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Horizon scanning prioritising summary

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Affymetrix DNA microarrays

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Enquiries about the content of this summary should be directed to:

HealthPACT Secretariat
Department of Health and Ageing
MDP 106
GPO Box 9848
Canberra ACT 2606
AUSTRALIA

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This *Horizon scanning prioritising summary* was prepared by Adriana Parrella, Linda Mundy and Janet Hiller from the National Horizon Scanning Unit, Adelaide Health Technology Assessment, Discipline of Public Health, Mail Drop 511, University of Adelaide, South Australia, 5005.

UPDATE PRIORITISING SUMMARY

REGISTER ID: **0000004**

NAME OF TECHNOLOGY: **DNA MICROARRAYS**

PURPOSE AND TARGET GROUP: **ABILITY OF DNA MICROARRAYS TO PREDICT CANCER OUTCOMES**

STAGE OF DEVELOPMENT (IN AUSTRALIA):

☐	Experimental	☐	Established
☒	Investigational	☐	Established *but* changed indication or modification of technique
☐	Nearly established	☐	Should be taken out of use

INTERNATIONAL UTILISATION:

COUNTRY	LEVEL OF USE		
	Trials Underway	Limited Use	Widely Diffused
Systematic review, Greece	✓		
Case series, breast cancer outcomes, Taiwan	✓		
Case series, breast cancer, Sweden	✓		

IMPACT SUMMARY:

DNA microarrays are a relatively new technology that is widely used in the research environment. They allow the simultaneous, rapid, characterisation of thousand of genes and as such are useful tools in categorising diseases such as cancer by determining the presence or absence of particular genes, which may provide important biological, diagnostic and prognostic information. It is believed that DNA microarrays may be able to predict individual outcomes in cancer patients and research in this area is being undertaken (Haviv & Campbell 2002, Ntzani & Ioannidis 2003). DNA microarrays are not currently used for *diagnostic* purposes in Australia.

There are two microarray technologies currently in use: spotted arrays, first developed at Stanford University in 1996, and oligonucleotide arrays, pioneered by the US based company Affymetrix. The Affymetrix system is based on the use of short oligonucleotides produce in-situ by a photolithographic process, with each gene represented by many oligonucleotides. Specificity is increased using the Affymetrix system (Haviv & Campbell 2002).

The spotted array system involves the binding of the target DNA, either a gene or a portion of the gene sequence of interest, to a glass slide in a grid-like arrangement (Figure 1). These grids may contain hundreds or thousands of genes but are presently

unlikely to contain the entire human genome (Haviv & Campbell 2002). Target DNA may be a "home-made" polymerase chain reaction (PCR) product or may be purchased commercially as sets of up to 20,000 human genes (personal communication Dr Greg Goodall, Institute of Medical and Veterinary Science, Microarray Facility). In the spotted array system, mRNA is isolated from 2 samples and converted into fluorescently tagged cDNA (single stranded DNA complementary to mRNA): the sample of interest may be tagged red (eg breast cancer sample) and a normal sample, tagged green. The 2 populations are mixed and hybridised to the DNA microarray. Unbound cDNA is washed away and a laser scanner measures the intensity of the remaining spot bound with cDNA. The data is translated to give a relative level of a particular gene transcript in both the test and normal samples (Haviv & Campbell 2002, Campbell 2003).

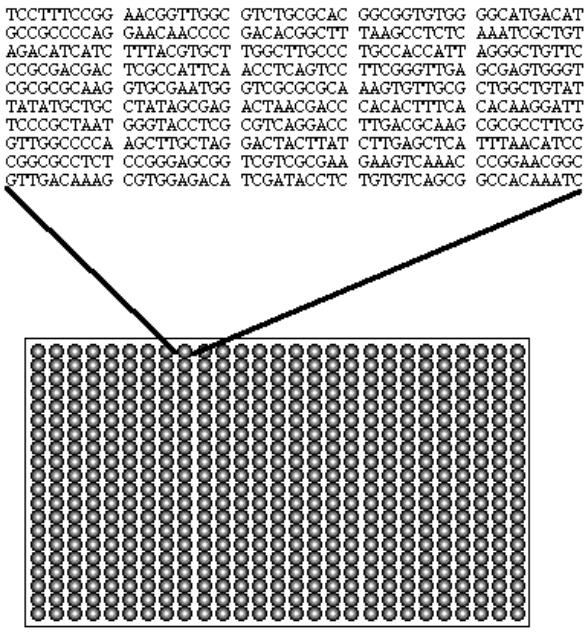

Figure 1. Example of a DNA microarray or chip, with a single gene sequence highlighted (Campbell 2003)

The systematic review by Ntzani & Ioannidis (2003) assessed the ability of DNA microarrays to predict major clinical outcomes such as death, recurrence and response to therapy, in malignant disease. They assessed 84 case series studies and found the predictive performance of microarrays to be variable due to a lack of validation and clinical design. However, they did find that significant associations were 3.5 (95% CI 1.5-8.0) times more likely with a doubling of the sample size and 9.7 (95% CI 2.0-47.0) times more likely with a 10-fold increase in microarray probes.

Huang et al (2003) used DNA microarrays in a case series to predict breast cancer outcomes. Breast cancer is highly heterogeneic due to the genetic complexity of individual tumours, therefore lymph node status at diagnosis is used as a surrogate measure for predicting breast cancer recurrence. Unfortunately lymph node status is not always detectable in all patients and 22-33% of these patients may go on to develop a recurrence of breast cancer. Huang et al (2003) identified patterns of gene

expression, associated with lymph node status, and used DNA microarrays to detect these meta-genes. They were 90% accurate in predicting outcomes in individual patients.

The AIHW reported 85,231 new cases of cancer (excluding skin cancers other than melanoma) in Australia in the year 2000. Of these, there were 11,400 new cases of breast cancer in Australia with a crude mortality rate of 26.1 per 100,000 in the year 2000. Breast cancer is the most common registered cancer and the leading cause of mortality for women in Australia (AIHW 2004).

Estimated costs for spotted DNA microarrays are estimated to be A$200 per DNA chip. DNA chips are produced by a robotic spotter and read by a laser scanner, which costs approximately $280,000 and A$80,000 respectively. A complete Affymetrix system would cost approximately A$250,000 with each Affymetrix slide costing A$700 (personal communication Dr Greg Goodall).

DECEMBER 2003 RECOMMENDATION:

Based on the availability of this technology in the Australian research environment, the level of clinical need and the low level evidence on prognostic accuracy, HealthPACT thereforer recommended that this technology be monitored.

DECEMBER 2003 SOURCES OF FURTHER INFORMATION:

Campbell, M. A. (2003). *Introduction to DNA microarrays* [Internet]. Department of Biology, Davidson College. Available from: http://www.bio.davidson.edu/Biology/macampbell/strategies/chipsintro.html [Accessed 14th January 2004].

Gruvberger, S. K., Ringner, M. et al (2003). 'Expression profiling to predict outcome in breast cancer: the influence of sample selection', *Breast Cancer Res,* 5 (1), 23-26.

Haviv, I. & Campbell, I. G. (2002). 'DNA microarrays for assessing ovarian cancer gene expression', *Mol Cell Endocrinol,* 191 (1), 121-126.

Huang, E., Cheng, S. H. et al (2003). 'Gene expression predictors of breast cancer outcomes', *Lancet,* 361 (9369), 1590-1596.

Ntzani, E. E. & Ioannidis, J. P. (2003). 'Predictive ability of DNA microarrays for cancer outcomes and correlates: an empirical assessment', *Lancet,* 362 (9394), 1439-1444.

Pusztai, L., Ayers, M. et al (2003). 'Clinical application of cDNA microarrays in oncology', *Oncologist,* 8 (3), 252-258.

Schena, M. (1996). 'Genome analysis with gene expression microarrays', *Bioessays,* 18 (5), 427-431.

Schena, M., Heller, R. A. et al (1998). 'Microarrays: biotechnology's discovery platform for functional genomics', *Trends Biotechnol,* 16 (7), 301-306.

Vigano, A., Dorgan, M. et al (2000). 'Survival prediction in terminal cancer patients: a systematic review of the medical literature', *Palliat Med,* 14 (5), 363-374.

SEARCH CRITERIA TO BE USED:

Neoplasms/*genetics/pathology/surgery/*mortality/diagnosis
Gene Expression Profiling/methods

*Oligonucleotide Array Sequence Analysis/*methods
Predictive Value of Tests
Prognosis
Survival Rate
Gene Expression Profiling
Gene Expression Regulation

HEALTH PACT DECISION:

- ☐ Horizon Scanning Brief
- ☒ Monitor
- ☐ Refer
- ☐ Full Health Technology Assessment
- ☐ Archive

JANUARY 2007 – UPDATE - EFFECTIVENESS AND SAFETY ISSUES

Since the original prioritising summary was published in 2003, Affymetrix, in partnership with Roche Diagnostic, have developed and released the CYP450 AmpliChip Test (personal communication Millenium Science, 21st September 2006). The AmpliChip CYP 450 test is the first FDA approved *in vitro* diagnostic test to use microarray technology for the comprehensive analysis of two genes that can influence drug efficacy and adverse drug reactions (FDA 2006). The The AmpliChip CYP450 detects genetic variations in the cytochrome P450 2D6 and 2C19 (CYP2D6 and CYP2C19) genes and provides the associated predictive phenotype (poor, intermediate, extensive, or ultra-rapid metaboliser of drugs).

Many drugs, including: antidepressants, anti-pyschotics, anti-arrhythmics, analgesics, β-blockers, anticonvulsants, proton pump inhibitors and some anti-cancer drugs, are primarily metabolised by the enzymes encoded by these two genes. It is suggested that predictive phenotype information may be used to aid clinicians in determining individual dosages for therapeutics metabolised by the CYP2D6 or CYP2C19 genes. This may improve patient outcomes by reducing adverse drug reactions and improving drug efficacy (Jain 2005). The potential benefit of the AmpliChip CYP450 is that it may override the need for lengthy trial-and-error approaches for optimising drug therapy and physicians may achieve earlier success using their patient's metabolic profile as a guide to dosage.

All available publications and abstracts obtained at the time of preparing this update either discuss the *reliability* of the test compared to other methods for genotyping or in the *potential* clinical applications in the following 5-10 years (de Leon 2006, de Leon et al 2006a, de Leon et al 2006b, Jain 2005 and Juran 2006). To date, there are no published studies that demonstrate improved patient outcomes as a result of the Amplichip determining phenotype status. A recent review found no evidence that testing for CYP450 polymorphisms in adults entering selective serotonin reuptake inhibitor (SSRI) treatment for depression leads to improvement in patient outcomes versus not testing, or in influencing depression management decisions by patients and clinicians (AHRQ 2006a).

Two studies were identified that examined the accuracy of the test in detecting genetic variations compared to more standard methods of genetic testing (Chou et al 2003 and FDA 2005). In one of these studies (level III-1 diagnostic evidence) the genotype reliability of CYP450 test (an earlier version of the AmpliChip) was compared with allele-specific PCR (AS-PCR) methods at five major allelic sites on the CYP2D6 *3, *4, *6, & and *9 gene alleles in 236 healthy people (Chou et al 2003). The alleles showed a high degree of concordance (>99%) between the CYP450 Genechip® and AS-PCR methods.

In addition, the study combined CYP2D6 genotype from both methods to divide the samples into the four phenotypes, poor, intermediate, extensive and ultra-rapid metabolisers, assessed by urinary elimination of dextromethorpan (DXT) over 8 hours to ascertain urinary metabolic ratio (MR).

The concordance between the AmpliChip CYP450 Test and established methods of gene detection (allele-specific PCR, PCR-RFLP and DNA sequencing) for CYP2C19 alleles in the FDA studies (level III-2 diagnostic evidence) was 99.6% (United States Food and Drug Administration 2005). The specificity of the AmpliChip CYP450 test was 100% for the CYP2C19 gene.

OTHER ISSUES

Affymetrix is also developing other genetic tests using microarray technology for applications in disease diagnosis and stratification. Two recent studies (level III-2 diagnostic evidence) reported that the Affymetrix arrays accurately distinguished Burkitt's lymphoma from diffuse large B-cell lymphoma compared to diagnosis by a pathologist (Dave et al 2006 and Hummel et al 2006). The authors suggest the ability to accurately classify Burkitt's lymphoma and diffuse large B-cell lymphoma is difficult to do by standard methods. Correct classification may improve clinical decision making as Burkitt's lymphoma and diffuse large B-cell lymphoma require different treatments. In both of these studies 12 and 34 percent of cases were misclassified by pathologists following established criteria for diagnosis. The microarray method correctly identified and classified these patients.

The manufacturer is currently exploring future applications of the microarray technology in detecting genetic causes of diseases. Early unpublished studies examine its use in diseases such as autism, diabetes and Alzheimer's (Technology Review 2006).

Roche Diagnostics are currently testing the AmpliChip in large, international multi-centre clinical trials to classify and correlate outcomes in leukaemia patients (AHRQ 2006).

JANUARY 2007 – CONCLUSION:

These studies highlight the question of whether the classifications defined by gene-expression are more clinically homogenous than those defined by standard clinical criteria and at this stage a definitive answer is not possible. All of the studies report that the Affymetrix technology has not widely diffused into clinical practice due to cost and practical implications but do emphasise clinical potential.

HEALTHPACT ACTION:

Although this prioritising summary is based on a single DNA microarray technology, the Affymetrix, a number of DNA microarrays are currently being developed and trialled in clinical situations. Many of these technologies are concerned with the over expression of genes and may provide information into the prognosis of particular diseases including breast and colon cancer. Therefore HealthPACT has recommended that an Emerging Technology Bulletin be conducted into all DNA microarrays.

LIST OF STUDIES INCLUDED

Level III-1 diagnostic evidence	1
Level III-2 diagnostic evidence	1

JANUARY 2007 - SOURCES OF FURTHER INFORMATION:

AHRQ (2006a). *Testing for Cytochrome P450 Polymorphisms in Adults With Non-Psychotic Depression Treated With Selective Serotonin Reuptake Inhibitors (SSRIs)* [Internet] Agency for Healthcare Research and Quality. Available from: http://www.ahrq.gov/downloads/pub/evidence/pdf/cyp450/cyp450.pdf [Accessed 8th January 2007].

AHRQ (2006b). Genetic Tests for Cancer, Agency for Healthcare Research and Quality, Maryland, United States, http://www.ahrq.gov/clinic/ta/gentests/gentests.pdf.

Chou, W. H., Yan, F. X. et al (2003). 'Comparison of two CYP2D6 genotyping methods and assessment of genotype-phenotype relationships', Clin Chem, 49 (4), 542-551.

Dave, S. S., Fu, K. et al (2006). 'Molecular diagnosis of Burkitt's lymphoma', N Engl J Med, 354 (23), 2431-2442.

de Leon, J. (2006). 'AmpliChip CYP450 test: personalized medicine has arrived in psychiatry', Expert Rev Mol Diagn, 6 (3), 277-286.

de Leon, J., Armstrong, S. C. & Cozza, K. L. (2006a). 'Clinical guidelines for psychiatrists for the use of pharmacogenetic testing for CYP450 2D6 and CYP450 2C19', Psychosomatics, 47 (1), 75-85.

de Leon, J., Susce, M. T. & Murray-Carmichael, E. (2006b). 'The AmpliChip CYP450 genotyping test: Integrating a new clinical tool', Mol Diagn Ther, 10 (3), 135-151.

Food and Drug Administration. (2006). *Amplichip CYP450 Test for CYP2C19 510k Summary* [Internet]. Food and Drug Administration. Available from: http://www.fda.gov/cdrh/pdf4/k043576.pdf [Accessed 8th November 2006].

Hummel, M., Bentink, S. et al (2006). 'A biologic definition of Burkitt's lymphoma from transcriptional and genomic profiling', N Engl J Med, 354 (23), 2419-2430.

Jain, K. K. (2005). 'Applications of AmpliChip CYP450', Mol Diagn, 9 (3), 119-127.

Juran, B. D., Egan, L. J. & Lazaridis, K. N. (2006). 'The AmpliChip CYP450 test: principles, challenges, and future clinical utility in digestive disease', Clin Gastroenterol Hepatol, 4 (7), 822-830.

Technology Review. (2006). *On Autism's Trail.* [Internet] Technology Review. Available from: http://www.technologyreview.com/BioTech/17699/ [Accessed 13th December 2006].