Protein microarrays in clinical microbiology

MA Abdo &
PJ Hudson

CSIRO Molecular and Health Technologies
343 Royal Parade, Parkville VIC 3052
Tel: (03) 9662 7233
Fax: (03) 9662 7101
E-mail: michael.abdo@csiro.au

Clinical microbiology laboratories have, in the past, broadly adopted new molecular biology techniques and automation. In the near future, the adoption of protein microarray technology has the potential to revolutionise the field in a manner similar to that of polymerase chain reaction (PCR). With the advantages of far greater sensitivity, parallel experimentation, reduced sample consumption and cost-per-test [1], the development of protein microarrays has come about through the realisation that mRNA levels do not necessarily correlate with protein expression [2].

Protein microarrays were described more than a decade ago [3], when it was demonstrated that a microarray using only a small quantity of a capture molecule and sample could be more sensitive than existing immunoassays, specifically the enzyme linked immunosorbant assay (ELISA) [4]. Typically, there are two approaches to protein microarray production – immobilisation of antibodies as capture reagents to detect antigens (a forward-phase microarray) and immobilisation of antigens as a reverse-phase microarray. However, there are many more iterations of protein-protein interactions and, as such, many possible applications of the technology (Figure 1).

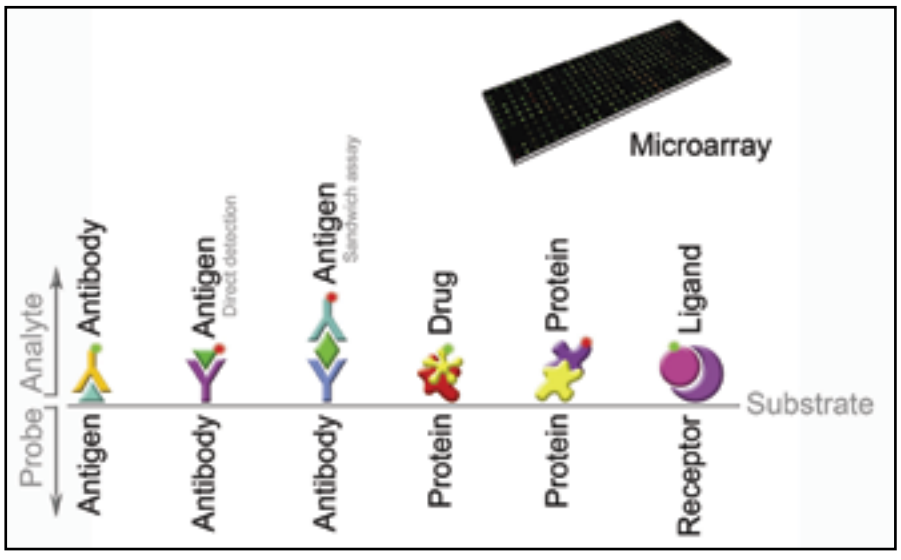


Figure 1. Protein microarray design and application.
Proteins arrayed on a solid support (probes) are used to interrogate a sample (analyte), typically sera or plasma, for the presence or altered expression of a cognate binder. A number of permutations in addition to those illustrated, are possible, including enzyme-substrate, antibody-carbohydrate and protein-liposome [1]. Protein-protein interactions are detected by conjugation of fluorescent reporters either directly to the analyte or following secondary detection steps. The specificity of the assay can be improved through the use of surrogate markers and capture reagents directed against a different epitope to that of the probe–sandwich assay.

The reported improvement in sensitivity of protein microarrays is dependent on the kinetics of the protein-protein interaction which requires optimised and flexible methods of microarray production, sample preparation and data analysis. The capture molecules of the microarray are deposited, or rather printed, in a small area or spot (typically 100-140μm in diameter) in an ordered array on a solid support or substrate; this assay format maximises the signal density generated by the binding protein and incorporates true parallelism, miniaturisation and high-throughput [5].

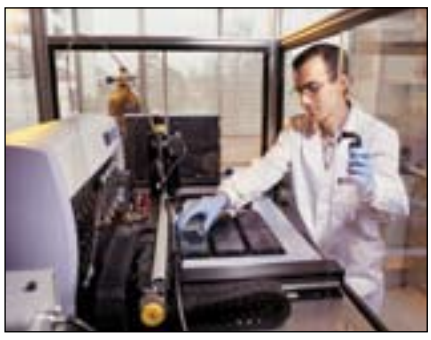
Microarray printing requires specialised robotics and understanding of the interaction between the capture molecule or probe and the substrate. DNA microarrays are produced through so-called contact printing; however, protein microarrays are more commonly produced using non-contact or ink-jet printing. The use of non-contact printing allows for greater flexibility in the substrates used, especially three-dimensional supports which account for the physical and chemical heterogeneity of the capture molecule [6].

Protein microarrays have been used in the drug discovery process [6] and utilised to screen expression libraries for binding to target molecules; however, they are more commonly used as a comparative fluorescence assay [1]. Reverse-phase antigen arrays have been employed to interrogate clinical samples for serodiagnosis of specific antibodies directed against pathogenic antigens such as *Toxoplasma gondii*, cytomegalovirus, rubella virus, and herpes simplex virus types 1 and 25. Work in our laboratory has also demonstrated the application of forward-phase antibody arrays in the detection of clinical biomarkers in human sera [7].

Similarly, other immune response markers could be used as surrogates for the diagnosis of pathogenic insult; for example, the accurate measurement of tumour necrosis factor – alpha (TNFα) could be achieved through measurement of the soluble form of the TNFα receptor 1 as opposed to TNFα itself[8]. This is feasible since, unlike the ELISA, microarray assays incorporate parallel standard curves for each protein of interest, thereby compensating for variability in the sample and standards and reducing a known source of bias[5,9].

In most documented assays, the sensitivity of protein microarrays is still within the range of validated ELISA assays[9]. However, increased sensitivity is possible through the amplification of the detection signal, through either conjugation of a greater number of signalling molecules to the binding protein or amplification of the signalling molecules themselves.

Protein microarrays are primarily a fluorescence-based technology (although chemiluminescent detection is also used); commonly organic dyes such as the cyanine dyes (GE Healthcare) are directly conjugated through NHS esters to the protein sample[10]. Whilst the dye to protein ratio is maintained at 2:1 (minimum standard), this is directly affected by differences in the available or exposed amine groups of the proteins in a sample. However, alternate dyes and conjugation methods are now available, such as the Alexa Fluor (Invitrogen) dyes and universal linkage system (Kreatech);



so too are fluorescent nanoparticles (Quantum Dots) and dendrimers (Genisphere) which have improved fluorescent properties.

Several additional amplification protocols have also been developed to reveal subnanomolar concentrations of target proteins. Rolling circle amplification (RCA) which utilises the covalent attachment of an oligonucleotide to a secondary antibody, and tyramide signal amplification (TSA), which uses the catalytic activity of horseradish peroxidase (HRP)[11], have dramatically increased the performance of protein microarray assays to the femtomolar range[5].

The most significant challenge in detecting protein-protein interactions in complex solutions such as whole blood, serum or plasma is the dynamic range of pathogen load or protein concentration. Protein levels can differ by nine orders of magnitude, whilst most current analytical instrumentation can measure only three to four orders of magnitude[12]. Since antibody-binding constants are commonly in the nanomolar range, an antibody microarray is unlikely to have the sufficient sensitivity to directly measure low abundance targets without sample enrichment.

A great deal of care must be used in the collection and preparation of clinical samples where the target molecules are in low abundance or may in fact degrade. This in itself is perhaps the greatest hurdle in the adoption of protein-based assays as clinical diagnostics; especially in the veterinary field where samples are often collected under sub-optimal conditions in remote locations. Of course, the signal amplification protocols outlined might overcome this; however, an alternative is to again take advantage of stable surrogate protein markers[12].

Due to the complexity of the proteome, in both diversity and dynamic range, the development of clinical protein microarrays is likely to be slow and troublesome. However, protein microarrays could provide a practical means to quantify thousands of different proteins in clinical and research applications. The current body of knowledge clearly demonstrates the enormous potential of protein microarrays as clinical diagnostics, and it is most likely that these will be adopted by microbiology laboratories in due time.

References

1. Zhu H & Snyder M. Protein chip technology. *Curr Opinion Chem Biol* 2003; 7:55-63
2. Hanash S. Disease proteomics. *Nature* 2003; 422:226-32
3. Ekins RP. Multi-analyte immunoassay. *J.Pharm Biomed Anal* 1989; 7:155-68.
4. Walter G, Bussow K, Cahill DJ, Lueking A & Lehrach H. Protein arrays for gene expression and molecular interaction screening. *Curr Opinion Microbiol* 2000; 3:298-302.
5. Bacarese-Hamilton T, Bistoni F & Cristani A. Protein microarrays: from serodiagnosis to whole proteome scale analysis of the immune response against pathogenic microorganisms. *BioTechniques* 2002; 33:S24-S29.
6. Zhou FX, Bonin J & Predki PF. Development of functional protein microarrays for drug discovery: Progress and challenges. *Comb Chem High Throughput Screening* 2004; 7:539-46.
7. Abdo M, Irving B, Hudson P & Zola H. Development of a cluster of differentiation antibody-based protein microarray. *J Immunol Methods* 2005; 305:3-9.
8. Corti A, Poiesi C, Merli S & Cassani G. Tumor necrosis factor (TNF) alpha quantification by ELISA and bioassay: effects of TNF alpha-soluble TNF receptor (p55) complex dissociation during assay incubations. *J Immunol Methods* 1994; 177:191-8.
9. Mezzasoma L, Bacarese-Hamilton T, Di Cristini M, Rossi R, Bistoni F & Crisanti A. Antigen microarrays for serodiagnosis of infectious diseases. *Clin Chem* 2002; 48:121-30.
10. Espina V, Woodhouse EC, Wulfkuhle J, Asmussen HD, Petricoin EF & Liotta LA. Protein microarray detection strategies: focus on direct detection technologies. *J Immunol Methods* 2004; 290:121-33.
11. Haab BB. Methods and applications of antibody microarrays in cancer research. *Proteomics* 2003; 3:2116–22.
12. Campbell CJ & Ghazal P. Molecular signatures for diagnosis of infection: application of microarray technology. *J Appl Microbiol* 2004; 96:18-23.

MICRO-FACT

The majority of organisms cannot be seen with the naked eye.