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Review

Is Next Generation Sequencing the future of blood group testing?

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A B S T R A C T

Blood group genotyping has many advantages over conventional phenotyping for both blood donors and patients, and a number of high-throughput methods have now been developed. However, these are limited by a requirement for existing knowledge of the relevant blood group gene polymorphisms, and rare or novel mutations will not be detected. These mutations could be successfully identified by DNA sequencing of the blood group genes, and such an approach has been made feasible by the introduction of Next Generation Sequencing (NGS) technology. NGS enables many genes from multiple samples to be sequenced in parallel, resulting in sequencing information that could be used to obtain accurate blood group phenotype predictions in both blood donors and patients.

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1. Introduction

Accurate typing of both blood donors and recipients is essential to prevent alloimmunisation of patients following blood transfusion. This is particularly important when considering transfusion-dependent patients, who are at significant risk of making red-cell alloantibodies, rendering future transfusions increasingly problematic. Blood provision for such patients requires red cell units which have undergone extended serological typing for relevant minor antigens, in addition to those routinely typed. In NHS Blood and Transplant (NHSBT), the transfusion service in England, routine serological typing for all donors

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comprises ABO, D, C, c, E, e and K. This routine phenotyping is performed by large scale automation on the PK7300 platform (Beckman Coulter). This instrument identifies the sample by way of a 128bit barcode, performs the test, interprets the result and transfers this directly to the Laboratory Information Management System (LIMS). This highly automated process requires minimal human interaction, enabling a high degree of accuracy combined with high throughput (1000 samples per day per machine) and low cost.

Extended phenotyping is then performed on a subset of these samples. All R_0 donors, and Group O, A and B repeat donors who are R_1R_1, R_2R_2 and rr are selected. Samples from donors of unknown extended phenotype are selected to fill the run to a suitable size. These samples are tested for $Jk^{a/b}$, M, S and C^w, with some of the same samples also being tested on a HemOS SP (Bio-Rad) for some, or all, of the antigens $Fy^{a/b}$, s, k, Kp^a and Lu^a. In 2012 NHSBT performed extended phenotyping in this way on approximately 200,000 donations.

Genotyping is not currently used routinely in the UK for assignment of donor blood groups, although some other countries are beginning to implement this [1,2]. There are a number of well-documented reasons for considering a move away from dependence on donor phenotyping to molecular testing [3,4]. Extended phenotyping in NHSBT requires considerable human interaction to locate and separate the correct samples for testing, which invariably increases both cost and error rate. In some instances there is limited availability of good quality serological reagents, e.g. anti-Do^a and -Do^b. Some phenotypes such as hr^B negative and hr^S negative are difficult to accurately detect serologically and weakly expressed antigens such as Fy^X may not be detected at all.

An important caveat regarding the ABO system must be stated when discussing the use of molecular methods for blood group testing. At present, molecular methods are not sufficiently accurate for ABO typing due to the extreme complexity of the ABO system, where gene rearrangements are common. The interpretation of ABO genotyping is further complicated by the inability of genotyping methods to resolve cis/trans ambiguities. The potentially fatal consequences of mistyping ABO, coupled with the availability of rapid, reliable and cheap serological typing, means that genotyping is unlikely to displace ABO phenotyping in the near future.

Molecular techniques are rapidly becoming the method of choice for determination of fetal blood groups in pregnancies at risk of haemolytic disease of the fetus and newborn (HDFN). The use of cell free fetal DNA derived from maternal plasma eliminates the need for invasive chorionic villus or amniotic fluid sampling. Blood group genotyping is also becoming more widely used for patients – especially those who are multiply transfused. UK guidelines state that patients who are likely to become transfusion dependent should undergo a full cell phenotype prior to transfusion; however, this is not always possible. If phenotyping is not performed then these patients should be genotyped for the blood group antigens D, C, c, E, e, K/k, $Jk^{a/b}$, $Fy^{a/b}$, M, N, S, s and the FyGATA mutation. Transfusion dependent patients negative for D, C, c, E, e or K antigens should receive units that are correspondingly negative for these antigens. This is considered to limit the incidence of antibody production but, interestingly, these patients do not appear to make fewer antibodies when transfused with blood from donors of a similar ethnic background [5] presumably due to the high degree of heterogeneity of, primarily, the *RH* genes within the ethnic population. Increased use of genotyping in patients to more accurately determine extended blood groups leads, in turn, to increased demand for a wider, accurately typed pool of donors to provide better matched blood for these patients.

2. Molecular testing: current methodologies

There are a number of existing methods available for medium to high-throughput red cell genotyping, using various techniques to identify the nucleotide at the position known to be responsible for each blood group antigen under investigation. Methods include nanofluidic real-time PCR [6] multiplex ligation-dependent probe amplification [7] and MALDI-TOF mass spectrometry [8].

The limitation of most commonly used genotyping methods is that they are only capable of detecting those alleles included in the assay design. Novel alleles, which may alter serological expression of the antigen, are unlikely to be detected, and additional polymorphisms, within primer sites for example, might lead to allele drop-out. Known or unknown mutations not covered by the assay design may also influence antigen expression, so the genotyping result may not reflect the serological expression (for example, in the case of null alleles). DNA sequencing of the blood group genes can overcome many of these issues, as even novel alleles are likely to be detected. Mutations outside of the coding regions of the genes, which may influence expression of the corresponding proteins may be missed, but the assay can be designed to include and detect these where they are known. However, standard Sanger sequencing is a laborious, expensive and time-consuming process, certainly not applicable to high-throughput testing. Currently, in the International Blood Group Reference Laboratory (IBGRL, NHSBT, UK), Sanger sequencing may be used for samples which give aberrant serological typing results, or discrepant phenotype/standard genotype (SNP detection) results. It may also be used to aid in antibody identification cases and for the determination of rare blood groups. The blood group genes most commonly sequenced in this way are *RHD*, *RHCE*, *GYPA*, *GYPB* (MNS) and *KEL* and many novel alleles have been identified as a result [9–11]. However, this system of individually sequencing each exon of a gene is very time-consuming and requires a relatively large amount of sample DNA. In some cases, multiple genes need to be sequenced in turn before a mutation likely to cause the observed serology is identified, which is both costly and time inefficient. These problems could potentially be overcome by sequencing all relevant blood group genes simultaneously using Massively Parallel Sequencing, also known as Next Generation Sequencing (NGS).