

# The European perspective: Current genotyping in Spain

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Current blood group genotyping in Spain is mostly applied to those cases in which the antigen determination is difficult or not possible.

However, genotyping methods are foreseen as a valuable alternative to serological methods and strategies for other applications, like high-throughput blood group typing are being developed.

Blood group genotyping in Spain is performed in Reference Laboratories and includes:

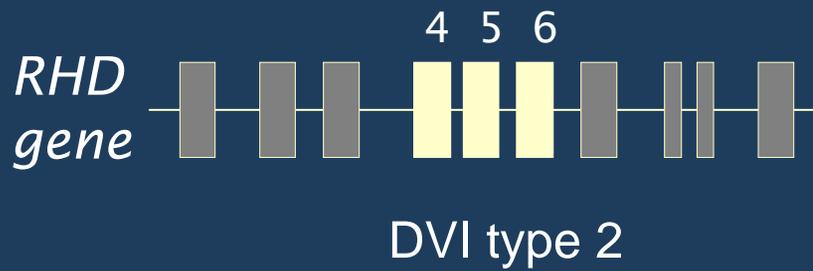
- RHD genotyping of donors (or patients) with an anomalous D antigen expression.

- RHD exon scanning

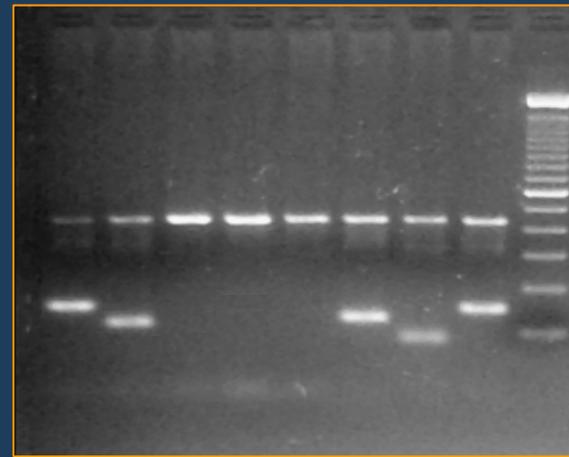
- RHCE specific polymorphisms (including intron 2)

- RHD pseudogen – PCR spanning the 37 bp insertion.

# RHD specific PCR-SSP

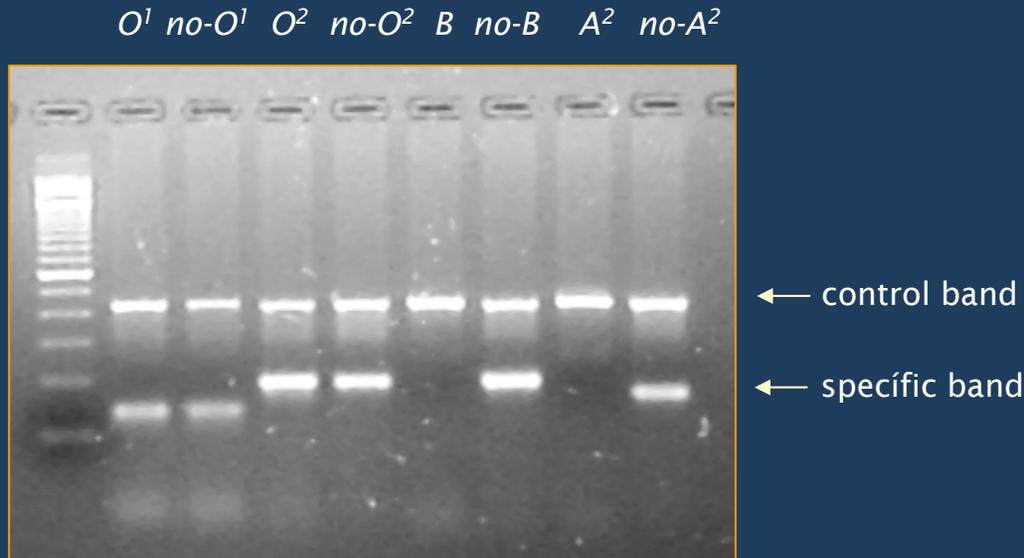


exons 2 3 4 5 6 7 9 10



Control band  
Specific band

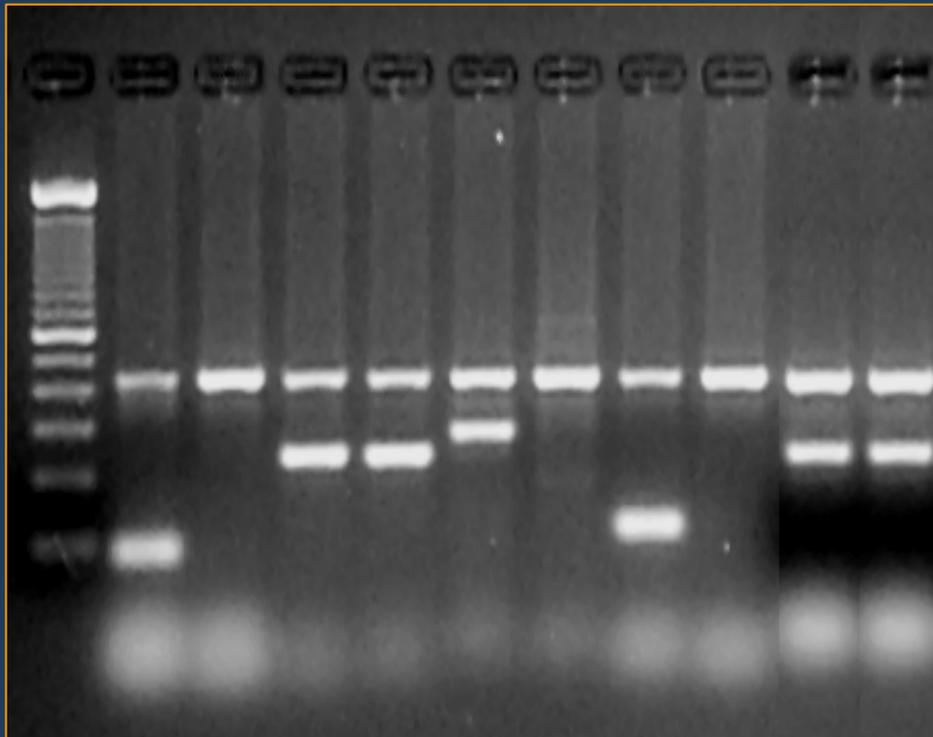
- ABO genotyping of donors (or patients) with a discrepancy in the forward and reverse grouping.



**ABO genotype: *O1/O2***

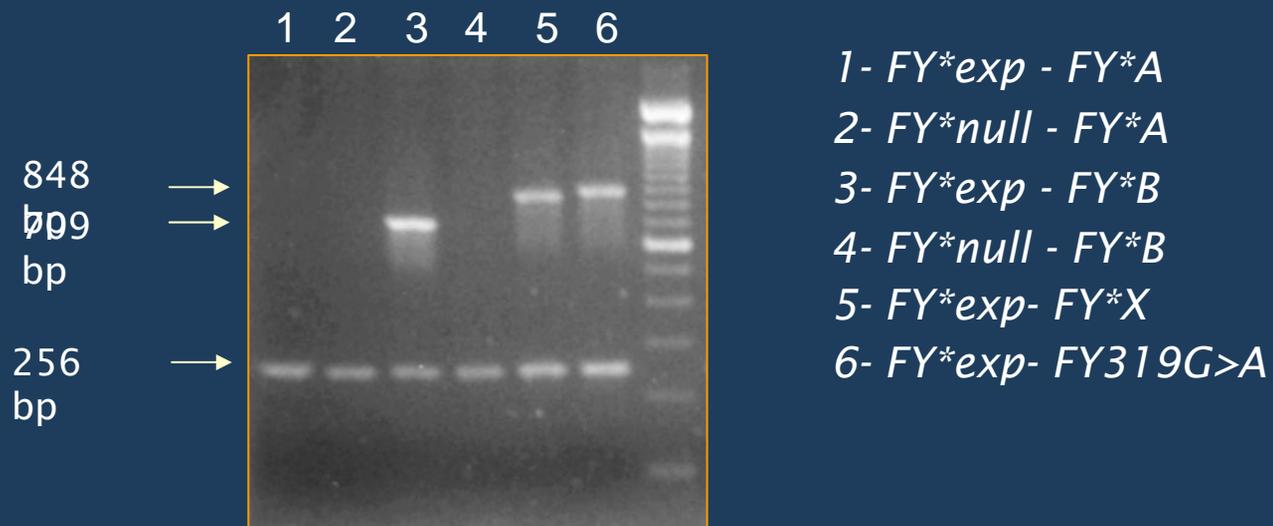
- HPA-1 typing to identify HPA-1a negative donors.

1a 1b 2a 2b 3a 3b 4a 4b 5a 5b



It is also used in blood group typing of red cell panel donors:

- For  $Fy^X$  confirmation in donors with weak  $Fy^b$  expression.

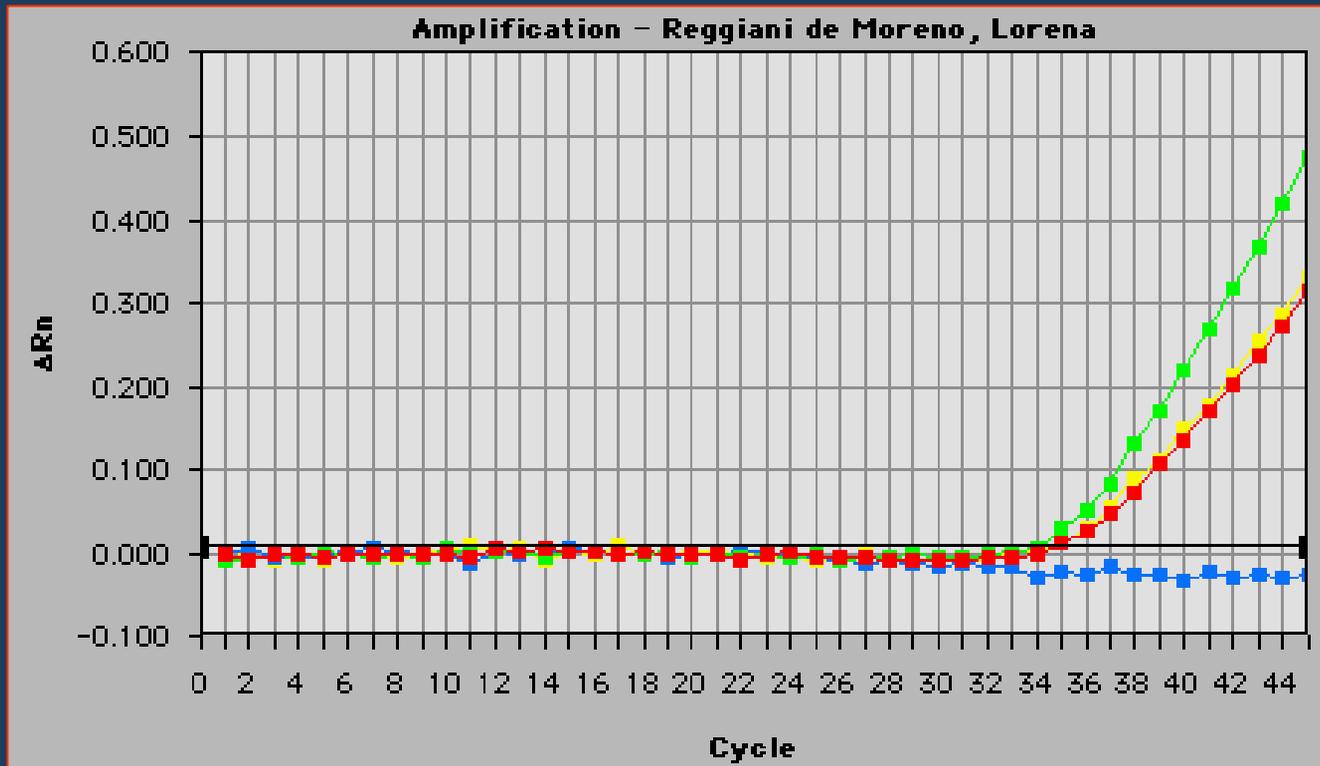


- For searching and confirming unfrequent phenotypes.

Ocasionally, it is applied to blood group typing of patients that have received multiple transfusions:

It is also used in the context of pregnancy, to determine feto-maternal incompatibility in sensitized pregnant women:

- Fetal blood group typing from amniotic fluid samples is available for RHD, RHCE, KEL, JK and FY.
- More recently, fetal RhD status can be determined from the cell-free DNA circulating in the maternal plasma.



RHD positive fetus

# What results have we got from the application of blood group genotyping methods?

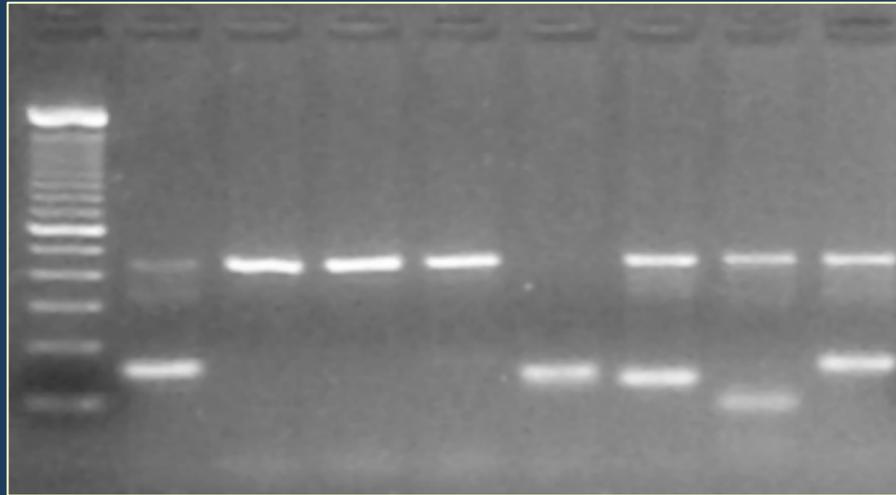
- 1) It has contributed to improve the quality and the accuracy of the typing results.
- 2) Molecular analysis of blood group alleles has allowed us to describe novel allelic variants, not reported in other populations.



D category VI type 4

# DVI type 4

RHD Exons 2 3 4 5 6 7 9 10



*RHD-CE(3-5)-D*

# Comparison with other DVI types:



RHD



RHD<sup>VI</sup> type 1



*RHD<sup>VI</sup> type 2*



RHD<sup>VI</sup> type 3



RHD<sup>VI</sup> type 4



RHCE

➤ DVI type 4 is the prevalent DVI type in the Spanish population

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| DVI<br>Type 1 | DVI<br>Type 2 | DVI<br>Type 4 |
|---------------|---------------|---------------|
| 4             | 2             | 11            |
| 23.5%         | 11.8%         | <b>64.7%</b>  |

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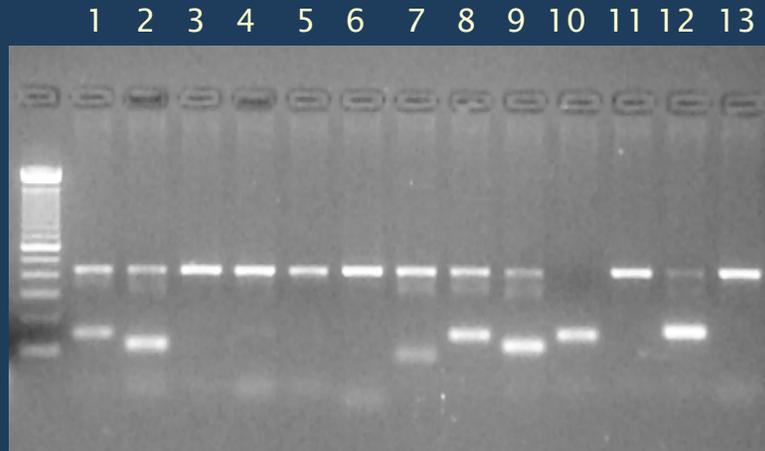
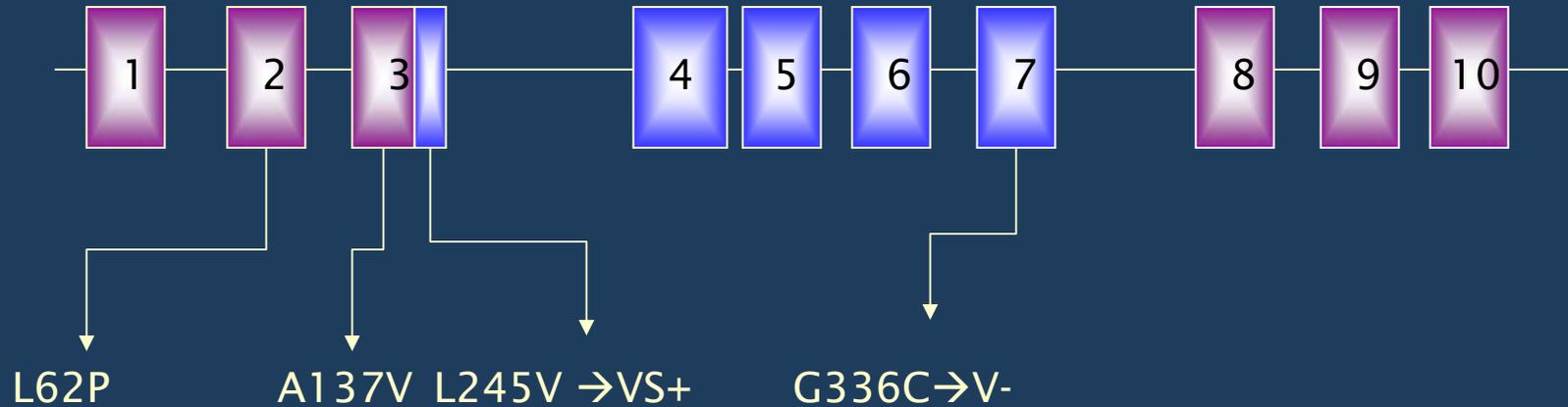
- In all cases, DVI type 4 is linked to the CDe haplotype.
- Serologically, this hybrid protein has a D-epitope expression pattern identical to the previously described DVI phenotypes.

# What results have we got from the application of blood group genotyping methods?

**3)** Molecular analysis of blood group alleles has led to the identification of allelic variants of african origin in our autochthonous population:

- The GATA-mutated FY allele  
→ carried by 4% of Spanish population.
- The hybrid RHD-CE-D<sup>S</sup> (r'<sup>S</sup> haplotype)

# *RHD-CE(3-7)-D. (C)de<sup>s</sup> allele*



Weak C expression

C-specific intron 2 PCR  
negative

We have studied:

225 D-negative samples (C or E positives)



22 carried positive RHD haplotypes



12 normal RHD gene by exon scanning

10 recombined RHD gene

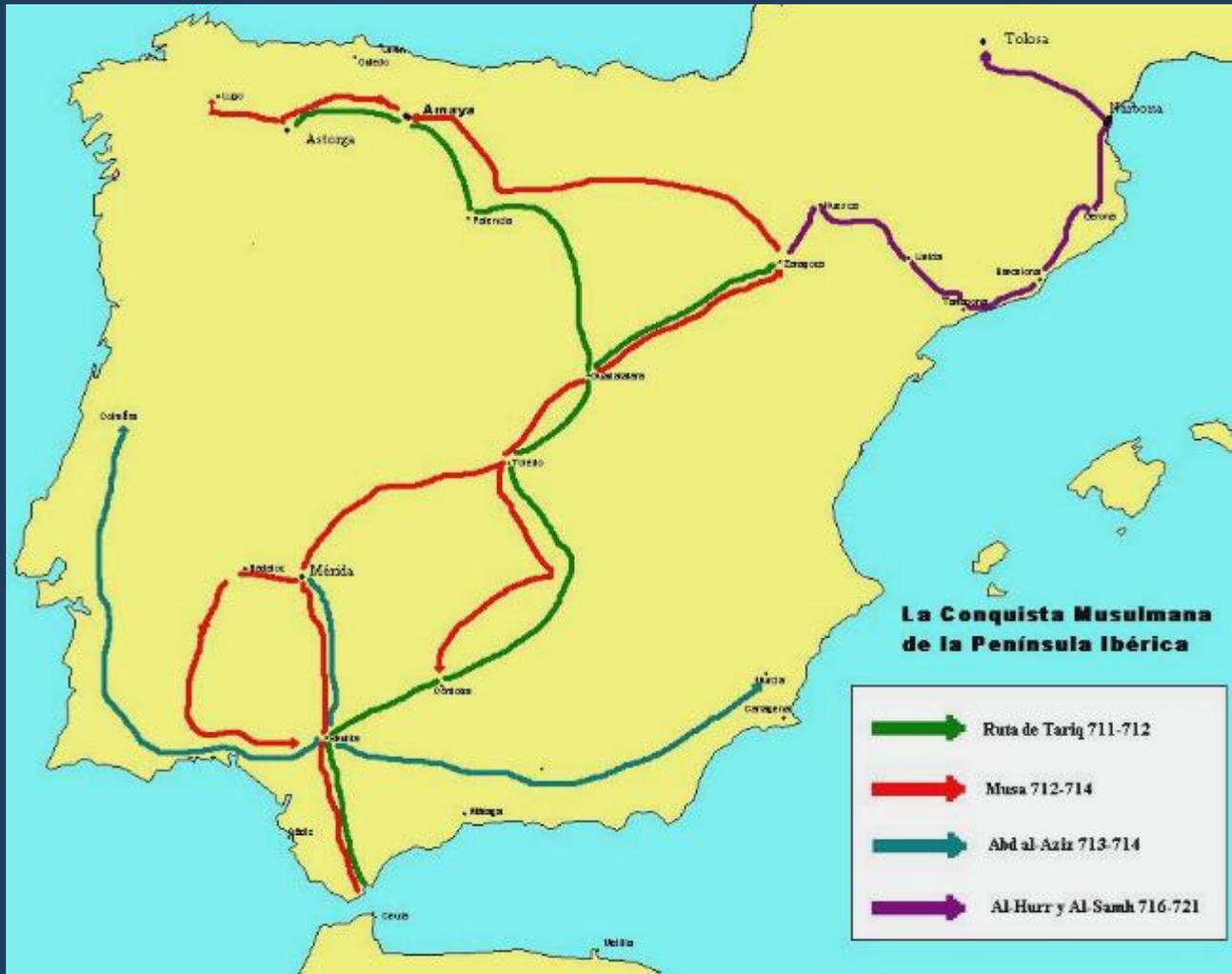
→ Half of the recombined RHD genes corresponded to the hybrid RHD-CE-D<sup>S</sup>.

→ In contrast, the RHD pseudogene is absent in the Spanish population.

**These observations are in agreement with the geographical proximity of Spain to the North of Africa and their historical background:**



▶ Several studies on HLA haplotypes and autosomal STRs support the hypothesis of a certain gene flow from the North of Africa to the Iberian peninsula.



⇒ Blood group genotyping is a helpful tool with a gradually increasing number of applications.

⇒ Genotyping studies are allowing us to have a better knowledge of the blood group distribution and molecular bases in our population.

⇒ This information will ultimately help us to improve blood group typing in our laboratories and blood banks.

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