes have at least three roles in clinical virology. These are: firstly, in diagnosis, to recognise the causative agent of an illness; secondly, for molecular typing for (i) patient management, (ii) epidemiological reasons (e.g. investigating routes of transmission), (iii) purposes related to vaccine use; and thirdly, in research, to investigate the interactions between the virus and the host cell. Microarrays intended for syndromic diagnostic purposes require genome specific probes to capture the unknown target viral sequences and thereby reveal the presence of that virus in a test sample. Microarrays intended for typing and patient management, e.g. monitoring antiviral drug resistant mutations require a set of probes representing the important sequence variants of one or more viral genes. Microarrays intended for research into virus–host interactions require probes representative of each individual gene or mRNA of either the virus or the host genome. Diagnostic microarrays are dependent for their utility and versatility on generic, multiplex or random polymerase chain reactions that will amplify any of several (unknown) viral target sequences from a patient sample. In this review, the existing and potential applications of microarrays in virology, and the problems that need to be overcome for future success, are discussed.
© 2003 Elsevier B.V. All rights reserved.

Keywords: Virology; DNA probes; Microarrays; DNA chips

1. Introduction and background

DNA arrays consist of oligonucleotide or PCR amplicon probes immobilised on a solid surface, usually glass or a nitrocellulose or nylon membrane. Membrane based arrays may be in the format of line probe blots. The results of hybridisation between the bound probe and labelled sequences in the sample applied and tested are revealed by scanning or imaging the array surface. High density arrays, which may have thousands of individual probes per cm^2, are referred to as microarrays. Arrays can be prepared in-house using one of the several arraying instruments available commercially. These will spot synthetic oligonucleotides or PCR amplicons onto glass slides at low or high density. Microarrays of oligonucleotides can also be synthesised on a silicon surface by photolithographic combinatorial chemistry methods similar to those used to make electronic chips. These microarrays on silicon surfaces are therefore known as 'DNA chips'. An example is the Affymetrix human immunodeficiency virus type 1 (HIV-1) antiretroviral drug mutation detection GeneChip which comprises a set of matched and mismatched probes designed to hybridise with protease and reverse transcriptase (RT) gene sequences.

Detailed technical information about arrays can be found in books (Schena, 1999) and Nature Genetics Reviews (1999, 2002), and the uses of arrays have been reviewed (Cummings and Relman, 2000; Kato-Maeda et al., 2001; Manger and Relman, 2000) see also Table 1. The usual nomenclature for arrays is that the element bound to the solid phase is the 'probe', while the 'target' is in solution. This is in contrast to Southern or dot blotting where the 'target' is bound and the 'probe' is in solution. In both cases, though, the target is DNA or RNA sequences derived from the test specimen. The process of making and using an array is illustrated in Fig. 1, and a check list of requirements for an arraying project is given in Table 2.

Reviewing the potential benefits that microarrays or DNA chips may be able to offer to the clinical virologist requires a certain amount of crystal ball gazing, and runs the risk of being overtaken by unforeseen developments. Nevertheless, it is possible to paint a broad picture of how microarrays might fit into the routine of the diagnostic laboratory in the near future:

- First, nucleic acid would be extracted from pathological specimens by means of an automated instrument.

* Tel.: +44-20-8200-4400x3245; fax: +44-20-8200-1569.
E-mail address: jonathan.clewley@hpa.org.uk (J.P. Clewley).

Table 1
Potential uses of DNA arrays

The genetic polymorphism of the virus
  (1) Detection of the presence or absence of specific viruses (e.g. influenza virus (Li et al., 2001) rotavirus (Chizhikov et al., 2002))
  (2) Confirmation of the identity of PCR products for:
    Diagnosis (e.g. Rota et al., 2003; Wang et al., 2002)
    Epidemiological surveillance and transmission
    Host range determination
    Monitoring molecular evolution and new strains
    Vaccine coverage surveillance
  (3) Antiviral drug resistance mutation screening (e.g. HIV-1 (Kozal et al., 1996; Wilson et al., 2000))
  (4) Vaccine quality control (e.g. poliovirus, mumps virus (Amexis et al., 2002; Cherkasova et al., 2003; Proudnikov et al., 2000))
  (5) Detection of the presence or absence of specific genes[a] (e.g. those associated with virulence and/or pathogenicity (Chizhikov et al., 2001))

The interaction of the virus and the host cell
  (6) Study of the RNA expression profile of virus following infection, including drug effects in vitro (e.g. HCMV (Chambers et al., 1999),
    KSHV (Jenner et al., 2001))
  (7) Study RNA expression profile of host following infection, including drug effects in vitro (e.g. HCMV (Zhu et al., 1998), PVM
    (Domachowske et al., 2002), HIV-1 (Corbeil et al., 2001; Geiss et al., 2000))
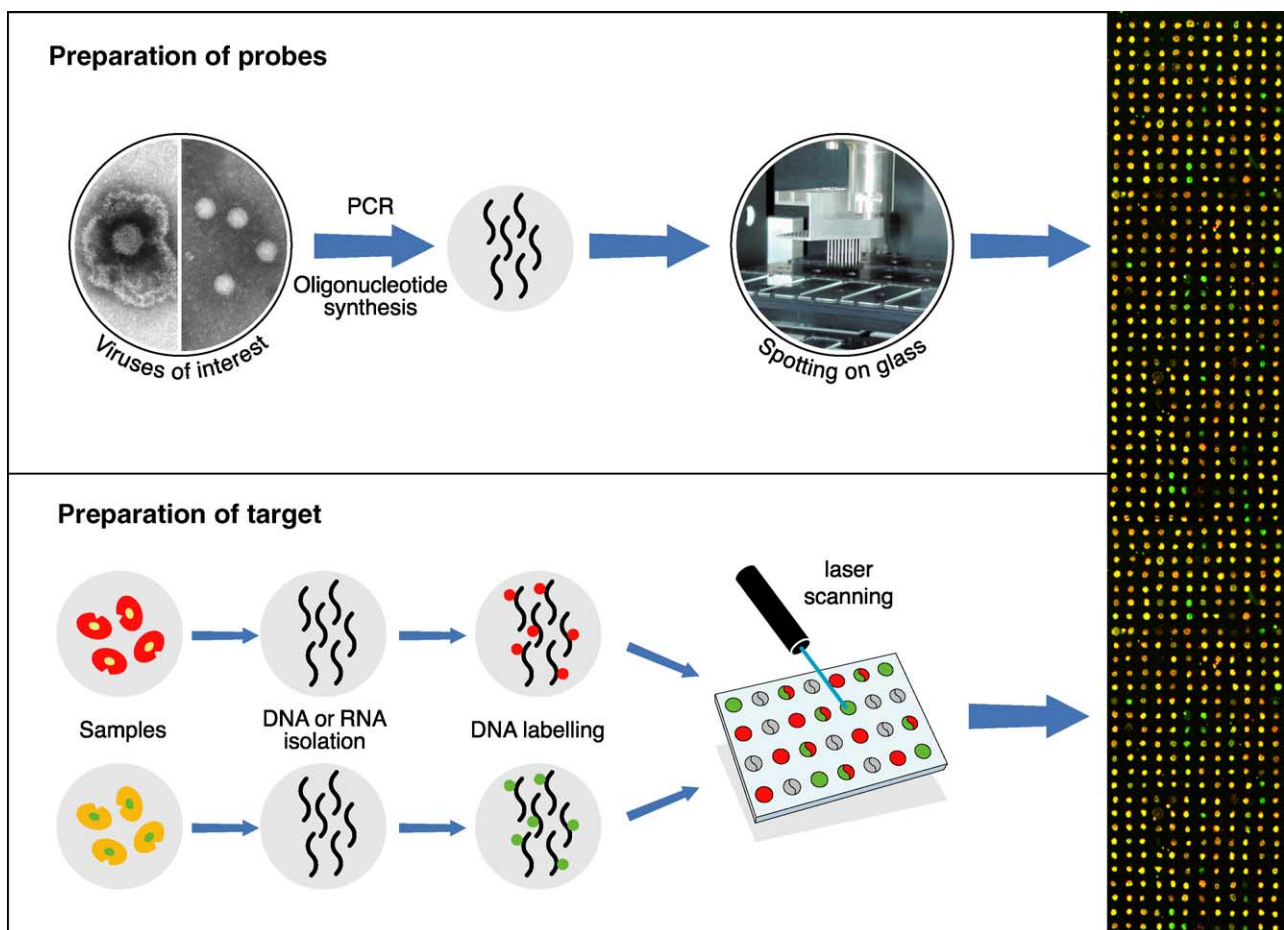
  [a] More useful for bacterial pathogens.

Fig. 1. An illustration of the processes involved in making and using an array. At the top is depicted the route from the virus of interest to the spotted arrays (herpesvirus and enterovirus particles are shown). At the bottom, DNA or RNA is extracted from samples, amplified and labelled with either Cy3-dCTP (green) or Cy5-dCTP (red). When applied to the array bearing the immobilised probes, the target binds to complementary sequences. An example of an array result is shown on the right: the green spots represent hybridisation of the probe only with target sequences labelled with Cy3-dCTP; the red spots represent hybridisation of the probe only with target sequences labelled with Cy5-dCTP; the yellow spots represent hybridisation with both target sequences.

Table 2
Requirements for a viral DNA arraying project

Genomic sequence data from several strains of the target viruses
Sets of PCR primers for making amplicons to be bound as probes to
   the solid phase or sets of synthetic oligonucleotides to be bound
   as probes to the solid phase
An arraying instrument
Generic and multiplex PCR assays for amplifying and fluorescently
   labelling target DNA/RNA
Hybridisation and washing chambers
A scanner to capture readout data from the array
Software to interpret array readout data

- Second, the RNA and/or DNA would be subject to an enzymatic process that selectively amplifies and fluorescently labels sequences from the genome of any virus that is present.
- Third, the amplified nucleic acid would be applied to a DNA chip and hybridize to complementary sequence probe(s) representing known pathogenic viruses.
- Fourth, detection instrumentation and software would interpret the fluorescent hybridisation signals on the chip and provide a readout that identifies the virus present in the original specimen.

How close is this to reality? In that it assumes the existence of a universal 'panviral' DNA chip, it is arguable that it makes little diagnostic or experimental sense. Pathological specimens, and viruses, come in all shapes and sizes and clinical judgement should be exercised to assess the probable causes of whatever condition the patient has, leading to an intelligent guess of which viruses to test for. It makes little sense, for example, to search for respiratory viruses in a gastrointestinal sample, or blood borne viruses in a throat swab. From this it follows that informed decisions need also to be made about which extraction protocol and amplification primers should be used. It seems likely, therefore, that rather than universal extraction and amplification methods and a panviral DNA, specialist chips will be developed for the diagnosis of similar clinical conditions. Proto-

types of such chips are already in use in many laboratories, but there is still a long way to go before they become routine in the way of, for instance, diagnostic ELISA kits. It is also probable that just as automated serological methods are the province of commercial suppliers, diagnostic viral DNA chips and accompanying instrumentation will have to come from that source, because of their cost and the need for consistency and quality control. Research and reference laboratories will, however, be involved in much of the bio-technical development necessary before such commercially supplied chips become available. These laboratories will also need to develop and maintain their own in-house methods for confirmatory purposes, for detecting more obscure viruses, and to monitor the performance of commercial equipment.

2. Molecular diagnostic arrays

The development of arrays that will be useful for diagnostic purposes in the clinical virology laboratory is dependent in part on the stage of development of PCRs for the detection of individual viral genomes. Generally, any DNA array system will need to be used in conjunction with a nucleic acid amplification method and, specifically, PCR methods capable of amplifying a wide range of viral genomes will be required. For example, if an investigator wishes to develop a microarray that would be useful in the diagnosis of viral infections of the CNS, it would be necessary to be able to amplify DNA sequences from a range of herpesviruses, enteroviruses, adenoviruses and retroviruses as well as JC, influenza, measles, mumps, rubella and West Nile viruses. The ability to do this is dependent on generic (Table 3), multiplex (Table 4) and random PCR amplification procedures (Bohlander et al., 1992; Elnifro et al., 2000; Wang et al., 2002).

2.1. Generic and multiple sequence amplification

Generic PCR tests are designed so that only one or two pairs of primers are necessary to amplify a target sequence

Table 3
Examples of generic primer PCR tests

Virus	Target	Reference
Papillomaviruses	L1 region	(Coutlee et al., 2002; Kleter et al., 1998; Nelson et al., 2000)
Enteroviruses	5′-NCR	(Clewley, 1995; Shih et al., 2003; Zoll et al., 1992)
Herpesviruses	DNA polymerase	(Calvario et al., 2002; Ehlers et al., 1999; Minjolle et al., 1999; VanDevanter et al., 1996; Yamamoto and Nakamura, 2000)
Retroviruses	*Pol* region	(Burmeister et al., 2001)
Lentiviruses (HIV/SIV)	*Pol* region	(Clewley et al., 1998; Courgnaud et al., 2001; Gelman et al., 1992; Masciotra et al., 2002; Miura et al., 1990; Yang et al., 2000)
Positive stranded viruses	RNA polymerase	(Chen and Plagemann, 1995)
Influenza viruses	NP gene	(Coiras et al., 2003)
RSV	Fusion protein gene	(Coiras et al., 2003)
Adenoviruses	Hexon protein gene	(Coiras et al., 2003)
Dengue virus/flaviviruses	3′-NCR, NS3 protein gene	(Chow et al., 1993; Houng et al., 2001; Seah et al., 1995)
Arenaviruses	3′-NCR	(Lozano et al., 1997)
Rotaviruses	VP7 segment	(Chizhikov et al., 2002)

Table 4
Examples of multiplex PCR tests[a]

Viruses	References
Respiratory	
Enterovirus, influenza viruses, RSV, parainfluenzaviruses, adenoviruses, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*	(Coiras et al., 2003; Daum et al., 2002; Echevarria et al., 2000; Grondahl et al., 1999; Li et al., 2001; Na et al., 2002; Stockton et al., 1998; Xu et al., 2000)
Neurological	
EBV, VZV, HHV-6, HSV-1, HSV-2, CMV, HHV-7, adenoviruses, enteroviruses, BK, JC, SV40	(Calvario et al., 2002; Casas et al., 1999; Fedele et al., 1999; Markoulatos et al., 2001; Quereda et al., 2000; Rahaus et al., 2003; Read et al., 2001; Robert et al., 2002; Valassina et al., 2002)
Blood borne	
HIV-1, HCV, HBV, HTLV-I and II	(Heredia et al., 1996; Meng et al., 2001; Vet et al., 1999)
Exanthemas	
Measles, B19, rubella	(Mosquera Mdel et al., 2002)
Gastroenteritis	
Adenoviruses, rotaviruses, Norwalk, enteroviruses, HAV	(Li et al., 2002; O'Neill et al., 2002)
STDs	
Papillomaviruses	(Gravitt et al., 2000; Zheng et al., 1995)

[a] See, Elnifro et al., 2000.

from a range of related viruses, for example those from the same genus or family. To develop this type of test, regions of conserved amino acid and nucleotide sequence are identified in the target genome. This is accomplished through multiple alignment of genome sequences from as many strains of the virus as possible. Suitable conserved sequences are found in protein encoding regions (e.g. at the active site of the enzyme) or untranslated regions that fold up to form self-annealed tertiary structures (e.g. ribosome binding sites and the 5′ and 3′ non-coding regions of viral genomes). Generic PCRs have been designed in this way for a conserved region of the DNA polymerase gene of the herpesviruses and for the 5′-NCR of the enteroviruses (Table 3). The primers for generic PCRs are often degenerate, having mixed bases at positions of redundancy in the target sequence, or they may utilise the base inosine at positions of maximum degeneracy (e.g. see, Clewley et al., 1998). The amplification reagents and thermal cycling conditions usually have to be empirically established for successful generic PCR.

In contrast to generic PCR, multiplex PCR involves the combination of several primer sets targeting different sequence regions in one amplification reaction (Table 4). In a PCR for neurological viruses, this might include generic primers for amplifying herpesvirus and enteroviruses together with more specific primers for measles, mumps, rubella and West Nile viruses. In a similar manner to generic PCRs, multiplex PCRs require careful optimisation, and even then it is difficult to make them as sensitive as specific single primer pair amplification reactions. To attain sufficient sensitivity, it may be necessary to split any sample into several aliquots for amplification with selected subsets of the full range of primers. Thus either one complex or several simpler multiplex PCRs containing a mixture of

generic and specific probes would be used to amplify and label DNA extracted from CNS or brain tissue.

The microarray that would then be used to detect the product of a multiplex PCR for neurological viruses would contain probes for each individual virus of interest that is, for example, probes for specific EBV, VZV, HHV-6, HSV-1, HSV-2, CMV, and HHV-7 sequences in the gene region between the generic primers. It would also have, if possible, a generic 'catch-all' probe so that uncharacterised herpesvirus sequences not recognised by the specific probes would not be missed. Similarly, specific and generic enterovirus probes would be included on the array, as well as specific probes for measles, mumps, rubella and West Nile viruses. Control probes on the array would consist of unrelated viral and human cellular DNA sequences.

2.2. Comprehensive diagnostic arrays and random PCR

The most promising and impressive arrays to date (August 2003) for use in diagnostic virology have been described by DeRisi and colleagues at the Howard Hughes Medical Institute (Wang et al., 2002). They initially constructed a microarray of 1600 unique 70-mer long oligonucleotide probes, designed from about 140 viral genome sequences, including entero-, rhino-, adeno-, orthomyxo-, paramyxo-, nido-, retro-, hepadna-, and papillomaviruses. As can be seen from that list, the viruses targeted were primarily, but not exclusively, respiratory tract pathogens. The microarray was initially validated using fluorescently labelled Kaposi's sarcoma-associated herpes virus (KSHV), respiratory syncytial virus (RSV), parainfluenza 3, poliovirus 1, adenovirus 12 and rhinovirus RNA prepared from both infected and uninfected cell cultures. More in depth analyses were performed with RNA from different serotypes of rhinoviruses,

as well as with RNA extracted from nasal lavage specimens from patients with common colds. The RNA was amplified to DNA and labelled using a random PCR procedure (Bohlander et al., 1992), a step that is essential if arrays are to be used successfully for the detection of pathogens of unknown or unrecognised sequence.

A more comprehensive version of this microarray was used in the identification of the SARS virus as a coronavirus (Rota et al., 2003). This microarray contained about 11,000 70-mer oligonucleotides, representing all complete viral genome sequences. The SARS material RNA was amplified and labelled by the random PCR approach, and then hybridised against the microarray. The DNA that hybridised to the array was recovered with a micromanipulator and further PCR-amplified, cloned and sequenced.

2.3. Detection of agents of bioterror

A microarray for the detection of five viruses, 11 prokaryotes and two eukaryotes considered to be pathogens that bioterrorists might employ has been designed by researchers at the Lawrence Livermore National Laboratory, USA (Wilson et al., 2002). Their strategy employed multiplex PCR amplification with primers targeting multiple regions of the genome of each pathogen, and the use of overlapping 20-mer oligonucleotide probes complementary to the entire diagnostic region. The microarray they made in this way had a total of 53,660 probes.

2.4. Other diagnostic arrays

A low density spot array has recently been described for the specific detection of enterovirus 71 (Shih et al., 2003). This array is designed to capture PCR amplicons produced from the 5′-NCR and the *VP2* gene of the enterovirus 71 genome.

The development of diagnostic microarrays is not confined to human virology: their use is being investigated for the detection of plant viruses (Boonham et al., 2003; Bystricka et al., 2003). Arrays have also been described for two viruses of economic importance for shrimp farmers, white spot syndrome virus and infectious hypodermal and haematopoietic necrosis virus (Quéré et al., 2002). These latter workers used a membrane based miniarray system with bound PCR amplicons for the capture of complementary strands which were labelled with digoxgenin. Hybridisation of the digoxgenin labelled target DNA to the bound PCR amplicon probes was demonstrated colorimetrically.

Arrays that are used for diagnostic purposes may be considered to be the least discriminatory in the sense that they need to be designed so that they are able to pick up all members of a virus group, and thereby identify novel pathogens as, for instance, was shown by the identification of the SARS agent as a coronavirus (Rota et al., 2003). It is complicated, however, to develop and use this type of array and, at present, there are more examples of arrays that are used

for strain typing viral genomes. For example, for typing influenza virus A and B strains, human group A rotaviruses, human papillomaviruses (HPV), hepatitis B and C viruses (HBV and HCV), and human immunodeficiency virus type 1 (HIV-1). Typing arrays exist in the format of glass slides, line probes or blots, and high density chips.

3. Molecular typing arrays

3.1. Influenza virus

An influenza virus microarray based on four haemagglutinin (HA), three neuraminidase (NA) and two matrix protein (MP) gene targets from five different influenza virus strains has been described (Li et al., 2001). Twenty-four PCR products (each of about 500 bp) were made from these gene targets and were spotted in duplicate onto glass slides, together with control *E. coli* amplicons and buffer spots to give an 8×8 array. Thus each gene was represented by two or three amplicons. Three multiplex PCRs based on combinations of two to four primers for the four HA, three NA and two MP gene targets were used to generate amplicons labelled with a fluorescent dye (Cy3-dCTP). When tested with fluorescently labelled DNA from different influenza virus strains, the expected correct homologous hybridisation patterns were observed on the array.

3.2. Rotaviruses

A rotavirus microarray based on 47 genotype-specific oligonucleotides designed in variable regions within the conserved VP7 segment, which is used to classify the G genotype of rotaviruses, has been described (Chizhikov et al., 2002). Each rotavirus was identified by an average of nine different specific probes (for the clinically relevant G1 to G4 and G9 genotypes), which were spotted on glass (via an aminolink) in an ordered way. PCR primers in regions of VP7 common to all group A rotaviruses were used to make amplicons fluorescently labelled with Cy5-dCTP. One of the primers was biotinylated, which allowed separation of the two amplicon DNA strands by streptavidin binding. In this way the test sequence could be applied to the array as fluorescently labelled single stranded DNA. When tested with 20 rotavirus isolates, the expected correlation with genotype results determined by sequencing was seen. As with the influenza virus array, details of the routine use of the rotavirus array in a diagnostic laboratory environment have yet to be published.

3.3. Line probe arrays and human papillomaviruses

The immediate precursor of a DNA array suitable for use for typing viral genomes was the reverse hybridisation line probe or blot, developed for HLA genotyping by scientists at Cetus Corporation, where PCR was invented (Bugawan et al., 1994; Saiki et al., 1989). Subsequently,

the first viral application was for the typing of HCV, developed at Innogenetics (Stuyver et al., 1993, 1996). Besides HCV, line probe/blot assays have been described for the detection of mutations in the HIV-1 reverse transcriptase gene (Stuyver et al., 1997), for genotyping and detecting lamivudine-resistance HBV (Aberle et al., 2001; Swenson et al., 2001), and for typing HPV (Gravitt et al., 1998; Kleter et al., 1999; Levi et al., 2002; van Doorn et al., 2002).

Although the details may vary, in general line probe blots or strips are made by immobilizing capture (i.e. probe) oligonucleotides onto nitrocellulose or nylon membranes. The oligonucleotides are identified from multiple sequence alignments of the target viral genes. After synthesis of each oligonucleotide probe, it is tailed with dT residues enzymatically or conjugated with BSA, and bound to the membrane. The dT tail or BSA acts as a spacer between the solid phase and the bases of the oligonucleotide probe that are complementary to the target viral sequence. This target is prepared by a PCR in which a specific product is amplified with a biotin residue incorporated at the end of it through the use of a biotinylated primer. After denaturation into single stranded DNA by treatment with alkali, hybridisation between the probe and the biotinylated DNA strand is revealed by a streptavidin-alkaline phosphatase colorimetric detection step.

There are two descriptions of line probe/blot assays for HPV, which provide a good example of how this technology may be used. One of these is known as a line blot assay (LBA) and makes use of a 450 bp amplicon in the L1 region of HPV using a primer set called PGMY (Gravitt et al., 1998, 2000). This primer set is a mixture (pool) of 5 upstream and 13 downstream oligonucleotides. The other assay is known as a line probe assay (LiPA) and it uses primers called SPF that amplify a small 65 bp amplicon within the L1 region (Kleter et al., 1998, 1999). The SPF primers are not a mixture like the PGMY primers, but are consensus primers based on highly conserved sequences. Both primer sets can detect similar numbers (25–27) of HPV anogenital genotypes. A comparison of these two methods has been carried out with a panel of 400 cervical scrapes and, although minor differences in the detection of some HPV types was seen, overall they were found to match and be equally suitable for diagnostic use (van Doorn et al., 2002). Either of these two assays could be readily adapted to a microarray format: the same oligonucleotides would be used but applied to glass slides rather than to membranes; the PCR products would be labelled with a fluorescent dye (e.g. Cy3 or Cy5); hybridisation would be using slide hybridisation chambers; and an array scanner and software would be used to obtain the results, instead of colorimetric visualisation.

The two available HPV line probe assays have thus been shown to be equally useful, though there have been few published evaluations of line probe assays against other methods of PCR product characterisation. For this, the gold standard method against which they must be compared is sequencing of the amplicon. One evaluation of a line probe assay compared to sequencing was for detection of drug resistance mutations in the reverse transcriptase gene of HIV-1. It concluded that for 40% of the specimens the line probe assay was insufficiently reliable for use on its own (Puchhammer-Stockl et al., 1999). One in five samples gave results that could not be interpreted so that the antiviral drug genotype had to be determined by sequencing. However, another study found that although there were some discrepancies in the results obtained by line probe assay, it was suitable for use in a clinical virology laboratory for HIV-1 antiretroviral drug mutation detection as an alternative to sequencing (Wilson et al., 2000). This latter evaluation also found the Affymetrix GeneChip, which detects mutations in the protease and reverse transcriptase genes of HIV-1, to be a satisfactory method.

3.4. DNA chips and HIV-1

The Affymetrix HIV-1 GeneChip was the first commercial microarray offered for use in clinical virology. It was initially developed for genotyping the protease gene, and later also for the reverse transcriptase gene (Kozal et al., 1996). Evaluation of its performance compared to DNA sequencing has been done by several laboratories and, in general, it has been found that genotyping by the chip is highly concordant with sequencing (Gunthard et al., 1998; Hanna et al., 2000; Wilson et al., 2000; Young et al., 1998). The Affymetrix HIV-1 protease GeneChip consisted of a high density array of over 12,000 oligonucleotides made by light-directed combinatorial chemical synthesis on a glass surface (Lipshutz et al., 1999). This array of 15-base overlapping oligonucleotides was designed so as to be able to interrogate each position of the HIV-1 subtype B protease gene through the means of a set of four oligonucleotides differing only at one base position for each nucleotide of the protease gene. The target was prepared from HIV-1 RNA by reverse transcriptase PCR with primers bearing T3 and T7 RNA polymerase promoters so that the amplicon produced by PCR in this way had these promoter sequences at each end. It could then be transcribed into RNA with T3 or T7 RNA polymerase in the presence of fluorescein-labelled rUTP. After fragmentation of this RNA, it was hybridised against the DNA chip so that the pattern of hybridisation could be read out as the sequence of the protease gene. This is a powerful method which illustrates two of the challenges that arise in using oligonucleotide microarrays to fully characterise viral genes. Firstly, the design and synthesis of the full overlapping set of redundant oligonucleotides is technically difficult and beyond the resources of many clinical virology laboratories. Secondly, the sequences of the oligonucleotides used on the chip must be based on a single known sequence, in this case that of subtype B of HIV-1. This means that separate chips would need to be made for each of the other subtypes of HIV-1, for example for subtype C which comprises about half of all worldwide infections today. The more rapidly and extensively the target genome mutates, the more diffi-

cult it will be to design sequence specific oligonucleotide chips that are able to catch all of the genome variants that might be present in individuals as HIV-1 quasispecies or already prevalent in the community. Obviously, this is more of a problem for a fast changing genomic target like HIV-1 than for a slowly evolving one like HPV.

3.5. Vaccine quality control

Microarrays, perhaps in the form of generic oligonucleotide microchips, have the potential to be used to examine batches of vaccine to ensure that they are free of viral genomic sequences (Amexis et al., 2002; Cherkasova et al., 2003; Petricoin et al., 2002; Proudnikov et al., 2000). One of the initial hopes for oligonucleotide microarrays was that they could be used for DNA sequencing by hybridisation (Southern, 1996). Although this has not been realised on large scale, small PCR amplicons (about 100 bp) can be characterised with arrays of all possible 8-mer oligonucleotides (a total of 4096 probes). The use of an array of this type has been described for detection and quantification of neurovirulent mutants in oral poliovirus vaccine (Proudnikov et al., 2000). A short sequence region of less than 100 bp was amplified from the 5′ end of the poliovirus genome from vaccine preparations, labelled with Texas Red fluorescent dye and applied to a microarray containing 4096 chemically immobilised oligonucleotides. The pattern of spots that was observed following hybridisation and washing was characteristic for the sequence applied. In practice this meant that if there were as few as 10% revertants in the vaccine preparation they could be recognised. Further studies using microarrays bearing 9 or 10 base long oligonucleotides are needed to determine whether this approach is applicable for longer PCR products. The alternative to using microarrays of generic oligonucleotides for characterising virus stocks is to use arrays of strain specific oligonucleotides, and this approach is also being investigated for the analysis of vaccine-derived polioviruses, as well as for analysis of the Jeryl Lynn strain of mumps virus (Amexis et al., 2002; Cherkasova et al., 2003).

4. RNA expression arrays

Microarrays have been used in two other ways in virology, firstly, to study the RNA expression profile of the virus following infection, including drug effects in vitro (Chambers et al., 1999; Jenner et al., 2001) and secondly, to study the RNA expression profile of the *host* following infection, including drug effects in vitro (Corbeil et al., 2001; Domachowske et al., 2002; Geiss et al., 2000; Iizuka et al., 2002; Zhu et al., 1998). The applications of these two complementary approaches in microbiology have been reviewed by (Cummings and Relman, 2000; Kato-Maeda et al., 2001; Manger and Relman, 2000). The RNA expression of HCMV in cell culture in the presence or absence of cycloheximide or

ganciclovir was analysed with an array of oligonucleotides representing HCMV ORFs made on glass slides (Chambers et al., 1999). Similarly, an array of 288 amplicons representing 88 HHV-8/KSHV ORFs was made on nylon membranes, and used to compare the expression of viral mRNAs during latency and TPA-induced lytic replication (Jenner et al., 2001). Other groups are studying poxvirus expression in this way (Laassril et al., 2002) (Evans, personal communication).

Gene expression can be investigated with the help of microarrays of probes (oligonucleotides or cDNA) representing the mRNAs—i.e. the expressed genes—of the host cell (Schena et al., 1995). This type of host cell gene array is most conveniently obtained commercially, e.g. from Affymetrix. To study HCMV infection, the role of cellular genes, before the onset of viral DNA replication, was analysed by hybridising labelled mRNA from infected cells to Affymetrix gene chips containing over 6600 human gene probes (Zhu et al., 1998). The level of 258 mRNAs was found to change by a factor of 4 or more. Similarly, the expression of mouse cellular mRNAs after infection with the paramyxovirus, pneumonia virus of mice, was analysed using an Affymetrix mouse gene microarray chip (Domachowske et al., 2002). Gene expression was compared in hepatocellular carcinomas induced by either HBV or HCV (Iizuka et al., 2002). Messenger RNA from HIV-1 infected T-cells was hybridised to 1500 cellular cDNAs to identify genes involved in T-cell signalling, subcellular trafficking and transcriptional regulation (Geiss et al., 2000). In the same manner, the effects of HIV-1 infection on T-cell transcription was examined using Affymetrix gene chips, and up and down regulation, that is differential expression of cellular genes, was observed (Corbeil et al., 2001).

It has been suggested that the hybridisation of labelled target DNA derived from patients infected with pathogens could be used diagnostically (Cummings and Relman, 2000). These authors wrote: "the unique constellation of virulence factors expressed by a specific pathogen will elicit a unique transcription response in the host". If this unique transcription pattern could be captured as a readout from an array it could be used as a diagnostic signature. Simplistically, this might be done by hybridising labelled cDNA/mRNA from PBMCs from a patient to an array of sequences representing human genes, giving an array profile diagnostic of a specific pathogen. The particular advantages of this approach, if successful, would be that only one sample would be needed for several pathogens, and that it would allow the detection of early and late infections and of uncultivable pathogens. In addition, it might be possible to infer time since exposure to the pathogen, if the transcription pattern were temporally characteristic (Cummings and Relman, 2000).

5. Practical problems

Aside from their costs and the difficulties associated with designing and making a suitable array, the most obvious

problems with their use are ones of quality control, because of the difficulties of standardisation and reproducibility associated with the large number of probes on an array, and the large number of slides that need to be made. Although it is possible to rehybridize some arrays, this is unlikely to be acceptable for diagnostic use because of the increase in noise and loss of signal that comes with reuse. It is also difficult to relate the signal from an array back to the amount of virus genome that was present in the original specimen, making quantification a problem.

Arrays of oligonucleotides or PCR amplicons spotted on glass slides can be made in-house, but there may be contamination if PCR amplicons are used, as large amounts of these products will be made. There may also be problems with the hybridisation reaction, for example, sequences not hybridising together for any obvious reason, and the probes or the target self-annealing because of internal complementary regions in their sequences. Commercially bought arrays may be inflexible because they are based on just one target sequence, e.g. the Affymetrix GeneChip for HIV-1 subtype B protease mutations (Kozal et al., 1996).

6. The future: protein arrays and biosensors

From the foregoing discussion it can be seen that microarrays have gained a foothold in virology and have the prospect of serving as a useful diagnostic tool. Other similar technologies that are less ready to be able to fulfil this diagnostic role include protein arrays and biosensors. Protein arrays are being prepared with antigens or antibodies bound to a solid phase (analogous to oligonucleotide or amplicon probes) and used to capture specific antibodies or antigens (de Wildt et al., 2000; Emili and Cagney, 2000; Holt et al., 2000; Walter et al., 2000). Essentially these are immunoassays in a microarray format (Schweitzer and Kingsmore, 2002). A protein array has been described for ToRCH screening whereby Toxoplasma gondii, rubella virus, CMV and HSV-1 and 2 antigens were printed on glass slides. The slides were first incubated with serum samples and subsequently with fluorescently labelled secondary antibodies. Human IgG and IgM bound to the printed antigens were detected by confocal scanning microscopy. Good concordance was obtained between the microarray results and those of ELISAs (Mezzasoma et al., 2002). A microarray of oligosaccharides on nitrocellulose has been developed for capturing carbohydrate-recognizing proteins, and it might be possible to develop this for viral diagnosis (Fukui et al., 2002).

Biosensors have been defined as "small devices which utilize biological reactions for detecting target analytes" (Wang, 2000). There are two ways of doing this, either by bioaffinity or by biocatalysis. Bioaffinity arrays involve the target analyte binding to a ligand immobilised on a solid phase (e.g. an oligonucleotide) and, instead of there being a necessity for detection of hybridisation of the probe and target by colorimetric or radioactive means, the hybridisation is detected by electronic means. This can be achieved via a transducer at the point where the probe is attached to the solid phase. This class of biosensors also includes hybridisation biosensors which rely on a conformational change induced by hybridisation, for example molecular beacons bearing quencher and fluorophore dyes. Biocatalytic arrays involve an immobilised enzyme being used to recognise the substrate of the enzyme, which is, in this case, the target of the array. A signal is generated when the enzyme catalyses a specific reaction because of the presence of the target in the analyte applied to the array. The reaction may be recognised either colorimetric ally or via an electronic transducer at the surface of the array.

7. Conclusions

Microarrays that are useful in the clinical virology laboratory will be developed both in the public and commercial sectors. Those in public laboratories will probably fit in with the research interests of the laboratory that develops them, and may not be easily transferable to other laboratories. More likely, it is commercial suppliers who will deliver quality controlled viral diagnostic arrays, just as line probe assays are made available by them at present. Whether their performance will match their probable high cost remains to be seen. They will be fast and easy to automate, but if they are to supplant the present range of genomic and serological diagnostic tests they must also be able to match them in terms of sensitivity and specificity. That may prove a stiffer challenge.

References

Aberle SW, Kletzmayr J, Watschinger B, Schmied B, Vetter N, Puchhammer-Stockl E. Comparison of sequence analysis and the INNO-LiPA HBV DR line probe assay for detection of lamivudine-resistant hepatitis B virus strains in patients under various clinical conditions. J Clin Microbiol 2001;39:1972–4.

Amexis G, Rubin S, Chizhikov V, Pelloquin F, Carbone K, Chumakov K. Sequence diversity of Jeryl Lynn strain of mumps virus: quantitative mutant analysis for vaccine quality control. Virology 2002;300:171–9.

Bohlander SK, Espinosa 3rd R, Le Beau MM, Rowley JD, Diaz MO. A method for the rapid sequence-independent amplification of microdissected chromosomal material. Genomics 1992;13:1322–4.

Boonham N, Walsh K, Smith P, Madagan K, Graham I, Barker I. Detection of potato viruses using microarray technology: towards a generic method for plant viral disease diagnosis. J Virol Meth 2003;108:181–7.

Bugawan TL, Apple R, Erlich HA. A method for typing polymorphism at the HLA-A locus using PCR amplification and immobilized oligonucleotide probes. Tissue Antigens 1994;44:137–47.

Burmeister T, Schwartz S, Thiel E. A PCR primer system for detecting oncoretroviruses based on conserved DNA sequence motifs of animal retroviruses and its application to human leukaemias and lymphomas. J Gen Virol 2001;82:2205–13.

Bystricka D, Lenz O, Mraz I, Dedic P, Sip M. DNA microarray: parallel detection of potato viruses. Acta Virol 2003;47:41–4.

Calvario A, Bozzi A, Scarasciulli M, Ventola C, Seccia R, Stomati D, et al. Herpes Consensus PCR test: a useful diagnostic approach to the screening of viral diseases of the central nervous system. J Clin Virol 2002;25:S71–8.

Casas I, Pozo F, Trallero G, Echevarria JM, Tenorio A. Viral diagnosis of neurological infection by RT multiplex PCR: a search for entero- and herpesviruses in a prospective study. J Med Virol 1999;57:145–51.

Chambers J, Angulo A, Amaratunga D, Guo H, Jiang Y, Wan JS, et al. DNA microarrays of the complex human cytomegalovirus genome: profiling kinetic class with drug sensitivity of viral gene expression. J Virol 1999;73:5757–66.

Chen Z, Plagemann PG. Detection of related positive-strand RNA virus genomes by reverse transcription/polymerase chain reaction using degenerate primers for common replicase sequences. Virus Res 1995;39:365–75.

Cherkasova E, Laassri M, Chizhikov V, Korotkova E, Dragunsky E, Agol VI, et al. Microarray analysis of evolution of RNA viruses: evidence of circulation of virulent highly divergent vaccine-derived polioviruses. Proc Natl Acad Sci USA 2003;100:9398–403.

Chizhikov V, Rasooly A, Chumakov K, Levy DD. Microarray analysis of microbial virulence factors. Appl Environ Microbiol 2001;67:3258–63.

Chizhikov V, Wagner M, Ivshina A, Hoshino Y, Kapikian AZ, Chumakov K. Detection and genotyping of human group A rotaviruses by oligonucleotide microarray hybridization. J Clin Microbiol 2002;40:2398–407.

Chow VT, Seah CL, Chan YC. Use of NS3 consensus primers for the polymerase chain reaction amplification and sequencing of dengue viruses and other flaviviruses. Arch Virol 1993;133:157–70.

Clewley JP. PCR and sequencing to type enteroviruses and rhinoviruses. In: Becker Y, Darai G, editors. PCR protocols for diagnosis of human and animal virus disease. Berlin: Springer-Verlag; 1995. p. 173–81.

Clewley JP, Lewis JCM, Brown DWG, Gadsby EL. A novel simian immunodeficiency virus (SIVdrl) pol sequence from the drill monkey Mandrillus leucophaeus. J Virol 1998;72:10305–9.

Coiras MT, Perez-Brena P, Garcia ML, Casas I. Simultaneous detection of influenza A, B, and C viruses, respiratory syncytial virus, and adenoviruses in clinical samples by multiplex reverse transcription nested-PCR assay. J Med Virol 2003;69:132–44.

Corbeil J, Sheeter D, Genini D, Rought S, Leoni L, Du P, et al. Temporal gene regulation during HIV-1 infection of human CD4+ T cells. Genome Res 2001;11:1198–204.

Courgnaud V, Pourrut X, Bibollet-Ruche F, Mpoudi-Ngole E, Bourgeois A, Delaporte E, et al. Characterization of a novel simian immunodeficiency virus from guereza colobus monkeys (Colobus guereza) in Cameroon: a new lineage in the nonhuman primate lentivirus family. J Virol 2001;75:857–66.

Coutlee F, Gravitt P, Kornegay J, Hankins C, Richardson H, Lapointe N, et al. Use of PGMY primers in L1 consensus PCR improves detection of human papillomavirus DNA in genital samples. J Clin Microbiol 2002;40:902–7.

Cummings CA, Relman DA. Using DNA microarrays to study host-microbe interactions. Emerg Infect Dis 2000;6:513–25.

Daum LT, Canas LC, Schadler CA, Ujimori VA, Huff WB, Barnes WJ, et al. A rapid, single-step multiplex reverse transcription-PCR assay for the detection of human H1N1, H3N2, and B influenza viruses. J Clin Virol 2002;25:345–50.

de Wildt RMT, Mundy CR, Gorick BD, Tomlinson IM. Antibody arrays for high-throughput screening of antibody-antigen interactions. Nat Biotechnol 2000;18:989–94.

Domachowske JB, Bonville CA, Easton AJ, Rosenberg HF. Differential expression of proinflammatory cytokine genes in vivo in response to pathogenic and nonpathogenic pneumovirus infections. J Infect Dis 2002;186:8–14.

Echevarria JE, Erdman DD, Meissner HC, Anderson L. Rapid molecular epidemiologic studies of human parainfluenza viruses based on direct sequencing of amplified DNA from a multiplex RT-PCR assay. J Virol Meth 2000;88:105–9.

Ehlers B, Borchers K, Grund C, Frolich K, Ludwig H, Buhk HJ. Detection of new DNA polymerase genes of known and potentially novel herpesviruses by PCR with degenerate and deoxyinosine-substituted primers. Virus Genes 1999;18:211–20.

Elnifro EM, Ashshi AM, Cooper RJ, Klapper PE. Multiplex PCR: optimization and application in diagnostic virology. Clin Microbiol Rev 2000;13:559–70.

Emili AQ, Cagney G. Large-scale functional analysis using peptide or protein arrays. Nat Biotechnol 2000;18:393–7.

Fedele CG, Ciardi M, Delia S, Echevarria JM, Tenorio A. Multiplex polymerase chain reaction for the simultaneous detection and typing of polyomavirus JC, BK and SV40 DNA in clinical samples. J Virol Meth 1999;82:137–44.

Fukui S, Feizi T, Galustian C, Lawson AM, Chai W. Oligosaccharide microarrays for high-throughput detection and specificity assignments of carbohydrate-protein interactions. Nat Biotechnol 2002;20:1011–7.

Geiss GK, Bumgarner RE, An MC, Agy MB, van't Wout AB, Hammersmark E, et al. Large-scale monitoring of host cell gene expression during HIV-1 infection using cDNA microarrays. Virology 2000;266:8–16.

Gelman IH, Zhang J, Hailman E, Hanafusa H, Morse SS. Identification and evaluation of new primer sets for the detection of lentivirus proviral DNA. AIDS Res Hum Retroviruses 1992;8:1981–9.

Gravitt PE, Peyton CL, Apple RJ, Wheeler CM. Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by a single-hybridization, reverse line blot detection method. J Clin Microbiol 1998;36:3020–7.

Gravitt PE, Peyton CL, Alessi TQ, Wheeler CM, Coutlee F, Hildesheim A, et al. Improved amplification of genital human papillomaviruses. J Clin Microbiol 2000;38:357–61.

Grondahl B, Puppe W, Hoppe A, Kuhne I, Weigl JA, Schmitt HJ. Rapid identification of nine microorganisms causing acute respiratory tract infections by single-tube multiplex reverse transcription-PCR: feasibility study. J Clin Microbiol 1999;37:1–7.

Gunthard HF, Wong JK, Ignacio CC, Havlir DV, Richman DD. Comparative performance of high-density oligonucleotide sequencing and dideoxynucleotide sequencing of HIV type 1 pol from clinical samples. AIDS Res Hum Retroviruses 1998;14:869–76.

Hanna GJ, Johnson VA, Kuritzkes DR, Richman DD, Martinez-Picado J, Sutton L, et al. Comparison of sequencing by hybridization and cycle sequencing for genotyping of human immunodeficiency virus type 1 reverse transcriptase. J Clin Microbiol 2000;38:2715–21.

Heredia A, Soriano V, Weiss SH, Bravo R, Vallejo A, Denny TN, et al. Development of a multiplex PCR assay for the simultaneous detection and discrimination of HIV-1, HIV-2, HTLV-I and HTLV-IIid. Clin Diag Virol 1996;7:85–92.

Holt LJ, Büssow K, Walter G, Tomlinson IM. By-passing selection: direct screening of antibody-antigen interactions using protein arrays. Nucleic Acids Res 2000;28:e72.

Houng HS, Chung-Ming Chen R, Vaughn DW, Kanesa-thasan N. Development of a fluorogenic RT-PCR system for quantitative identification of dengue virus serotypes 1–4 using conserved and serotype-specific 3′ noncoding sequences. J Virol Meth 2001;95:19–32.

Iizuka N, Oka M, Yamada-Okabe H, Mori N, Tamesa T, Okada T, et al. Comparison of gene expression profiles between hepatitis B virus- and hepatitis C virus-infected hepatocellular carcinoma by oligonucleotide

microarray data on the basis of a supervised learning method. Cancer Res 2002;62:3939–44.

Jenner RG, Alba MM, Boshoff C, Kellam P. Kaposi's sarcoma-associated herpesvirus latent and lytic gene expression as revealed by DNA arrays. J Virol 2001;75:891–902.

Kato-Maeda M, Gao Q, Small PM. Microarray analysis of pathogens and their interaction with hosts. Cell Microbiol 2001;3:713–9.

Kleter B, van Doorn L-J, ter Schegget J, Schrauwen L, van Krimpen K, Burger M, et al. Novel short-fragment PCR assay for highly sensitive broad-spectrum detection of anogenital human papillomaviruses. Am J Pathol 1998;153:1731–9.

Kleter B, van Doorn L-J, Schrauwen L, Molijn A, Sastrowijoto S, ter Schegget J, et al. Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. J Gen Microbiol 1999;37:2508–17.

Kozal MJ, Shah N, Shen N, Yang R, Fucini R, Merigan TC, et al. Extensive polymorphisms observed in HIV-1 clade B protease gene using high-density oligonucleotide arrays. Nat Med 1996;2:753–9.

Laassril M, Chizhikov C, Shchelkunov S, Chumakov K. Detection and discrimination of orthopoxviruses by oligonucleotide microarray hybridization. Am Soc Virol 2002;203:P34–41.

Levi JE, Kleter B, Quint WGV, Fink MCS, Canto CLM, Matsubara R, et al. High prevalence of human papillomavirus (HPV) infections and high frequency of multiple HPV genotypes in human immunodeficiency virus-infected women in Brazil. J Clin Microbiol 2002;40:3341–5.

Li J, Chen S, Evans DH. Typing and subtyping influenza virus using DNA microarrays and multiplex reverse transcriptase PCR. J Clin Microbiol 2001;39:696–704.

Li JW, Wang XW, Yuan CQ, Zheng JL, Jin M, Song N, et al. Detection of enteroviruses and hepatitis A virus in water by consensus primer multiplex RT-PCR. World J Gastroenterol 2002;8:699–702.

Lipshutz RJ, Fodor SP, Gingeras TR, Lockhart DJ. High density synthetic oligonucleotide arrays. Nat Genet 1999;21:20–4.

Lozano ME, Posik DM, Albarino CG, Schujman G, Ghiringhelli PD, Calderon G, et al. Characterization of arenaviruses using a family-specific primer set for RT-PCR amplification and RFLP analysis. Its potential use for detection of uncharacterized arenaviruses. Virus Res 1997;49:79–89.

Manger ID, Relman DA. How the host 'sees' pathogens: global gene expression responses to infection. Curr Opin Immunol 2000;12:215–8.

Markoulatos P, Georgopoulou A, Siafakas N, Plakokefalos E, Tzanakaki G, Kourea-Kremastinou J. Laboratory diagnosis of common herpesvirus infections of the central nervous system by a multiplex PCR assay. J Clin Microbiol 2001;39:4426–32.

Masciotra S, Yang C, Pieniazek D, Thomas C, Owen SM, McClure HM, et al. Detection of simian immunodeficiency virus in diverse species and of human immunodeficiency virus type 2 by using consensus primers within the *pol* region. J Clin Microbiol 2002;40:3167–71.

Meng Q, Wong C, Rangachari A, Tamatsukuri S, Sasaki M, Fiss E, et al. Automated multiplex assay system for simultaneous detection of hepatitis B virus DNA, hepatitis C virus RNA, and human immunodeficiency virus type 1 RNA. J Clin Microbiol 2001;39:2937–45.

Mezzasoma L, Bacarese-Hamilton T, Di Cristina M, Rossi R, Bistoni F, Crisanti A. Antigen microarrays for serodiagnosis of infectious diseases. Clin Chem 2002;48:121–30.

Minjolle S, Michelet C, Jusselin I, Joannes M, Cartier F, Colimon R. Amplification of the six major human herpesviruses from cerebrospinal fluid by a single PCR. J Clin Microbiol 1999;37:950–3.

Miura T, Sakuragi J, Kawamura M, Fukasawa M, Moriyama EN, Gojobori T, et al. Establishment of a phylogenetic survey system for AIDS-related lentiviruses and demonstration of a new HIV-2 subgroup. AIDS 1990;4:1257–61.

Mosquera Mdel M, de Ory F, Moreno M, Echevarria JE. Simultaneous detection of measles virus, rubella virus, and parvovirus B19 by using multiplex PCR. J Clin Microbiol 2002;40:111–6.

Na BK, Kim JH, Shin GC, Lee JY, Lee JS, Kang C, et al. Detection and typing of respiratory adenoviruses in a single-tube multiplex polymerase chain reaction. J Med Virol 2002;66:512–7.

Nature Genetics Reviews. The chipping forecast. Nat Genet 1999;21:1–60.

Nature Genetics Reviews. The chipping forecast II. Nat Genet 2002;32:461–552.

Nelson JH, Hawkins GA, Edlund K, Evander M, Kjellberg L, Wadell G, et al. A novel and rapid PCR-based method for genotyping human papillomaviruses in clinical samples. J Clin Microbiol 2000;38:688–95.

O'Neill HJ, McCaughey C, Coyle PV, Wyatt DE, Mitchell F. Clinical utility of nested multiplex RT-PCR for group F adenovirus, rotavirus and Norwalk-like viruses in acute viral gastroenteritis in children and adults. J Clin Microbiol 2002;25:335–43.

Petricoin 3rd EF, Hackett JL, Lesko LJ, Puri RK, Gutman SI, Chumakov K, et al. Medical applications of microarray technologies: a regulatory science perspective. Nat Genet 2002;32:474–9.

Proudnikov D, Kirillov E, Chumakov K, Donlon J, Rezapkin G, Mirzabekov A. Analysis of mutations in oral poliovirus vaccine by hybridization with generic oligonucleotide microchips. Biologicals 2000;28:57–66.

Puchhammer-Stockl E, Schmied B, Mandl CW, Vetter N, Heinz FX. Comparison of line probe assay (LIPA) and sequence analysis foe detection of HIV-1 drug resistance. J Med Virol 1999;57:283–9.

Quéré R, Commes T, Marti J, Bonami J, Piquemal D. White spot syndrome virus and infectious hypodermal and hematopoietic necrosis virus simultaneous diagnosis by miniarray system with colorimetry detection. J Virol Meth 2002;105:189–96.

Quereda C, Corral I, Laguna F, Valencia ME, Tenorio A, Echeverria JE, et al. Diagnostic utility of a multiplex herpesvirus PCR assay performed with cerebrospinal fluid from human immunodeficiency virus-infected patients with neurological disorders. J Clin Microbiol 2000;38:3061–7.

Rahaus M, Desloges N, Wolff MH. Development of a multiplex RT-PCR to detect transcription of varicella-zoster virus encoded genes. J Virol Meth 2003;107:257–60.

Read SJ, Mitchell JL, Fink CG. LightCycler multiplex PCR for the laboratory diagnosis of common viral infections of the central nervous system. J Clin Microbiol 2001;39:3056–9.

Robert PY, Traccard I, Adenis JP, Denis F, Ranger-Rogez S. Multiplex detection of herpesviruses in tear fluid using the "stair primers" PCR method: prospective study of 93 patients. J Med Virol 2002;66:506–11.

Rota PA, Oberste MS, Monroe SS, Nix WA, Campagnoli R, Icenogle JP, et al. Characterization of a novel coronavirus associated with Severe Acute Respiratory Syndrome. Science 2003;300:1394–7.

Saiki RK, Walsh PS, Levenson CH, Erlich HA. Genetic analysis of amplified DNA with immobilized sequence-specific oligonucleotide probes. Proc Natl Acad Sci USA 1989;86:6230–4.

Schena M, editor. DNA microarrays. A practical approach. Oxford: Oxford University Press; 1999.

Schena M, Shalon D, David RW, Brown PO. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 1995;270:467–70.

Schweitzer B, Kingsmore SF. Measuring proteins on microarrays. Curr Opin Biotechnol 2002;13:14–9.

Seah CL, Chow VT, Tan HC, Can YC. Rapid single-step RT-PCR typing of dengue viruses using five NS3 gene primers. J Virol Meth 1995;51:193–200.

Shih SR, Wang YW, Chen GW, Chang LY, Lin TY, Tseng MC, et al. Serotype-specific detection of enterovirus 71 in clinical specimens by DNA microchip array. J Virol Meth 2003;111:55–60.

Southern EM. DNA chips: analysing sequence by hybridization to oligonucleotides on a large scale. Trends Genet 1996;12:110–5.

Stockton J, Ellis JS, Saville M, Clewley JP, Zambon MC. A multiplex PCR for typing and subtyping influenza and respiratory syncytial viruses. J Clin Microbiol 1998;36:2990–5.

Stuyver L, Rossau R, Wyseur A, Duhamel M, Vanderborght B, Van Heuverswyn H, et al. Typing of hepatitis C virus isolates and

characterization of new subtypes using a line probe assay. J Gen Virol 1993;74:1093–102.

Stuyver L, Wyseur A, van Arnhem W, Hernandez F, Maertens G. Second-generation line probe assay for hepatitis C virus genotyping. J Clin Microbiol 1996;34:2259–66.

Stuyver L, Wyseur A, Rombout A, Louwagie J, Scarcez T, Verhofstede C, et al. Line probe assay for rapid detection of drug-selected mutations in the human immunodeficiency virus type 1 reverse transcriptase gene. Antimicrob Agents Chemother 1997;41:284–91.

Swenson PD, Van Geyt C, Alexander ER, Hagan H, Freitag-Koontz JM, Wilson S, et al. Hepatitis B virus genotypes and HBsAg subtypes in refugees and injection drug users in the United States determined by LiPA and monoclonal EIA. J Med Virol 2001;64:305–11.

Valassina M, Valentini M, Valensin PE, Cusi MG. Fast duplex one-step RT-PCR for rapid differential diagnosis of entero- or toscana virus meningitis. Diagn Microbiol Infect Dis 2002;43:201–5.

VanDevanter DR, Warrener P, Bennett L, Schultz ER, Coulter S, Garber RL, et al. Detection and analysis of diverse herpesviral species by consensus primer PCR. J Clin Microbiol 1996;34:1666–71.

van Doorn L-J, Quint W, Kleter B, Molijn A, Colau B, Martin M-T, et al. Genotyping of human papillomavirus in liquid cytology cervical specimens by the PGMY line blot assay and the SPF10 line probe assay. J Clin Microbiol 2002;40:979–83.

Vet JA, Majithia AR, Marras SA, Tyagi S, Dube S, Poiesz BJ, et al. Multiplex detection of four pathogenic retroviruses using molecular beacons. Proc Natl Acad Sci USA 1999;96:6394–9.

Walter G, Bussow K, Cahill D, Lueking A, Lehrach H. Protein arrays for gene expression and molecular interaction screening. Curr Opin Microbiol 2000;3:298–302.

Wang J. From DNA biosensors to gene chips. Nucleic Acids Res 2000;28:3011–6.

Wang D, Coscoy L, Zylberberg M, Avila PC, Boushey HA, Ganem D, et al. Microarray-based detection and genotyping of viral pathogens. Proc Natl Acad Sci USA 2002;99:15687–92.

Wilson JW, Bean P, Robins T, Graziano F, Persing DH. Comparative evaluation of three human immunodeficiency virus genotyping systems: the HIV-GenotypR method the HIV PRT GeneChip assay, and the HIV-1 RT line probe assay. J Clin Microbiol 2000;38:3022–8.

Wilson WJ, Strout CL, DeSantis TZ, Stilwell JL, Carrano AV, Andersen GL. Sequence-specific identification of 18 pathogenic microorganisms using microarray technology. Mol Cellular Probes 2002;16:119–27.

Xu W, McDonough MC, Erdman DD. Species-specific identification of human adenoviruses by a multiplex PCR assay. J Clin Microbiol 2000;38:4114–20.

Yamamoto T, Nakamura Y. A single tube PCR assay for simultaneous amplification of HSV-1/-2, VZV, CMV, HHV-6A/-6B, and EBV DNAs in cerebrospinal fluid from patients with virus-related neurological diseases. J Neurovirol 2000;6:410–7.

Yang C, Dash BC, Simon F, van der Groen G, Pieniazek D, Gao F, et al. Detection of diverse variants of human immunodeficiency virus-1 groups M, N, and O and simian immunodeficiency viruses from chimpanzees by using generic *pol* and *env* primer pairs. J Infect Dis 2000;181:1791–5.

Young B, Johnson S, Bahktiari M, Shugarts D, Young RK, Allen M, et al. Resistance mutations in protease and reverse transcriptase genes of human immunodeficiency virus type 1 isolates from patients with combination antiretroviral therapy failure. J Infect Dis 1998;178:1497–501.

Zheng PS, Li SR, Iwasaka T, Song J, Cui MH, Sugimori H. Simultaneous detection by consensus multiplex PCR of high- and low-risk and other types of human papilloma virus in clinical samples. Gynecol Oncol 1995;58:179–783.

Zhu H, Cong JP, Mamtora G, Gingeras T, Shenk T. Cellular gene expression altered by human cytomegalovirus: global monitoring with oligonucleotide arrays. Proc Natl Acad Sci USA 1998;95:14470–5.

Zoll GJ, Melchers WJG, Kopecka H, Jambroes G, van der Poel HJA, Galama JMD. General primer-mediated polymerase chain reaction for detection of enteroviruses: application for diagnostic routine and persistent infections. J Clin Microbiol 1992;30:160–5.