



A New Paradigm for Transfusion Medicine HEA BeadChip™

Human Erythrocyte Antigen Genotyping

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Red blood cell antigen Genotyping using Bioarray Solutions BeadChip™ DNA Microarray

Introduction

The association of the majority of red blood cell (RBC) antigens with single-nucleotide polymorphisms (SNPs)¹ provides the basis for determining blood group antigen expression at the deoxyribonucleic acid (DNA) level. Molecular immunohaematology, by invoking DNA analysis, not only reduces the need for increasingly rare serologic reagents, but also permits the reliable determination of a phenotype in situations that are difficult to resolve by serologic methods, especially when the available antibody reagents are only weakly reactive. These applications of DNA analysis include the determination of antigens with weak or altered expression, the analysis of patients in a variety of conditions including, e.g., chemotherapy or transfusion therapy, and the identification of foetuses at risk for haemolytic disease of the newborn². In the context of immunohaematology, DNA analysis has been used previously to screen for allele combinations by amplification of selected mutations and polymorphisms by sequence specific primers and to analyse specific alleles by allele specific polymerase chain reaction (PCR), restriction fragment length polymorphism analysis, or real-time PCR³. Although these methods have led to advances in the identification of alleles, they are not ideal for a large-scale clinical application given their complexity, labour-intensive format, and low throughput. Most recently, when implemented in an array format, high-throughput DNA analysis was shown to permit the rapid determination of entire sets of designated SNPs associated with platelet and minor blood group antigens^{3,6}. The BeadChip™ format (BioArray Solutions, Warren, NJ) presented here adopts a bead assay format, long established in diagnostic applications^{7,8}, to optimise assay performance and combines it with a novel method of bead array assembly to produce random encoded microparticle arrays that have been shown to permit rapid customisation and to provide reliable performance in the clinical setting. Thus, the BeadChip™ format has been applied to nucleic acid as well as to protein analysis for a variety of applications, including immuno-haematology, the analysis of both donor and patient samples, transplantation medicine, genetic disease carrier screening, including the application to neonatal screening, and autoantibody profiling.

<http://www.BioArrayS.com>

Over the past two years, BioArray Solutions (BAS) has established a significant and growing presence in immuno-haematology as a direct result of the enthusiastic adoption and support of the BeadChip™ system, and now have in place approximately 40 installations in leading medical institutions and transfusion centres in the US and overseas, for research use only.

The first UK based evaluation was undertaken at the Scottish Blood Transfusion Service, Aberdeen in collaboration with the ATMU, University of Aberdeen. The data obtained were presented at several UK based meetings but most recently at the AABB 2007⁹. The overall concordance rate for the Human Erythrocyte Antigens and Haemoglobinopathies (HEA) kit was 99.4% and concluded that the Bioarray Solutions System is a feasible and reproducible integrated flexible array solution for high complexity transfusion testing. The results have led to two further UK based transfusion services interest in evaluations; the Irish Blood Service, Dublin and the Welsh Blood Service, Cardiff. These will take place in April/May 2008 and May/June 2008, respectively.

The emerging presence of BAS in the market has attracted the attention of Immucor Gamma, a leading player in the transfusion diagnostics business, and has recently signed a merger agreement with BAS. Immucor Gamma's proven capabilities in providing automated platform solutions, and its FDA licensing expertise, established distribution network, and financial resources will collectively facilitate a more rapid and extensive introduction of the BeadChip™ products to the diagnostic market. Current kits in the BloodChip™ range include; HEA, RhD Variant, RhCE Variant, HPA and HLA.

The BeadChip™ Format

Making BeadChip™ platform

BeadChip™ manufacturing entails three fundamental operations, namely: producing collections ("libraries") of encoded microparticles ("beads") functionalised with allele-specific oligonucleotides (or other capture moieties); pooling aliquots of bead suspensions and assembling arrays by forming and immobilising monolayers of beads in designated areas of a patterned silicon wafer (or wafer section); singulating the wafer section into individual chips; and placing sets of chips into a desired configuration, e.g., a 1 x 8 configuration, or an 8 x 12 configuration on a carrier (Fig.1).

Using BeadChip™ platform

The BeadChip™ assay protocol, as previously described⁶, includes a multiplexed PCR reaction, post-PCR processing to generate single-stranded targets, and on-chip allele identification by way of enzyme-mediated probe elongation. That is, allele-specific probes containing variable 3' termini matching either the normal or a variant allele act as nested primers for the

simultaneous elongation of matching probes by a DNA polymerase. Each bead produces an assay signal reflecting the incorporation of fluorescently labelled dNTPs into the elongation products displayed on that bead. Using an automated array imaging system provided by BioArray Solutions, assay signal intensity patterns are recorded from sets of chips and passed to software for automated analysis. This analysis produced a reference table for genotype-to-phenotype conversion, showing the combinations of SNPs at one or more variable sites probed by the BeadChip™ panel, to antigen expression state (Table 2).

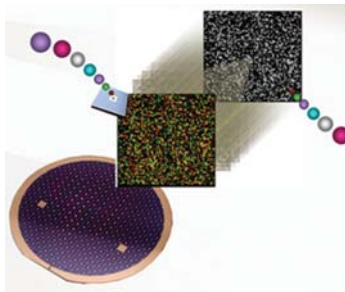
The signal intensities produced by paired probes are converted into discrimination ratios, and these in turn are converted to phenotypes by reference to look-up tables, thereby eliminating manual transcription. The assay signal intensities produced for each SNP by the corresponding pair of elongation probes provides the basis for allele discrimination as described previously⁶. The BeadChip™ system thus includes kits providing complete assay protocols, and a proprietary assay delivery system providing automated "snapshot" array imaging and web-hosted data management <http://www.BioArrays.com>

Discussion

The potential for saving on expensive and declining stocks of rare antisera with limited sensitivity, labour/time costs associated with current serological testing make this microarray based technology a more attractive alternative for the immuno-haematology service laboratory. The ability to test a large number of donors simultaneously for several antigens, in combination with computerised analysis and interpretation of data, makes it feasible to maintain a diverse inventory of well characterised donors. For patients, the ability to reliably determine antigen expression corresponding to the phenotype, regardless of clinical condition and therapeutic regimen, holds the potential to substantially reduce delays (for patients having multiple antibodies) incurred under the current "Type & Screen" paradigm in procuring compatible blood units for transfusion¹⁰. As with the current paradigm, compatibility is to be confirmed by an antiglobulin crossmatch. With the inclusion of polymorphisms to identify RhCcEe haplotypes and corresponding antigen expression, the current HEA panel permits the simultaneous analysis of 28 antigens in 11 blood group systems.

The routine determination of an extended HEA phenotype by DNA analysis of donors and recipients will make it possible to consider a new paradigm for the selection of donors for given recipients on the basis of the respective antigen repertoires.

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 Quest Biomedical Limited



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TABLE 1.

Composition of HEA BeadChip™ format

Blood factor	Analyte	Polymorphism
Rh	Cc	203A>G, Int 2
	Ee	676G>C
Kell	K/k	698T>C
Kidd	Jk ^a /Jk ^b	838G>A
Duffy	Fy ^a /Fy ^b	FY125G>A
Duffy-GATA	Silencing FY	FY-33T>C
	Fy ^a (Fy[b+ ^w])	265C>T
MNS	GYPA (M/N)	60C>T
	GYPB (S/s)	143T>C
	GPB Silencing	230C>T, +5 g>t
Lutheran	Lu ^a /Lu ^b	230A>G
Diego	Di ^a /Di ^b	DI2561T>C
Colton	Co ^a /Co ^b	CO134C>T
Dombrock	Do ^a /Do ^b	DO793A>G
	Jo(a+ ⁻)/Jo(a- ⁻)	DO350C>T
	Hy+ ⁻ /Hy- ⁻	DO323G>T
Landsteiner-Wiener	LW ^a /LW ^b	LW308A>G
Scianna	Sc1/Sc2	SC169G>A
Hemoglobin S	HbS	173A>T

TABLE 2.

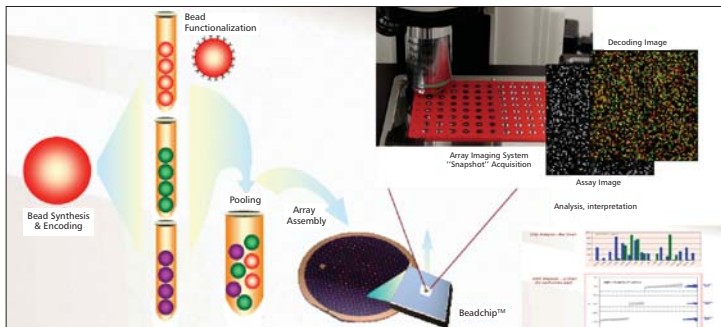
Example of genotype-to-phenotype conversion tables

	KEL(698T>C)	JK(838G>A)	DI(2561T>C)	CO(134C>T)
AA	K+k-	Jk(a+b-)	Di(a+b-)	Co(a+b-)
AB	K+k+	Jk(a+b+)	Di(a+b+)	Co(a+b+)
BB	K-k+	Jk(a-b+)	Di(a-b+)	Co(a-b+)

	LW(308A>G)	LU(230A>G)	SC(169G>A)	HGBS(173A>T)
AA	LW(a+b-)	Lu(a+b-)	Sc:1,2	SCT-
AB	LW(a+b+)	Lu(a+b+)	Sc:1,2	SCT+
BB	LW(a-b+)	Lu(a-b+)	Sc:-1,2	HgbSS

* SCT = sickle cell trait.

Fig. 1. Illustration of BeadChip™ system showing the bead assembly protocol on silicon wafer, array imaging system, and decoding and assay images. Also shown is an example of HEA data analysis charts generated automatically by BeadChip™ analysis software using web-based software (wHEA; BioArray Solutions, Warren, NJ), showing 28 antigen states derived from DNA analysis, linked to a BeadChip™ carrier-ID and sample-ID, stored in a database.



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