Investigating the prospect of applying *RHD* genotyping to develop optimal transfusion strategies for weak D patients in Ireland

Paula Holton[1], Diarmaid O'Donghaile[2], Sorcha Ni Loingsigh[2], Mark Lambert[1]
[1] Blood Group Genetics, Irish Blood Transfusion Service, Dublin, Ireland.
[2] Red Cell Immunohaematology, Irish Blood Transfusion Service, Dublin, Ireland

The RhD antigen and *RH* genetics

- The RhD antigen is highly immunogenic and clinically significant from a transfusion and obstetric perspective.
- Since the cloning of the *RHD* and *RHCE* genes, the true complexity of *RH* genetics has been revealed.
- A single amino acid substitution, even within a membrane spanning domain, can create a new antigen or affect the existing antigen's expression, increasing diversity.
- In *RHD* positive individuals, >275 alleles have been identified, exceeding the number of antigens classified by serology.
- RhD variants are composed of weak D, partial D and DEL phenotypes. RhD variants differ by ethnicity with weak D frequently encountered in Caucasians, partial D in African Blacks and DEL in the Asians.

Molecular basis of weak D

- In 1999, Wagner *et al*. gave us an insight into the molecular basis of weak D.
- Two main molecular mechanisms:
  1. One or more nucleotide changes in *RHD* resulting in RhD amino acid substitutions, e.g. weak D type 2.
  2. A genetic recombination event, possibly a gene conversion creating a *RHD-CE-D* gene and hybrid protein, e.g. weak D type 4.
- Weak D individuals were not considered at risk of producing alloanti-D. Therefore, could receive RhD+ blood products.
- Some weak D types were discovered to stimulate immunisation events.
- The International Society of Blood Transfusion established a working group to characterise D variants.
- Approximately 87% (69-100%) of Caucasian weak D individuals are weak D type 1, 2 or 3, with population distributions varying for each type (Van Sandt *et al.* 2015).
- Weak D types are associated with certain Rh phenotypes, i.e. Weak D type 1 and 3 with RhC+ and weak D type 2 with RhE+.

How should a weak D patient be treated?

- Growing international consensus that weak D type 1-3 patients should be treated as RhD+ and non-weak D 1-3 patients treated as RhD- (Daniels 2013; Sandler *et al.* 2015).
- Irish Transfusion Laboratories usually follow BCSH Guidelines, which currently do not recommend *RHD* genotyping for D variant patients (Milkins *et al.* 2013).
- In 2015, the College of American Pathologists recommended investigation of RhD anomalous results using *RHD* genotyping and concluded that implementation of tiered services may reduce cost.
- Molecular classification of weak D types 1-3 offers an alternative approach to serotyping in developing optimal transfusion strategies.
- Discovering the distribution of weak D alleles in Irish patients is fundamental to assess current techniques and future prospects.

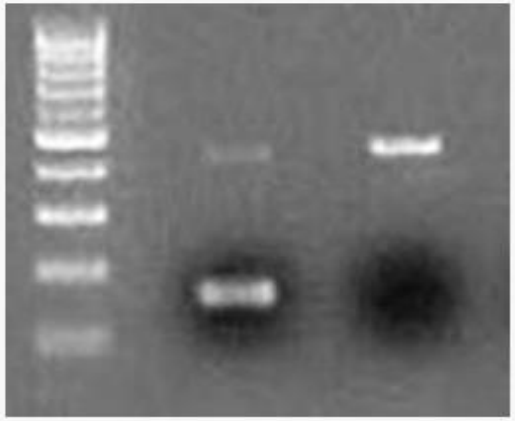
Study Design

- DNA was isolated from 240 patients referred for weak D investigation.
- DNA analysed for weak D alleles 1-5 by SSP-PCR and *RHD* exon 10, if not weak D types 1-5 (Muller *et al.* 2001).
- Demographical and serological data associated with the sample obtained from the laboratory information system (LIS):
  - Rh haplotype
  - Transfusion policy issued
  - Evidence of alloanti-D
- Cost analysis and turnaround time of serologic and molecular technique.

Results

WD1

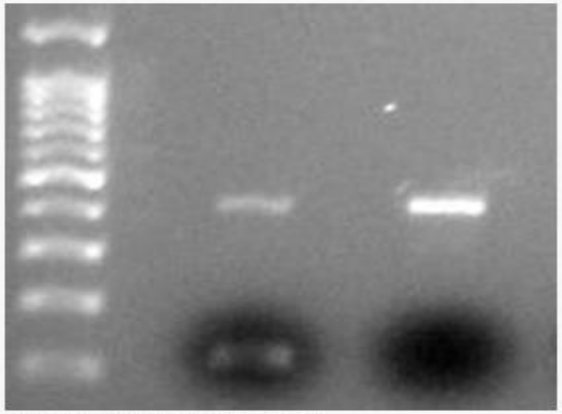
A



Weak D type 1, band at 153bp (lane 2)

WD2

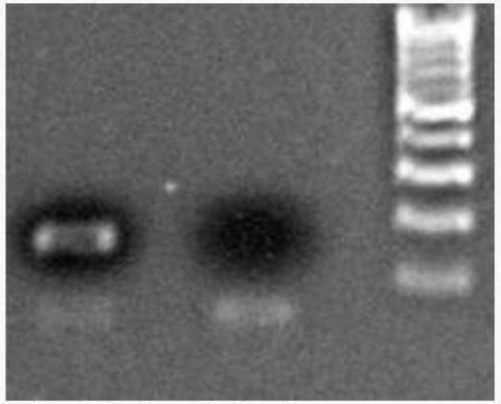
B



Weak D type 2, band at 126bp (lane 2)

WD3

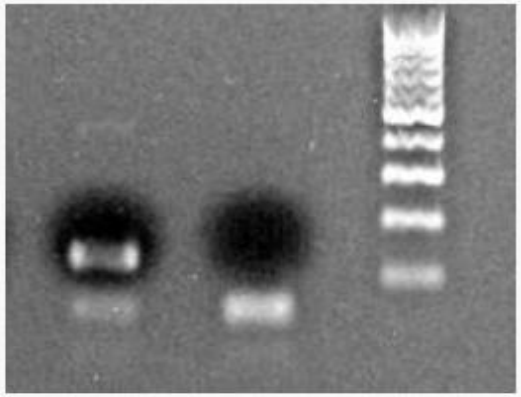
C



Weak D type 3, band at 166bp (lane 1)

WD4

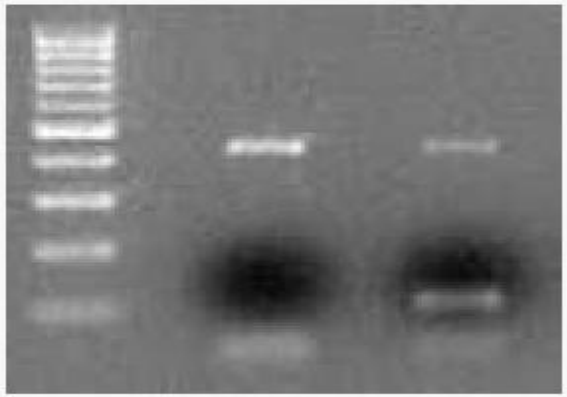
D



Weak D type 4, band at 138bp (lane 1)

WD5

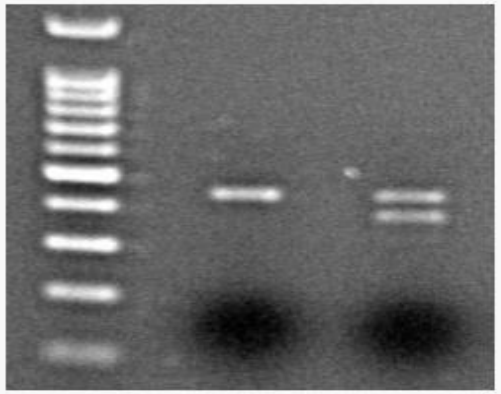
E



Weak D type 5, band at 112bp (lane 3)

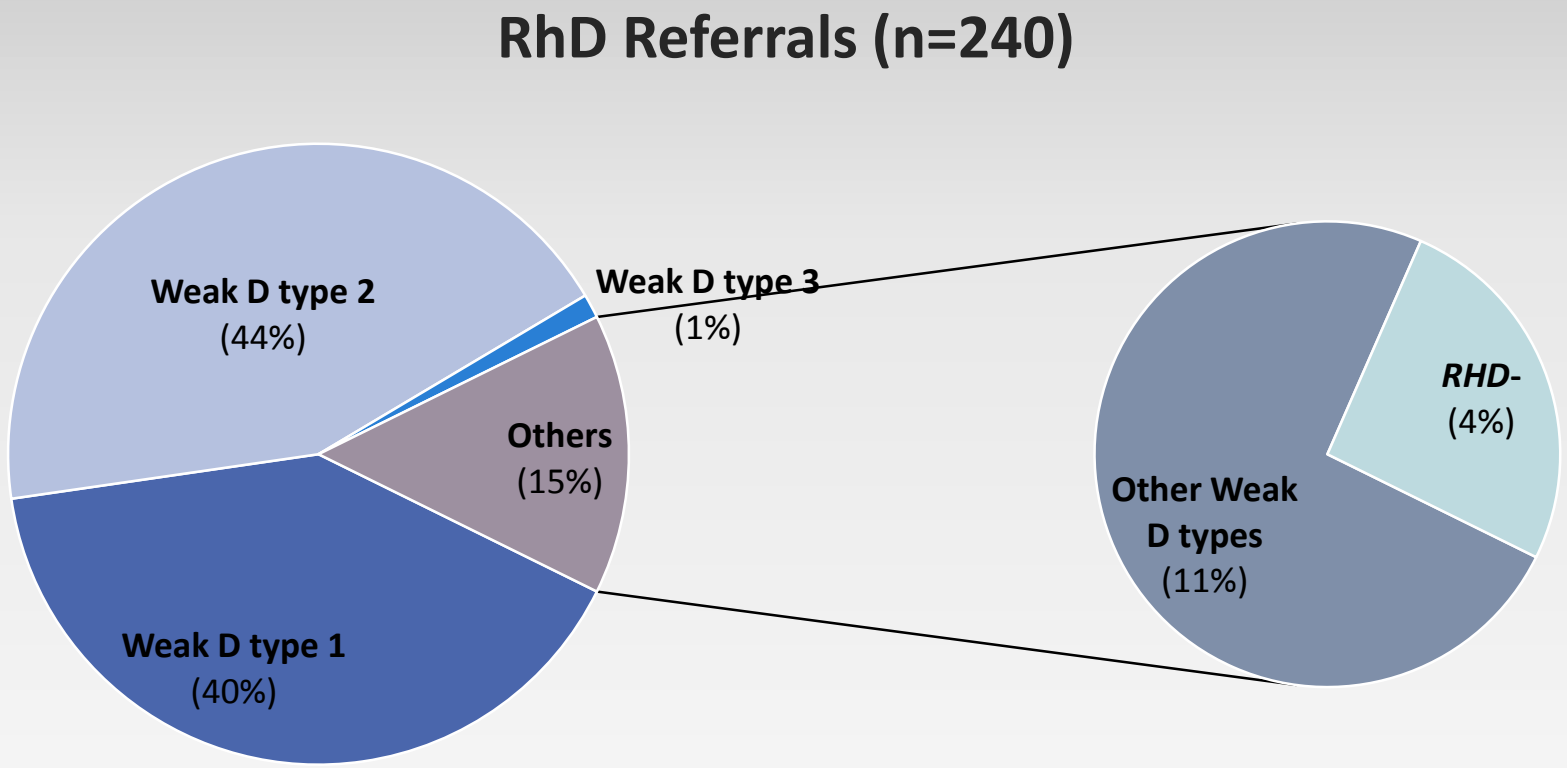
Exon10

F



Exon 10, band at 382bp (lane 3) and HGH, band at 434bp (lane 2 & 3)

Weak D Genotyping



If all weak D patients are transfused RhD+ red cells, 11% of weak D patients would receive RhD+ blood incorrectly, and would be at risk of developing alloanti-D.

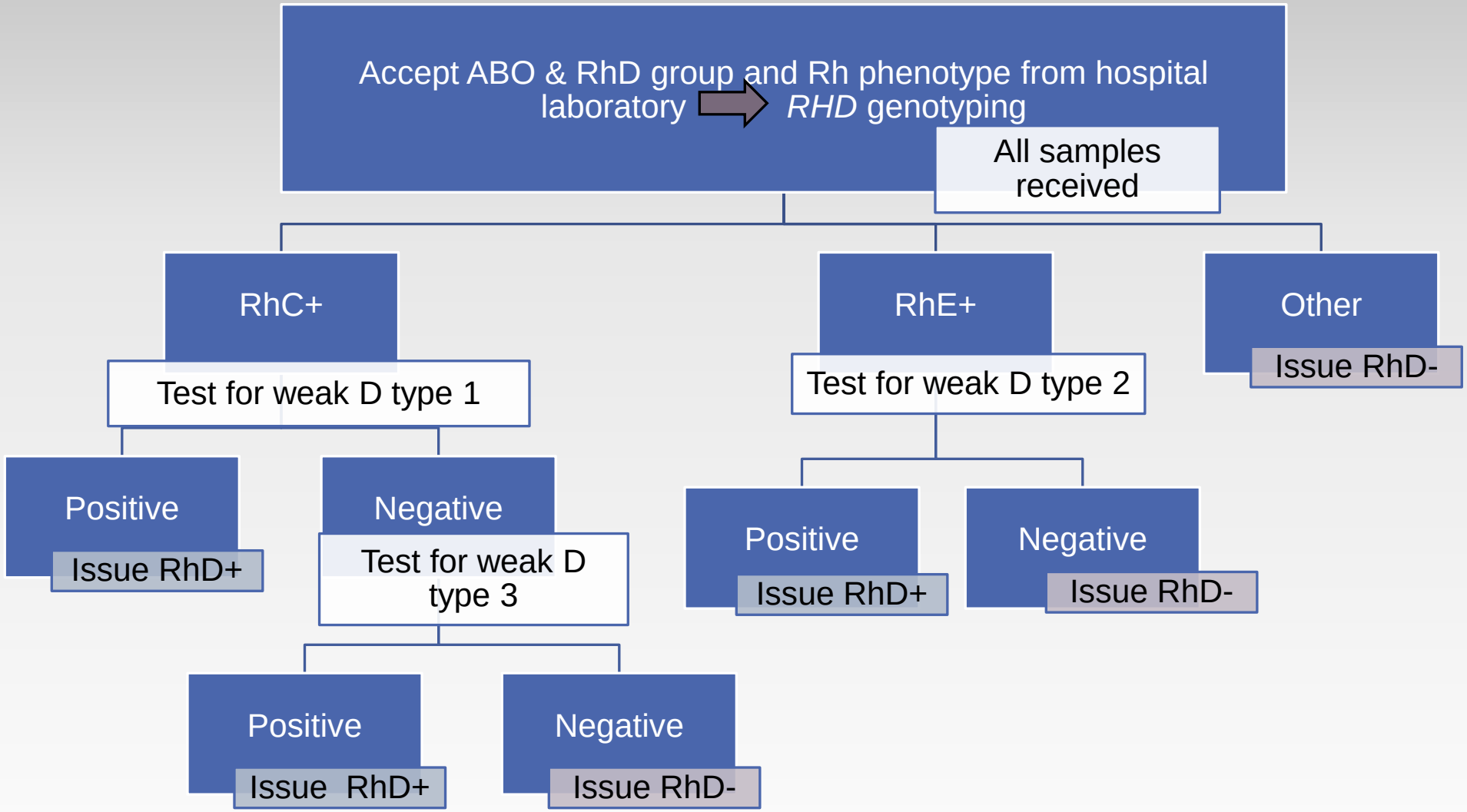
Where do we fit worldwide?

Territory	n=	WD1	WD2	WD3	Non-WD1-3
Belgium, Flanders	495	54%	29%	3%	14%
Germany, North	260	65%	17%	17%	1%
Ireland	**240**	**40%**	**44%**	**1%**	**15%**
France, West	230	40%	27%	5%	27%
Czech Republic	169	58%	10%	20%	12%
Croatia	167	38%	4%	46%	13%
Germany, Southwest	159	60%	27%	4%	9%
France, South	141	26%	42%	3%	29%
Austria, Tyrol	130	33%	8%	50%	9%
Austria, North	128	56%	23%	15%	6%
Portugal	99	16%	64%	14%	6%
Australia	89	43%	54%	3%	-
France	68	44%	31%	4%	21%
Russia	63	29%	14%	49%	8
Argentina	55	38%	16%	15%	31%
Spain, Catalonia	43	49%	33%	9%	9%
Canada, Ontario	32	50%	25%	3%	22%
Total	2505	47%	27%	12%	13%

Rh haplotype, cost and policy analysis

- Weak D type 1:
  - 98.9% association with a *DCe* haplotype
  - One individual was (we believe for the first time) associated with a *Dce* haplotype.
- Weak D type 2:
  - 100% association with a *DcE* haplotype.
- Cost analysis showed a saving of €5/sample by implementation of *RHD* genotyping with a slightly prolonged turnaround time (2 hours).
- No individuals with an alloanti-D present in their serum at the time of testing were identified, two weak D type 1 with an autoanti-D.
- 100% of weak D type 1-3 individuals received RhD+ and 100% of RhD- and weak D type 4 individuals received RhD-.
- Six percent of the unknown cohort received policies recommending transfusion of RhD+ blood products.
  - Six percent increased utilisation in RhD- blood products and Rh immunoglobulin.

Testing strategy for the Blood Group Genetics laboratory



Thank you!

References

- Daniels G (2013). Variants of RhD – current testing and clinical consequences. *British Journal of Haematology* **161,** 461-470.
- Milkins C, Berryman J, Cantwell C, Elliott C, Haggas R, Jones J, Rowley M, Williams M & Win N (2013). Guidelines for pre-transfusion compatibility procedures in blood transfusion laboratories. *Transfusion Medicine* **23,** 3-35.
- Müller TH, Wagner FF, Trockenbacher A, Eicher NI, Flegel WA, Schönitzer D, Schunter F & Gassner C (2001). PCR screening for common weak D types shows different distributions in three Central European populations. *Transfusion* **41,** 45-52.
- Sandler SG, Flegel WA, Westhoff CM, Denomme GA, Delaney M, Keller MA, Johnson ST, Katz L, Queenan JT, Vasallo RR & Simon CD (2015). It's time to phase in *RHD* genotyping for patients with a serologic weak D phenotype. *Transfusion* **55,** 680-689.
- Van Sandt VS, Gassner C, Emonds MP, Legler TJ, Mahieu S & Körmöczi GF (2015). *RHD* variants in Flanders Belgium. *Transfusion* **55,** 1411-1417.
- Wagner, F.F., Gassner, C., Müller, T.H., Schonitzer, D., Schunter, F., and Flegel, W.A. (1999) Molecular basis of weak D phenotypes. *Blood,* **93**(1), 385-393.