

Molecular Blood Grouping and it's applications in Transfusion Medicine

Dr Gautam Wankhede

**Presented at “Enhancing Blood Safety”, Transfusion Medicine CME
Government Medical College, Chandigarh. March 2012**

Molecular Biology in Blood Group typing

- Nearly 300 blood group specificities on red cells are known, many of which are polymorphic
- Molecular mechanisms responsible for these polymorphisms are diverse
Majority represent single nucleotide polymorphisms (SNPs), gene deletion; single nucleotide deletion & sequence duplication, which introduce reading-frame shifts; nonsense mutation, etc
- Knowledge of the molecular bases to blood group provides a means to predict blood group phenotype with a high degree of accuracy

Common terms used in Molecular Biology

Gene – sequence of nucleotides in the DNA that provides the coded instructions for RNA synthesis, which translated into protein, leads to expression of a blood group

Locus - the place a gene occupies on a chromosome

Allele - Mutually exclusive forms of the same gene, usually arising through mutation, that are responsible for variation in the blood group.

Exon: The region of a gene that contains the code for producing protein. Each exon codes for a specific portion of the complete protein. Exons are separated by introns, regions of DNA that have no function.

Polymorphism - the presence of two or more distinct phenotypes in a population due to the expression of different alleles of a given gene

Primer - short single-stranded DNA sequences that are synthesized to correspond to the beginning and ending of the DNA stretch to be copied or identified

Polymerase Chain Reaction

- The polymerase chain reaction (PCR) is a technique where in a single or a few copies of a piece of DNA segment are identified and amplified
- PCR is used to amplify a specific allelic region of a DNA strand
- With the Human Genome being completely decoded, the genes which encode most (or all) of the blood group systems
- Any variation in the genetic coding can be picked up, though the physical manifestation of that variation may or may not be evident

Molecular Biology techniques for Blood grouping

- **SSP-PCR** = To detect specific sequences using primers against the targets of nucleotide sequences (Alleles) which are amplified
- **SSOP-PCR** = Amplification of DNA followed by attachment with sequence-specific oligonucleotide probe
- **RT-PCR** = Amplify and simultaneously quantify a targeted gene in a DNA
- **Microarray-PCR** = Microscopic DNA spots on a solid surface act as probes, thus this can accomplish many genetic tests in parallel
- **Sequencers** = Differentiates light signals originating from fluorochromes attached to nucleotides, and detects the sequence

Applications in Transfusion Medicine

Detect Blood Group Antigens in Patients

- Multiple/recently transfused patients blood grouping
- To distinguish an alloantibody from an autoantibody (eg, anti-e)
- To identify alloantibody when a patient's RBCs type antigen-positive and a variant phenotype is suspected (eg, anti-D in a D-positive)
- To detect weakly expressed antigens where the patient is unlikely to make antibodies to transfused antigen-positive RBCs
- Identify molecular basis of unusual serological results
- When antibody is weak or not available (eg, anti-Doa, -Dob, -Jsa, -V/VS)

Applications in Transfusion Medicine

Detect Blood Group Antigens in Donors

- Mass screening to increase antigen-negative inventory (DNA Arrays)
- To find donors whose RBCs lack a high-prevalence antigen
- To resolve blood group discrepancies
- To detect genes that encode weak antigens
- Quality Control of Antisera
- To type donors for reagent RBCs for antibody screening cells and antibody identification panels, especially RhD Zygosity

Applications in Transfusion Medicine

Detect Blood Group Antigens in AIHA cases

- The strong DAT positive cases can not be grouped
- Reduce labor-intensive procedures that are required to detect underlying antibodies each time the patient requires blood transfusion
- Type ABO, RH and Kell (+Kidd) status of the patients using Lymphocytes
- Type donors using conventional methods
- ‘Least incompatible’, but significant antigen matched blood
- SSP-PCR is a faster method than conventional work up in such cases
- Should be seen as a treatment rather than a diagnostic test

Applications in Transfusion Medicine

Identify fetus at risk for hemolytic disease of the newborn (HDFN)

- In prenatal setting to identify the fetus who is NOT at risk of HDFN
- Should be considered when a mother's serum contains an IgG alloantibody that has been associated with HDFN and the father's antigen status for the corresponding antigen is heterozygous
- A semi-invasive method such as Amniocentesis can be used
- PCR using maternal plasma has also been used to find out the fetal blood group Genotype. It is a routine procedure in many countries now.

Applications in Transfusion Medicine

Work up of discrepant samples

- Regional referral laboratories can work on such samples
- SSP-PCR is a faster method than conventional work up for discrepant samples such as adsorption-elution or family studies
- Specially useful for D Variants
- Subgroups of A & B cause many problems; Medical, Ethical and Medicolegal
- Quality Control of Antisera (by detecting reactions against variants)

Human Platelet Antigen (HPA) Typing

Platelet antigen alloimmunization can induce

1. Neonatal alloimmune thrombocytopenia (NAIT)
2. Post-transfusion purpura
3. Platelet transfusion refractoriness

HPA systems are not only associated with organ transplantation rejection and cardiovascular disease, but are also frequently assessed in general population studies.

Platelet donors can also be typed and called for donation in case of any of the above conditions. The HPA typed panel of donors (registry) is already in place in certain countries.

Human Neutrophil Antigen (HNA) Typing

Neutrophil-specific antibodies are implicated in:

Febrile non-haemolytic transfusion reactions

Transfusion-related acute lung injury (TRALI)

Neonatal alloimmune neutropenia

Autoimmune neutropenia

Persistence of post-bone marrow transplant neutropenia

Transfusion-related alloimmune neutropenia (TRAIN)

Drug-induced neutropenia

The HNA system includes 7 antigens assigned to five groups

Traditionally, HNA phenotyping has been performed by human antibodies in agglutination test or the immunofluorescence test

Procedure – SSP PCR

- BAGene kits consist of prealiquoted and dried reaction mixtures consist of allele specific primers along with internal control primers

