

Performance Comparison of Medion-MDmulticard, ID-DiaMed and Scangel-BioRad RhD/ABO Serotyping Using a Multi-Variant, Caucasian-specific, Genotyped Donor Panel

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BACKGROUND Validations of routinely used serological typing methods usually require an intense performance evaluation typically including large numbers of samples. Additionally, certain mandatory typing results for variant bloodgroups, as e.g. for RHD category VI, need to be met by the tests. However, performance evaluations could be improved and still be simplified applying current knowledge about the occurrence of standard blood groups and their variants.

AIMS Taken the knowledge about Caucasian *RHD* and *ABO* population genetics, and using local blood group DNA-typing records of the last 12 years, a Caucasian-specific donor panel could be compiled for a performance comparison of the three serological methods MDmulticard (Medion Diagnostics), ID-System (DiaMed) and ScanGel (BioRad). With respect to D, one study on weak Ds and one on unexpressed *RHD* alleles were taken as guideline for the compilation [1, 2]. *RHD* alleles not included in the above mentioned studies, were considered depending on their prevalence in the DNA-typing records of the last 12 years (including a total of 705 samples for *RHD* "clarification"), assuming that even rare partial, or weak Ds would have been recognized within this time-interval. Composition of test panel is shown in table 1.

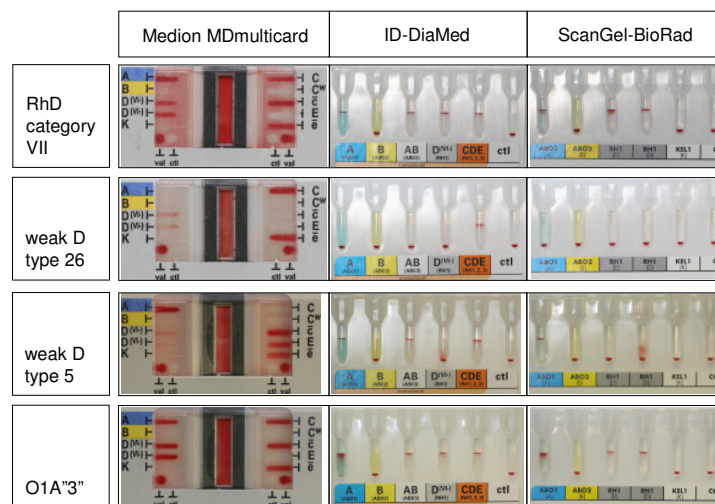


Figure 1. Comparative typings for the three serological methods for 3 variant RhD and 1 "weak A" are given. MDmulticard and ScanGel defined D category VII as weakly D+, ID-System showed regular D+. Weak D type 26 was reliably recognized as D+ by MDmulticard, whereas ID-System and ScanGel interpreted it as D-; this is of specific interest, since weak D type 26 has been shown to cause anti-D immunization when transfused to D- individuals [2]. Weak D type 5 was weakly detected as D+ by MDmulticard, but only very weakly by the ID-System and ScanGel. O1A"3" was detected at almost regular intensity by MDmulticard, ID-System and ScanGel.

Table 1. Shows the composition of the test panel including "regular" and "variant" D, and O, A2 and A3 phenotypes on the left side. The right part gives the results of serological investigation in % of expectancy (e.g. D+ = 100%, D- = 0%).

"REGULAR" pheno-types investigated	serologically	n investigated	phenotype FRQ (local)
ccdde	D -	1	17,400%
Ccddee	D -	2	1,260%
CCdde	D -	3	0,020%
ccddEe	D -	2	0,580%
ccddEE	D -	2	0,005%
ccDde	D +	2	1,656%
CcDde	D +	2	42,381%
CCDde	D +	3	22,029%
ccDEe	D +	2	12,355%
ccDEE	D +	2	2,308%
TOTAL REGULAR		21	100,000%

D1 MDmulti	D2 MDmulti	D1 ID	D2 ID	D1 ScanGel	D2 ScanGel
0%	0%	0%	0%	0%	0%
0%	0%	0%	100%	0%	0%
0%	0%	0%	100%	0%	0%
0%	0%	0%	88%	0%	0%
0%	0%	0%	100%	0%	0%
100%	100%	100%	100%	100%	100%
100%	100%	100%	100%	100%	100%
94%	94%	100%	100%	100%	96%
100%	100%	100%	100%	100%	100%
100%	100%	100%	100%	100%	100%

"VARIANT" pheno-types investigated	serologically	n investigated	"phenotype" FRQ (local)	references
RHD DNB	weak	3	n.a. (> 11)	3
RHD DFR	weak	4	n.a. (> 11)	4
Rh33	weak	1	n.a. (> 11)	4
RHD IV type IV	regular	1	n.a. (1)	4
RHD VI type I	weak	5	1 : 1494	1
RHD VI type II	weak	2	n.a. (> 11)	1
RHD VII	(weak)	4	1 : 22406	1
weak D type 1	weak	4	1 : 521	1
weak D type 2	weak	5	1 : 2240	1
weak D type 3	weak	4	1 : 344	1
weak D type 4.0 or 4.1	weak	3	1 : 5601	1
weak D types 4.2	weak	1	n.a. (> 11)	1
weak D type 5	weak	3	1 : 5601	1
weak D type 15	weak	3	n.a. (> 11)	5, 6
weak D type 26	weak	2	1 : 20988	2
RHD (M295I)	DEL	4	1 : 4198	2
RHD (V53+1G>A)	DEL	1	1 : 20988	2
RHD-CE(2;9)-D hybrid	D -	8	1 : 862	2
TOTAL VARIANT		58	n.a.	

D1 MDmulti	D2 MDmulti	D1 ID	D2 ID	D1 ScanGel	D2 ScanGel
72%	72%	100%	100%	75%	88%
21%	21%	6%	97%	0%	88%
33%	33%	75%	88%	25%	0%
67%	67%	100%	100%	81%	88%
0%	0%	0%	88%	0%	0%
0%	0%	0%	100%	0%	0%
63%	67%	97%	100%	75%	81%
33%	29%	41%	100%	34%	47%
33%	30%	40%	95%	20%	40%
58%	58%	81%	100%	56%	69%
67%	67%	92%	92%	79%	75%
50%	50%	75%	75%	63%	75%
33%	33%	17%	100%	0%	17%
0%	0%	0%	96%	0%	0%
33%	33%	0%	100%	0%	0%
0%	0%	0%	100%	13%	13%
0%	0%	0%	88%	0%	0%
0%	0%	0%	97%	0%	0%

ABO pheno-types investigated	serologically	n investigated	"phenotype" FRQ (local)
O1O2	O	8	n.a. (>11)
O1A3	A weak	3	1 : 1000
O1A2	A weak	3	n.a. (>11)
TOTAL VARIANT		6	n.a.

A MDmulti	A ID	A ScanGel
0%	0%	0%
100%	67%	92%
100%	100%	100%

METHODS Fresh blood was obtained from previously DNA-typed individuals. All samples were investigated with the above mentioned serological methods by two different technicians in a blinded way and retyped on DNA level. For ABO samples were used, mainly to interpret weak serological reactivity for blood group A specificity. All three A"3" alleles had an A302 specific DNA sequence, with one single additional substitution only.

RESULTS With respect to C, c, E, e, Cw, ABO and K, results of performance were as expected for all three methods (data shown partially only). However, focusing on specific variant RhD phenotypes, pronounced differences in reaction strengths were observed in between them. Exemplary results are shown in Figure 1. In case of weakened expression of certain antigens (D, A), percentage of total intensities of reaction strengths of the two independent reads and for all samples of one genotype were calculated and are given in table 1 (D+ (100%), D- (0%), for D and A (100%) and O (0%) for ABO).

SUMMARY / CONCLUSIONS Since the 79 sample panel for D serology is thought to include all regular phenotypes and all locally rare variants, it actually represents a much larger sample collective. The corresponding size can be calculated by the individual population frequency of a single variant allele multiplied by their number of samples investigated. Exemplarily, investigating 5 samples of weak D type 2 with their local phenotype frequency of 1 per 2.240 individuals, multiplies to a final weak D type 2 panel-coverage of 11.200 individuals – a reasonably higher number, than usually considered for test evaluations. Therefore, predefined multi-variant test panels allow for more significant performance evaluation of new serological test procedures compared to such based on randomly chosen samples only.

REFERENCES 1. Gassner C, Doescher A, Drnovsek TD et al. Presence of RHD in serologically D-, C/E+ individuals: a European multicenter study. *Transfusion*. 2005 Apr;45(4):527-38. 2. Muller TH, Wagner FF, Trockenbacher A et al. PCR screening for common weak D types shows different distributions in three Central European populations. *Transfusion*. 2001 Jan;41(1):45-52. 3. Wagner FF, Eicher NI, Jorgensen JR et al. DNB: a partial D with anti-D frequent in Central Europe. *Blood*. 2002 Sep 15;100(6):2253-6. 4. Wagner FF, Gassner C, Muller TH et al. Molecular basis of weak D phenotypes. *Blood*. 1999 Jan 1;93(1):385-93. 5. Avent ND, Reid ME. The Rh blood group system: a review. *Blood*. 2000 Jan 15;95(2):375-87. Review. 6. Gassner C. unpublished observation.

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