

QA - Quality assurance of D negative red cell units

Willy A. Flegel MD
Professor of Transfusion Medicine

Institut für Klinische Transfusionsmedizin und Immunogenetik Ulm
Institut für Transfusionsmedizin, Universitätsklinikum Ulm, Germany



Relevance of Rh blood group

Clinical

- D antigen always considered in transfusions
 - largest immunization rate of all > 260 blood group antigens
 - because RhD protein is completely missing in D neg.
- Leading cause of hemolytic disease of newborn (HDN)
 - anti D prophylaxis (RhG)
- antigens CcEe (and K), if
 - girl, woman < 45 years
 - multiple transfusions
 - immunohematologic problems

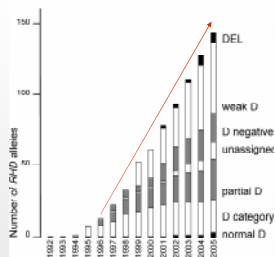
Biological

- RhAG
- RhCE und RhD
- large number in membrane
 - RhD 10.000 – 25.000 per RBC
- important for membrane integrity and RBC stability
- involved in transport

Rh blood group review 2007:
<http://www.aerzteblatt.de/v4/archiv/pdf.asp?lang=en&id=54759>



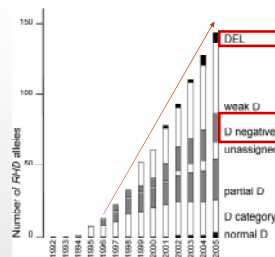
Type of D antigen variant correlates with the molecular type



- D antigen variants
 - D negative
 - DEL
 - weak D
 - partial D
 - D category
 - D category
 - normal D antigen



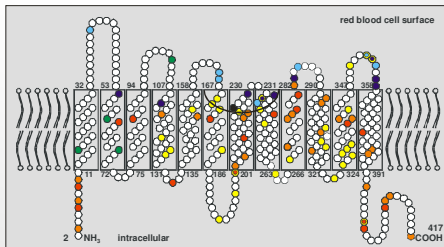
What to do about DEL versus D neg. in donors?



- D antigen variants
 - D negative
 - DEL
 - weak D
 - partial D
 - D category
 - D category
 - normal D antigen



Amino acid substitutions cause partial D and weak D



- RhD vs. RhCE (yellow)
- weak D (red/orange)
 - C/c (green)
 - E/e (black)
- partial D (blue/light blue)

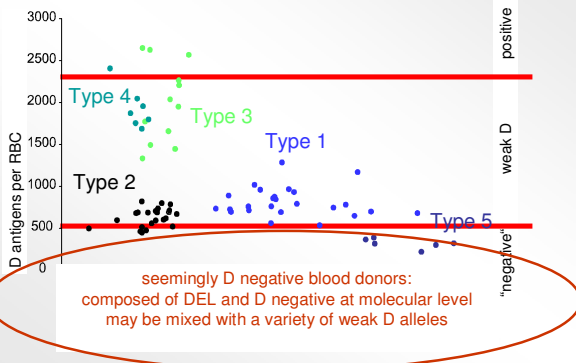


Definition of DEL

- Detection by adsorption/*elution* only
 - hence, formerly called D_{el}
 - negative even in antiglobulin test
 - D antigen density very low
 - e.g. < 50 D antigens per RBC
- | | |
|--------------------------|----------|
| • Compare weak D type 5: | 300 |
| • weak D type 2: | 500 |
| • weak D type 4.1: | 4,000 |
| • normal D in CcDee: | > 10,000 |



DEL and D neg. alleles > 40 to date

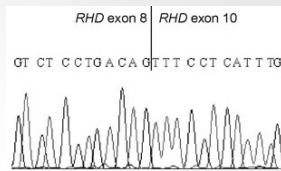


Molecular basis of DEL

- *RHD*(IVS1+1G>A)
- *RHD*(IVS3+1G>A)
- *RHD*(K409K)
- *RHD*(L153P)
- *RHD*(M295I) in *CDe*
- *RHD*(W408R)
- *RHD*(M1I,L84P)
- *RHD*(147delA)
- *RHD*(X418L)
- *RHD* in DEL
- modified splice site
 - most frequent cause
 - cause of most frequent DEL
- missense mutation
 - loss of start codon
- frameshift mutation
 - unusual: D neg. expected
- loss of stop codon
- no known change

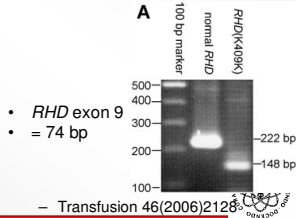
RHD(K409K)

- Most frequent worldwide
 - clinically most important
- 10% – 30% of D neg.
 - shown in Chinese Japanese Koreans
 - occurs in Europe, but rare



- *RHD*(delEx9) does not exist
- identical to *RHD*(K409K)

Exon 9---> Intron 9--->
 AGTTTTCTGGAAgtaagattttcacc
 TCAAAAGACCTTcatttctaaaaagtgg
 80 100

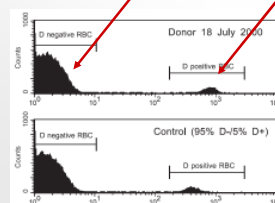


- *RHD* exon 9 = 74 bp

– Transfusion 46(2006)2128

D+/- chimera in ccddee donors

- Evidence for immunogenicity:
 - D+/- chimera RBC unit
 - donor carries two RBC populations: the second population may derive from (unknown) twin
 - 95% D neg. + 5% D pos.



- total of 13 donations
 - caused anti-D in all D neg. recipients tested

D+/- chimera RBC unit

- donor carried two RBC populations:
 - 95% 0 D neg.
 - 5% B D pos.
- 1 chimeric RBC unit
 - caused acute intravascular hemolysis
 - after latest transfusion

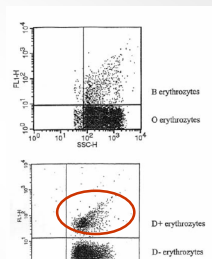


Fig. 2. ABO and D populations of the chimeric donor (EACS).

Transfusion 43(2003)1449

QA in D neg. RBC units

Why?

because of weak D, DEL & D+/- chimera donors found by screening of seemingly D neg. donors
 rationale: to improve RBC unit safety

Anti-D immunization by weak D and DEL RBC units is established

- primary immunization
 - by weak D type 2
- in pregnant woman
 - by weak D type 26
- secondary immunization
 - by the most prevalent DEL *RHD*(K409K)
- anti-D in all D neg. recipients tested
 - by a D+/- chimera RBC unit
 - various case reports published since 2000



Routine testing 1/02 – 12/05 at our blood service

- screening of all serologically D neg. first time donors for *RHD* gene
 - 29,823 in 4 years
- PCR screening of pools of 20 donors
 - PCR-SSP for *RHD* intron 4
- all novel *RHD* alleles characterized
 - PCR, sequencing, testing for DEL

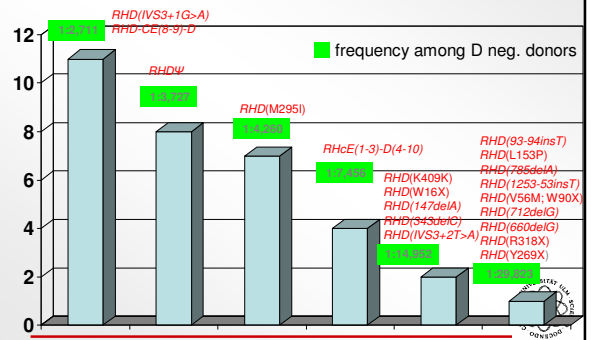


Distribution among Rhesus phenotypes

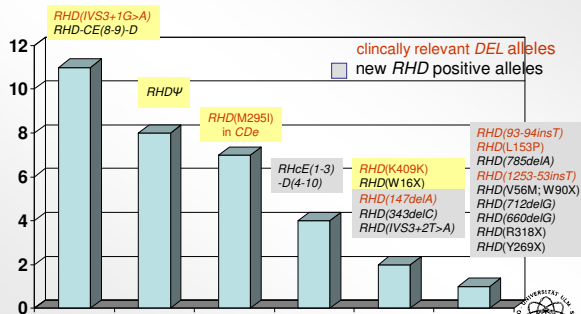
phenotype	n	<i>RHD</i> positive	DEL positive
ccddee	27,859	8	0
Ccddee	1,241	44	23
ccddEe	679	7	1
CCddeE	20	2	2
CcddEe	19	0	0
ccddEE	4	0	0
CCddEe	1	0	0
total	29,823	61	26



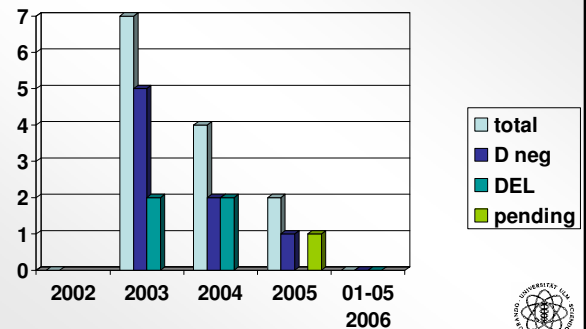
RHD alleles observed



RHD alleles in "D neg." donors



New *RHD* pos. alleles per year



Can we afford it?

- 3 PCR per day for screening in pools of 20 donors at the blood service in Southwestern Germany
- to serve all seemingly D neg. first time donors
 - 200,000 donations/yr in Wü (Ulm) since 1.1.2002
 - 500,000 donations/yr in Ba-Wü since 1.7.2002
 - 750,000 donations/yr in Ba-Wü–He since 1.1.2007
- **Molecular testing of D neg. donors:**
 - 1 test per donor's lifetime
 - will detect DEL as well as D+/- chimera



Cost savings

It would obviate the need to tightly control the sensitivity of anti-D testing of donors and its quality assurance, thus contributing to cost containment.

- Light cycler: *RHD* intron 4 and exon 7 PCR
- recently introduced practice at the blood center Linz, Austria
- antiglobulin for D neg. donors was dropped
- Transfusion 45(2006)466



Regulatory issues

Regulatory affairs are affected as well, and standards may have to be revised to allow retaining such donors in the donor pool, as is established practice in many European countries.

- generally not a big issue in Europe
- for instance, German guidelines: antiglobulin is mandatory & needs to be dropped before we can recover such cost savings
 - perhaps a topic for European standards?

• Transfusion 45(2005)466



What are the benefits?

anti-D immunization is of considerable clinical importance for girls and women of reproductive age. In the case of a D positive pregnancy, this would be likely to result in Rhesus hemolytic disease of the newborn.

- to avoid anti-D immunization
- of particular relevance for D neg. women

Up to 1% of all pregnant women have clinically significant anti erythrocyte antibodies (2, e1). Anti-D remains the main indication for phototherapy or exchange transfusions in newborns (2, e2), and pregnant women who are D negative show an above average incidence.

- Anti-D > 50% of all antibodies in HDN
- D neg. women are grossly overrepresented



Do you know what is in your D negative red cell unit?

- Screening in 1,700 D negative
- repeat donors with C and/or E
- collaborative study 2004:
- Austria, Germany, Switzerland, Slovenia, Russia
- 89 *RHD* gene positive with 15 DEL positive
- additional "D neg." blood donors:
 - 1 DVI type 1
 - 1 weak D type 1
 - 2 weak D type 5
 - 1 weak D type 26 (caused anti-D during pregnancy)
 - plus 9 "regular *RHD*?"

– Transfusion 45(2005)527



Do you know what is in your D negative red cell unit?

- Screening in 8,442 D negative
- repeat donors with all Rh phenotypes
- Single center study 2000
- Ulm
- 50 *RHD* gene positive with 15 DEL positive
- additional "D neg." blood donors:
 - 2 DVI type 1
 - 1 weak D type 2
 - 1 partial D DIM
 - 1 D+/- chimera (caused multiple anti-D)

– BMC Genet 2(2001)10



Adaptation to different populations required

TABLE 2. Molecular causes of D-, including DEL, in Korean persons

Type of RHD variation	RHD allele	Observed in this study		
		RhCE phenotype	Number of donors	Subtotal (number)
RHD deletion	RHD deletion	ccee	72	94
		Ccee	2	
		ccEe	18	
		ccEE	2	
		Ccee	11	
RHD-CE-D hybrid alleles	RHD-CE(2-9)-D ₂	CcEe	1	13
		Ccee	1	
		CcEe	15	
RHD alleles with SNP	RHD(K409K)	CcEe	1	16
	Weak D type 15	CcEe	1	
		CcEe	1	
	Weak D type 17	CcEe	2	
Total				126

- Transfusion 46(2006)2128
- applicable to all East Asian populations



RHD alleles expressing DEL differ among ethnic groups

Population	Prevalence in D-negative blood donors		Prevalent RHD alleles
	Any RHD allele	DEL phenotype	
Europeans	0.2%	1 in 1000	<u>RHD(IVS3+1G>A)</u> , RHD-CE(8-9)-D, RHD Ψ , weak <u>D type 11 in CDe^s</u>
Africans	10%	<1 in 100	RHD Ψ , Cde ^s
East Asians	30%	1 in 3	<u>RHD(K409K)</u> , <u>weak D type 15</u> , <u>weak D type 17</u>

– Curr Opin Hematol 13(2006)476-83



Hemovigilance: published evidence

- Anti-D in D neg. recipients
 - without known reason for immunization
 - trace back: evidence for weak D type 2 and 26
- Transfusion of RHD pos. D neg. RBC unit
 - proof or refutation of clinical relevance
 - classical look back: evidence for D+/- chimera
- Tx reaction in D neg. recipient with anti-D
 - any RHD pos. D neg. RBC unit involved?
 - no evidence to date



Donors typing D negative by serology ...

... screen for the presence of weak D, DEL and D+/- chimera by molecular methods.

It's so easy.




Blood group genotyping may equip transfusion medicine to provide personalized medication at reasonable cost:

we should seize this opportunity.

Transfusion 46(2006)1064
Rh blood group review 2007 online:
<http://www.aerzteblatt.de/v4/archiv/pdf.asp?lang=en&id=54759>



DRK-Blutspendedienst
Baden-Württemberg - Hessen 

Institut für Klinische
Transfusionsmedizin und
Immungenetik Ulm (IKT Ulm)