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Development and Validation of a Fully Automated Platform for Extended Blood Group Genotyping

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Thirty-five blood group systems, containing >300 antigens, are listed by the International Society of Blood Transfusion. Most of these antigens result from a single nucleotide polymorphism. Blood group typing is conventionally performed by serology. However, this technique has some limitations and cannot respond to the growing demand of blood products typed for a large number of antigens. The knowledge of the molecular basis of these red blood cell systems allowed the implementation of molecular biology methods in immunohematology laboratories. Here, we describe a blood group genotyping assay based on the use of TKL immobilization support and microarray-based *HIFI* technology that takes approximately 4 hours and 30 minutes from whole-blood samples to results analysis. Targets amplified by multiplex PCR were hybridized on the chip, and a revelation step allowed the simultaneous identification of up to 24 blood group antigens, leading to the determination of extended genotypes. Two panels of multiplex PCR were developed: Panel 1 (KEL1/2, KEL3/4; JK1/2; FY1/2; MNS1/2, MNS3/4, *FY*Fy* et *FY*X*) and Panel 2 (YT1/2; CO1/2; DO1/2, HY+, Jo(a+); LU1/2; DI1/2). We present the results of the evaluation of our platform on a panel of 583 and 190 blood donor samples for Panel 1 and 2, respectively. Good correlations (99% to 100%) with reference were obtained. *(J Mol Diagn 2016, 18: 144—152; http://dx.doi.org/10.1016/j.jmoldx.2015.09.002)*

Today, 35 blood group systems are listed by the International Society of Blood Transfusion, which represent >300 antigens and 1568 alleles listed in the National Center for Biotechnology Information Blood Group Gene Mutation Database.[1] Most of them were cloned and sequenced,[2,3] unraveling the molecular bases of these blood group systems. Most results from single nucleotide polymorphism (SNP). Red blood cells (RBCs) carrying a particular antigen may elicit an immune response if introduced in the blood circulation of a patient who lacks this antigen. It is the antibody produced during the immune response which is problematic and leads to donor/patient transfusion incompatibility, maternal-fetal incompatibility, and autoimmune hemolytic anemia. This immune response can be immediate or delayed and, in some cases, lethal. For these reasons, antigen-negative blood is required for a safe transfusion. For decades, the method of reference for testing blood group antigens was the hemagglutination technique. This is a simple and well-established technique available for all major blood groups, with specificity, sensitivity, and security appropriate for clinical diagnostic environment. However, this gold standard method has certain limitations (immunologic reagent availability and specificity) when it comes to the determination of minor or rare blood group antigens critical to fulfill a perfect matching between patient and donor.[4] Reagents are specialized and must be obtained from immunized patients or donors (polyclonal and monoclonal antibodies) or from immunized mice (monoclonal antibodies). Cost of immunologic reagents keeps increasing, and many antibodies are not available or weakly reactive, which is not suitable for the growing need of blood products typed for a large number of antigens. Consequently, only a relatively low number of donors are typed for larger number

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of antigens, which hinders the establishment of an antigen-negative inventory. In addition, antigen expression on the surface of the RBCs is often too weak to be detected by serology, and discrepancies in serologic activity can occur between different manufacturer's reagents.[5] Finally, even if all of the necessary antibodies required to determine all of the blood group systems were available, the following problems would remain: i) it is difficult to type recently transfused patients,[6] multitransfused patients such as those with sickle cell disease,[7] or those who RBCs are coated with IgG[5]; ii) D-typing is difficult because of the high number of antigens produced by the RHD gene[8]; iii) in some cases the phenotype does not exactly represent the genotype, because of silencing alleles, (eg, for the Duffy blood group[9]); and iv) hemagglutination only gives an indirect indication of the risk and severity of anemia or hemolytic disease of the fetus and newborn.[10]

The identification of the molecular bases of most of the blood group systems paved the way for DNA-based assays as typing tools. Techniques such as allele-specific PCR or sequence-specific primer PCR and PCR-restriction fragment length polymorphism cannot be used routinely in laboratories because of their low throughput. However, in the past few years, several blood group genotyping platforms for SNP identification were developed[4] and have helped to override the limitations of serology, replacing the immunochemical reagents by synthetic and reproducible probes. These methods usually associate a multiplex PCR step, followed by detection with specific probes that correspond to the analyzed polymorphisms. Indeed, two-thirds of all blood group antigens are defined by SNPs (approximately 200 SNPs).[11] In this situation, a technologic solution on the basis of multiplex PCR which can analyze several variables at the same time is required. A few high-throughput platforms are available, namely HEA BeadChip (BioArray Solutions Ltd., Warren, NJ),[12,13] BLOODchip (Progenika, Medford, MA)[14] and Genome Lab SNP Stream (Beckman Coulter, Brea, CA),[15,16] but process in steps of these platforms can be sometimes fastidious, and test duration can be long (5 hours after extraction for BLOODchip platform).

To overcome these disadvantages, we developed a new platform, with high-throughput capabilities, and which take only 3.25 hours after extraction (or 4.5 hours from whole blood to extended blood group report), because of the use of a fully robotized process flow. It is an emerging blood genotyping technique that takes advantage of the *HIFI* technology for the assembly of microarrays with standard 96-, 384- and 1536-well plate.[17,18] It associates fully automated steps from extraction to multiplex PCR, hybridization, and reading. The format of the test is based on classical 96- or 384-well plates with each well bottom modified with an array of probe oligonucleotides. Each array is composed of a probe set (16 probes associated with Panel 1 and 14 probes associated with Panel 2) and two controls.

The whole-blood samples (Figure 1) are first processed through automated DNA extraction and then amplified with the use of biotinylated gene-specific primers. Without any pretreatment or purification step, the PCR products are then hybridized on the array and labeled to produce stained positive spots that are directly detected and quantified with

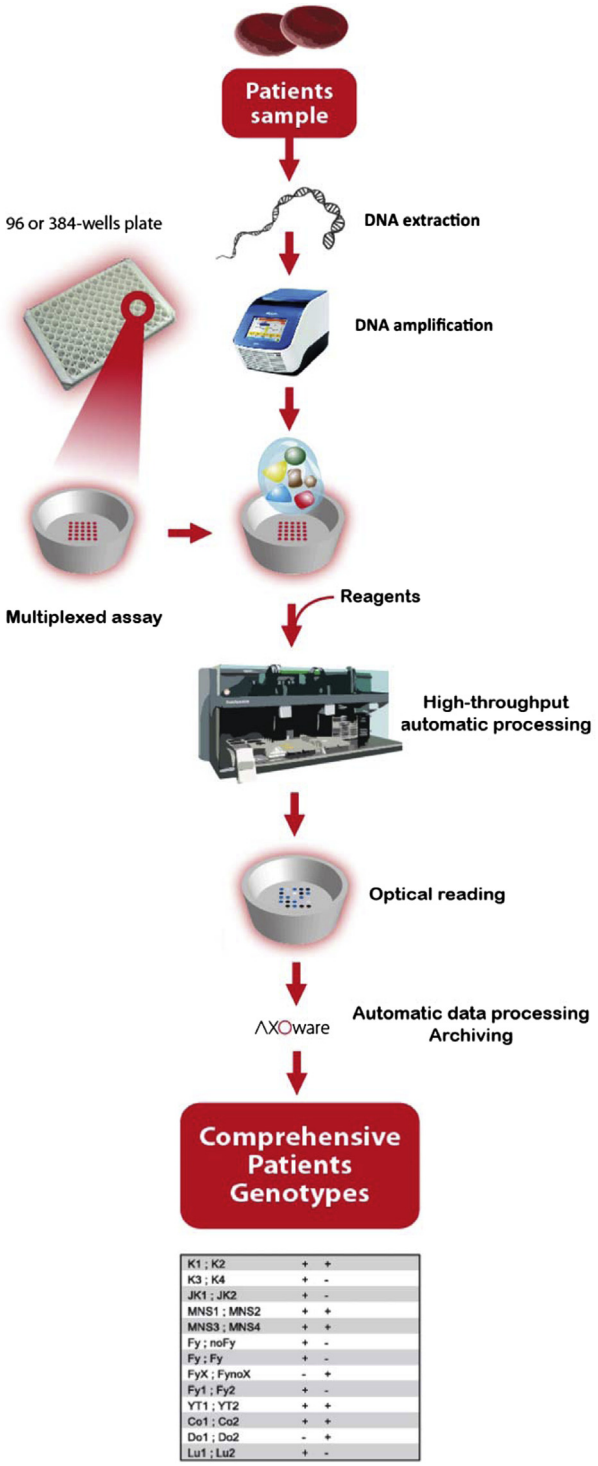

Figure 1 Principle of HIFI technology. Patient sample composed of whole blood is lyzed and used for DNA extraction before multiplex amplification. Amplified DNA sequences corresponding to blood group are then hybridized on the Microwell microarray, then labeled for signal generation with the use of colorimetric imaging. Genotypes are listed in the table.