Frequency of *DAU*, *RHDψ* and *Cde^s* in South African blood donors

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Background:

About 18% of the Caucasian population are phenotyped D-negative. In almost all of these D-negative individuals the D gene is deleted, whereas in other ethnic groups (Blacks, Asians) aberrant and non functional *RHD* alleles are much more frequent than than in Europeans. In these cases D negative individuals often carry *RHD* alleles like *RHDψ and Cde^S* (1-3, table 1). So far *RH* genotyping assays are mainly based on research in whites. These assays may not be reliable due to the genetic variation in *RH* among other ethnic groups. It is important to be aware of the differences in genetic sequences. In order to develop genotyping methods that are reliable in a multiracial society.

Purpose:

A new PCR-SSP system with prepipetted ready-to-use reagents was evaluated for the prediction of the D, C, c, E and e phenotype and the detection of variant *RHD* alleles from DNA of individuals from African descent.

Method:

From a South African donation centre we obtained anticoagulated whole blood from 439 Whites, 177 Asians, 53 Blacks and 45 individuals of mixed race, referred to as "Coloured" in South Africa. These samples were tested for ABO and D, stored at 4°C and sent to Germany. Here we determined the complete Rh formula with standard serological methods (gel centrifugation test). We used an automated protocol for DNA extraction (Agowa, Berlin, Germany) and tested with a multiplex PCR containing primers for *RHDψ*, *RHD* intron 7 and *RHD*(W16X). Additionally, we tested for variant *RHD* alleles like *RHDψ*, *DAU* and *Cde^S* and the *C, c E* and *e* alleles of *RHCE* with SSP kits (RH-TYPE, Partial-D-TYPE, BAG, Lich, Germany) in black and coloured donors.
The PCR conditions are shown in table 2.
Dried allele-specific primers, nucleotides as well as primers for the internal controll (HGH - 434 bp, Chromosom 1 - 659 bp in RH-Type reaction 2) are prepipetted in a specific combination of single tubes in plates. A mastermix of 10 µl consisting of PCR-buffer, DNA-solution, Taq-Polymerase and aqua dest. is to be added to the dried reaction mixes. After PCR and separation of the amplicons in the gel the specific bands can be evaluated, finally (fig.1 and 2).

Results:

After comparison of the amplicons of 714 samples with the evaluation diagrams (fig. 1 and fig. 2) following results could be obtained:
The percentage of D-negative individuals was 19.4 in Whites, 2.8 in Asians, 1.9 in Blacks and 2.2 in Coloured. *RHDψ* was not found in Whites and Asians. Five (9.4%) Blacks were tested positive for *RHDψ* (all *RHD*/*RHDψ*) and one (2.2%) Coloured individual carried the *RHDψ* allele (D-negative, homo- or hemizygous). In 97 of 98 tested individuals (Blacks plus Asians) the complete Rh phenotype and genotype was concordant. In one C-sample we obtained a positive result with the *Cde^s* SSP. Using published primers (4) for the *Cde^s* hybrid exon 3 the presence of the *Cde^s* allele was confirmed. The *Cde^s* allele was also found in 3 of 53 (5.7%) Blacks and in 1 of 45 (2.2%) Coloured donors. The variant *RHD* allele *DAU* was observed in 8 (15,1%) Black and 2 (3,8%) Coloured donors (table 3).

Conclusion:

The D negative phenotype in the presence of *RHD* has been elucidated recently in Africans. However, only small numbers of individuals have been tested yet. Molecular testing for *RHDψ*, *Cde^s* and *DAU* is not only relevant in Africa, but also in regions of Europe and America with large populations of people with African decent (4). In this study we add data to the published literature with respect to the frequency of *RHDψ*, *Cde^s* and *DAU*. The prediction of the D, c, E and e phenotype from DNA with SSP seems to be reliable in black and coloured South African blood donors. Since two independent SSP methods indicated the presence of the *Cde^s* allele in a donor who tested C-negative with stored red cells, a repeat serological test is required in order to interpret this single discrepancy in this study.

References:

1. **Wagner FF** et al. (2001) BMC Genetics 2:10.
2. **Singleton BK** et al. (2000) Blood 95:12-18.
3. **Wagner FF** (2003) BMC Genetics 24;4(1):14.
4. **Daniels G** (1997) 20;350 (9081):862-3.
5. **Tax MG** (2002) Transfusion;42(5):634-44.

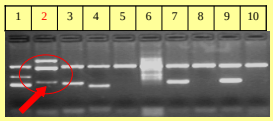
fig. 1

Evaluation diagram, BAGene RH-TYPE

Reaktion No.	1	2	3	4	5	6	7	8	9	10
PCR-Products	224 123	248 154 390	140	113	215	142	145	157	155	181
Genotype	D 4/4 D 7	W16X RHD Ψ D 7	D 4 RHD Ψ neg	D el neg	Cde^S	C	c	E	e	C^w
Reaktion pattern										
D-positive	+	+	+	+	+	-				
D-positive/RHDΨ	+	+	+	+	+	-				
D-negative/ RHDΨ	+	+	+	-	+	-				
D-positive/Cde^S	+	+	+	+	+	+				
Sample 1	+	+	+	+	+	-	-	+	-	+	-
Sample 2	+	+	-	+	+	+	+	+	-	+	-

Gel Pictures

Sample 1, D-pos. ccee *RHD ψ*



Sample 2, D-pos. Ccee Cde^S

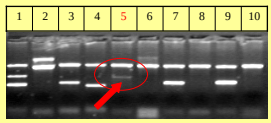


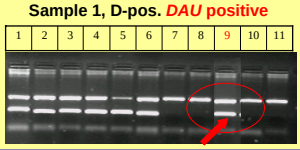
fig. 2

Evaluation diagram, BAGene Partial-D-TYPE

Reaktion No.	1	2	3	4	5	6	7	8	9	10	11
PCR-Products	146	118	135	132	132	120	166	117	140	107	113
RHD Exons	D2	D3	D4	D5	D6	D7	D2	D7	D8	D6	D9
Phenotypes											
D-positive	+	+	+	+	+	+					
DAU	+	+	+	+	+	+			+		
Sample 1	+	+	+	+	+	+	-	-	+	-	-
Sample 2	+	+	+	+	+	+	-	-	-	-	-

Gel Pictures

Sample 1, D-pos. *DAU* positive



Sample 2, D-pos. *DAU* negative

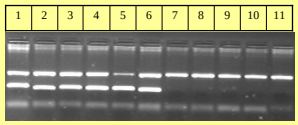


table 1

Base-Substitutions

DAU (Cluster: DAU-0 to DAU-4)	1136C>T (Exon 8)
RHD ψ	37 bp duplication at intron3/exon 4 junction, multiple missense mutations, stop codon in exon 6
Cde^s	RHD-CE (3-7)-D

table 2

PCR conditions:

Steps	Time	Temp.	No. Cycles
1st Denaturation	5 min	96°C	1 cyle
Denaturation	10 sec	96°C	5 cycles
Annealing + Extension	60 sec	70°C	
Denaturation	10 sec	96°C	10 cycles
Annealing	50 sec	65°C	
Extension	45 sec	72°C	
Denaturation	10 sec	96°C	15 cycles
Annealing	50 sec	61°C	
Extension	45 sec	72°C	
Last Extension	5 min	72°C	1 cycle

table 3

Results:

	Whites	Asians	Blacks	Couloured
Total	439	177	53	45
***RHD* negative**	19.4 %	2.8 %	1.9 %	2.2 %
RHD ψ	-	-	9.4 %	2.2 %
DAU	-	-	15.1 %	3.8 %
Cde^S	-	-	5.7 %	2.2 %