Etablissement Français du Sang

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# Flexible automated platform for blood group genotyping on DNA microarrays

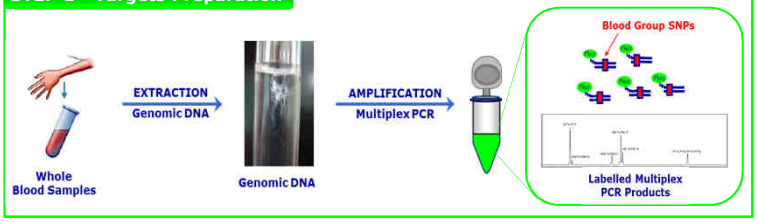

**S. Paris[1], D. Rigal[1], V. Barlet[1], M. Verdier[1], N. Coudurier[2], P. Bailly[3], J.-C. Brès*[1,4]**

[1] Établissement Français du Sang Rhône-Alpes, Lyon, France
[2] Établissement Français du Sang, La Plaine Saint Denis, France
[3] Établissement Français du Sang Alpes-Méditerranée, UMR 7268, Aix-Marseille Université, Marseille, France
[4] Établissement Français du Sang Pyrénées-Méditerranée, Laboratoire TransDiag - UMR 1058, Montpellier, France
*Contact : jean-charles.bres@efs.sante.fr

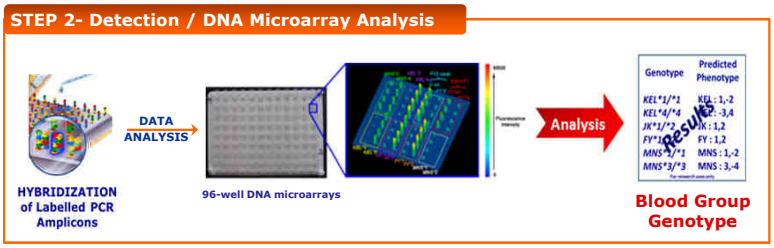

## Introduction

The standard method of testing for red blood cells (RBCs) antigens is phenotyping by antibody-based agglutination assays with automated equipment. ABO and Rhesus phenotyping of blood donors is systematically performed by blood banks, but minor blood group antigens are not currently tested on a regular basis. Part of the problem is that hemagglutination requires costly specific monoclonal reagents, and many antibodies are not available to test some minor antigens. Moreover, this serological test is a time-consuming method and is unsuited for large-scale automated screening, restricting blood banks to answer to the increasing demand of extensively typed blood components.
To overcome these limitations, we have developed an automated platform for genotyping, allowing multiplex determination of blood donor antigens, meeting Blood Bank screening laboratories requirements for testing blood donations: high-throughput, traceability and moderate cost of analysis.
Our automated DNA-based method using 96-well DNA microarrays allowed simultaneous detection of eight single nucleotide polymorphisms (SNPs) associated with clinically important blood group antigens (KEL1/KEL2, KEL3/KEL4, JK1/JK2, FY1/FY2, MNS1/MNS2, MNS3/MNS4, *FY*X* and *FY*Fy* alleles). The workflow process integrated two automated platforms, one for "pre-PCR" set-up and genomic DNA extraction, and one for the "post-PCR" procedures. The targets extracted from whole blood samples were amplified by a multiplex PCR of different blood group systems in one tube. The detection of hybridized amplicons on the microarray, via a fluorescence scanner, led to the determination of the donor genotype.

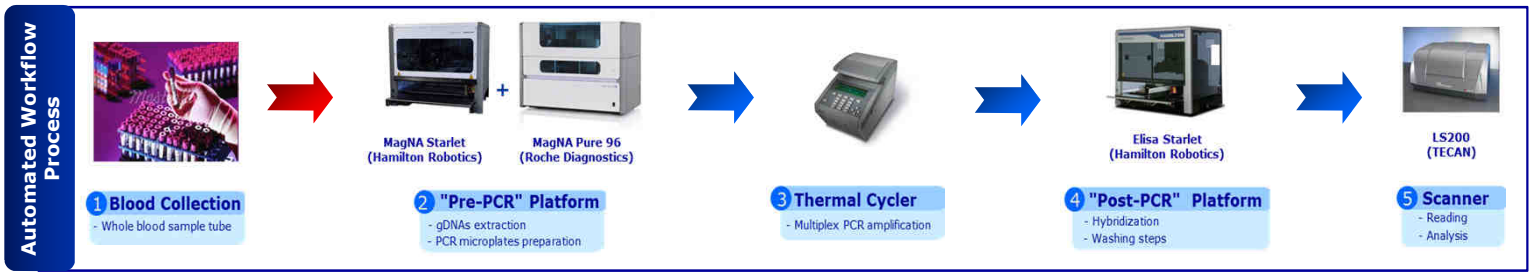
### STEP 1 - Targets Preparation




### STEP 2- Detection / DNA Microarray Analysis




## Automated Workflow Process for Multiplex Blood Group Genotyping



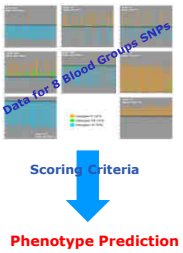

## Extended Blood Group Genotyping : Medium Scale Evaluation

### Determination of Scoring Criteria for Phenotype Prediction

- **Reference Panel : 192 blood donor samples** extensively phenotyped (Laboratoire de Qualification Biologique du Don, EFS - Rhône-Alpes)
- **4 Blood Group Systems – 12 blood group antigens :** Kell (KEL1/KEL2, KEL3/KEL4), Kidd (JK1/JK2), Duffy (FY1/FY2, *FY*X* and *FY*Fy* alleles), MNS (MNS1/MNS2, MNS3/MNS4)
- **Determination of Scoring Criteria for the 8 SNPs**

| System | Probes set | Genotype *A/*A | *A/*B | *B/*B |
|---|---|---|---|---|
| KEL | *KEL*01/KEL*02* | r > 0.59 | -0.11 < r < 0.10 | r < - 0.43 |
| | *KEL*03/KEL*04* | Undetermined* | -0.07 < r < 0.02 | r < -0.40 |
| JK | *JK*01/JK*02* | r > 0.33 | - 0.11 < r < 0.18 | r < - 0.51 |
| FY | *FY*01/FY*02* | r > 0.52 | - 0.19 < r < 0.26 | r < - 0.46 |
| | *FY*Cwt /FY*02M.01 (FY*X)* | r > 0.33 | 0.13 < r < 0.26 | Undetermined* |
| | *FY*wt /FY*02M.02 (FY*Ex)* | r > 0.21 | -0.13 < r < 0.08 | r < - 0.67 |
| MNS | *GYPA*01 (MNS*1)/GYPA*02 (MNS*2)* | r > 0.41 | 0.21 < r < 0.33 | r < - 0.20 |
| | *GYPB*03 (MNS*3)/GYPB*04 (MNS*4)* | r > 0.21 | -0.23 < r < 0.06 | r < - 0.68 |

*due to the absence of *FY*X/*X* and *KEL*3/*3* genotypes in the blood donors panel




### Results

- **819 Blood Donor Samples extensively phenotyped**
- **Use of Scoring Criteria for Genotype determination** ➡ **Phenotype Prediction**
- **Comparison between Predicted Phenotypes and Serological Phenotypes**

| System | Allele | Number of phenotyped samples | Number of invalid samples | Genotyped samples' | Concordance rate (95% confidence interval) | Number of Discordances | Discordances: Serological phenotype | Discordances: Predicted phenotype (from genotype) |
|---|---|---|---|---|---|---|---|---|
| KEL | *KEL*01/KEL*02* | 960 | 22 (2.3%) | 938 (97.7%) | 99.89 % (99.39-99.98%) | 1 | KEL : 1, -2 (*KEL*01/01*) | KEL : 1, 2 (*KEL*01/02*) |
| | *KEL*03/KEL*04* | 9 | 0 | 9 (100%) | 100% (70.09-100%) | 0 | | |
| JK | *JK*01/JK*02* | 960 | 40 (4.1%) | 920 (95.8%) | 99.89 % (99.39-99.98%) | 1 | JK : 1, -2 (*JK*01/01*) | JK : 1, 2 (*JK*01/02*) |
| FY | *FY*01/FY*02/ FY*02M.01/FY*02M.02* | 960 | 33 (3.4%) | 927 (96.6%) | 100% (99.59-100%) | 0 | | |
| MNS | *GYPA*01/GYPA*02* | 58 | 0 | 58 (100%) | 100 % (93.79-100%) | 0 | | |
| | *GYPB*03/GYPB*04* | 960 | 23 (2.4%) | 937 (97.6%) | 99.89 % (99.39-99.98%) | 1 | MNS : 3, 4 (*GYPB*03/04*) | MNS : 3, -4 (*GYPB*03/03*) |

➡ **HIGH CONCORDANCE RATE PHENOTYPE/GENOTYPE**

## Conclusion

Our results[1] show that our assay with a simple protocol, combining multiplex PCR and 96-well DNA-microarray, can provide a performant automated blood group genotyping with a low relative cost of analysis.
The predicted phenotypes from blood group genotypes showed a **high concordance rate of 99.93 %** with serological phenotypes determined by standard-agglutination assay. Only 3 discordances were found and were confirmed by another DNA-based assay (LIFECODES RBC, Immucor).
Due to the high flexibility of our method, these module of SNPs will be easily extended to other markers of interest for blood transfusion (RH, HPA, …) by creating a new combination of SNPs. The integration of this molecular diagnostic tool could improve the transfusion safety by providing fast and reliable multiparametric results while reducing the relative cost of analysis.


**Reference** [1] S. Paris, D. Rigal, V. Barlet, M. Verdier, N. Coudurier, P. Bailly, J.-C. Brès. Flexible automated platform for blood group genotyping on DNA microarrays. The Journal of Molecular Diagnostics. 2014 May; 16(3):335-342.



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