Lack of discrimination of D variants by multiple routine serology methods

Rh discrepancies can pose a problem for routine testing because of partial D and weak D phenotypes. Depending on the presence, absence and relative expression of D epitopes on red cells, individuals may type as D positive or D negative with commercially available anti-D reagents.[34,36,46] Serologic weak D phenotypes may give strong, weak or negative reactions depending on the method, anti-D reagent and whether potentiating reagents are used. Therefore, results may differ among laboratories.[47,48] For example, even in a single laboratory the results varied considerably among 47 samples tested by a panel of monoclonal anti-D (Table S1). The 3 negative results with the anti-D clone LHM 70/45 did not correlate with any distinct D variant and confused the guidance for a rational transfusion strategy. Our results exemplify how serologic typing results cannot discriminate clinically relevant D variants (Table S1). Only by using molecular tools to define the D variants involved, can clinicians keep distinct D variants apart and follow the established guidance for optimal clinical transfusion strategy.

Serologic and molecular blood group studies in the Arab population

The Arab population has only recently been studied for D variants, although comprising 422 million people living in 22 countries and territories of the Arab League between the Atlantic Ocean and the Arabian Sea, and between the Mediterranean Sea and the Horn of Africa. The largest such serologic study for the Arab population comprised 23,098 blood donors in Morocco, 10 of whom had the serologic weak D phenotype (0.04%),[49] similar to the prevalence in Europeans. Among 425 D negative samples, 4 were serologically found to carry the DEL phenotype (0.94%),[50] which is 10 times more common than in Europeans.[24,25] Besides 11 partial D samples, including 2 DVa (6%), 3 DVI (9%), and 6 DVII (19%), among 32 Moroccan blood donors with a serologic weak D phenotype, 21 samples (66%)[51] would require molecular testing to resolve the underlying *RHD* alleles.

Dawoud and collegues reported *RHD* data from Gaza, indicating surprisingly high prevalences of DVI (3%) and DNB (4%) among 102 samples.[6] The molecular data also implied a prevalence of 20% D negative, which contrasted with the known D negative rate of 10% among Palestinians.[52] Among samples with the serologic weak D phenotype in Egypt, weak D type 4.2 (32%[20] and 38% in Cairo[22] and 44% in Fayoum[21]) and weak D type 4.0 (16%,[20] 11%[22] and 20%[21]), were the most prevalent weak D types, with weak D type 4.1 not being excluded because the assay could not discriminate. The weak D type 15[20] and type 17,[21] which are often seen in Asian population,[53] were found at low prevalences (2%).[20,21] Besides 6 DV (25%) among 24 Egyptian individuals with serologic weak D phenotype,[20] 18 samples (75%) would require molecular testing to resolve the underlying *RHD* allele.

Table S1. Serologic results for 67 samples with a serologic weak D phenotype among 13,431 Tunisian blood donor samples*

			Reaction strength in serologic screening method †								Reaction strength with a panel of anti-D						Transfusion Strategy	
			Microtiter plate		Tube		Opaline plate											
RHD variant	Phenotype	(n)	anti-D mix 1	anti-D mix 2	anti-D mix 1	anti-D mix 2	anti-D mix 1	anti-D mix 2	Score ‡	(n)	LHM76/55	LHM77/64	LHM70/45	LHM59/19	LHM169/80	LDMI	Donor	Recipient
normal RHD sample 27	CcDee	1	+++	+++	+++	+++	+	+	14	1	+	+	+	+	+	+	D+	D+
DVII	CcDee	1	+++	+++	+++	++	+	+	13	1	nt	nt	nt	nt	nt	nt	D+	D+
weak D type 4.0	ccDee	1	+++	+++	+++	++	+	(+)	13	1	nt	nt	nt	nt	nt	nt	D+	D+
weak D type 4.0	ccDee	17	+++	+++	++	+	+	(+)	11	10	+	+	+	+	+	+	D+	D+
										7	nt	nt	nt	nt	nt	nt	D+	D+
normal RHD sample 43	CCDee	1	+++	+++	++	+	+	(+)	11	1	+	+	+	+	+	+	D+	D+
weak D type 4.0	ccDee	16 §	+++	+++	++	+	(+)	(+)	10	12 §	+	+	+	+	+	+	D+	D+
										4	nt	nt	nt	nt	nt	nt	D+	D+
weak D type 4.0	ccDee	3	+++	+++	+	+	+	(+)	9.5	3	+	+	+	+	+	+	D+	D+‖
weak D type 4.0	ccDee	2	+++	+++	+	+	(+)	(+)	9	2	+	+	+	+	+	+	D+	D+‖
normal RHD sample 32	CCDee	1	+++	+++	+	(+)	+	(+)	9	1	+	+	+	+	+	+	D+	D+‖
weak D type 4.0	ccDee	3	+++	+++	+	(+)	(+)	(+)	8.5	2	+	+	+	+	+	+	D+	D+‖
										1	nt	nt	nt	nt	nt	nt	D+	D-
weak D type 4.1	ccDee	1	+++	+++	+	(+)	(+)	(+)	8.5	1	nt	nt	nt	nt	nt	nt	D+	D-
weak D type 4.2.2	ccDee	2	+++	+++	+	(+)	(+)	(+)	8.5	1	+	+	+	+	+	+	D+	D+‖
										1	+	+	-	+	+	+	D+	D-
weak D type 3	CcDee	1	+++	+++	+	(+)	(+)	(+)	8.5	1	nt	nt	nt	nt	nt	nt	D+	D-
weak D type 4.0	CcDee	1	+++	+++	+	(+)	(+)	-	8	1	nt	nt	nt	nt	nt	nt	D+	D-
weak D type 4.0	ccDee	1	+++	+++	(+)	-	(+)	-	7	1	+	+	+	+	+	+	D+	D-
weak D type 4.0	ccDEe	1	++	++	+	+	(+)	(+)	7	1	+	+	+	+	+	+	D+	D+
weak D type 4.0	ccDee	2	++	++	+	(+)	(+)	(+)	6.5	2	+	+	+	+	+	+	D+	D+‖
weak D type 4.0	ccDee	2	+++	++	+	-	(+)	-	6.5	2	+	+	+	+	+	+	D+	D-
weak D type 100	ccDee	1	++	++	(+)	+	(+)	(+)	6.5	1	+	+	+	+	+	+	D+	D+‖
weak D type 4.0	ccDee	1	++	++	+	(+)	(+)	-	6	1	nt	nt	nt	nt	nt	nt	D+	D-
weak D type 4.2.2	ccDee	1	++	++	+	(+)	(+)	-	6	1	+	+	+	+	+	+	D+	D+‖
weak D type 4.2.2 /RHD⧩	ccDee	1	++	++	(+)	(+)	(+)	(+)	6	1	+	+	-	+	+	+	D+	D-
weak D type 4.0	ccDee	2	++	+	(+)	(+)	(+)	(+)	5	1	+	+	+	+	+	+	D+	D+‖
										1	nt	nt	nt	nt	nt	nt	D+	D-
weak D type 4.2.2	ccDee	1	++	++	(+)	(+)	-	-	5	1	+	+	+	+	+	+	D+	D-
weak D type 4.0	CcDee	1	+	+	+	(+)	(+)	(+)	4.5	1	+	+	-	+	+	+	D+	D-
weak D type 4.2.2	ccDee	1	+	+	(+)	(+)	(+)	(+)	4	1	nt	nt	nt	nt	nt	nt	D+	D-
weak D type 1	CcDee	1	(+)	(+)	+	(+)	-	-	2.5	1	+	+	+	+	+	+	D+	D-
Total		67								67								

* The remaining 11,974 D positive samples that were tested with the 3 serologic screening methods showed ≥ 3+ reactivity with both anti-D reagents in all the 3 techniques. The 1,390 D negative samples that were tested with the 3 serologic screening methods showed negative reactivity with both anti-D reagents in all the 3 techniques.

† Two monoclonal IgG/IgM mixtures were used: anti-D mix 1 (Fortrees with IgG/IgM clones) and mix 2 (Biomaghreb with clones P3x61 (IgM), P3x21223B10 (IgM), P3x290 (IgG) and P3x35 (IgG)).

‡ Score was calculated as the sum of the agglutination strengths: - = 0, (+) = 0.5, + = 1, ++ = 2, and +++ = 3.

§ 1 of 16 samples in screening was ccDEe, which was also 1 of 12 samples tested with the anti-D panel.

‖ Considered as D- if not tested by the ID-Partial RhD-Typing Set

nt - not tested

Table S2. *DVII* allele frequency in Tunisian and German blood donors

Population	Donors (n)	Observations (n)		Alleles (n)*		*DVII* allele frequency among all *RH* haplotypes		Reference
		DVII antigen (phenotype)	*DVII* allele (genotype)	*DVII* allele	Any *RH* haplotype	Mean	95% CI‡	
Tunisia	2,000	n.t.	16	16	4,000	0.40%	0.23% - 0.65%	[19]
Germany	78,156	80	n.t.	197†	156,312	0.13%	0.11% - 0.14%	[34]

* $p < 0.001$, χ^2 test, 2 sided

† Caluclated for all blood donors based on published *RH* haplotype frequencies in Germany.[36, Table 3]

‡ 95% confidence interval (CI), Poisson distribution

n.t. - not tested