Molecular Blood Group Typing: Methods and Indications

Christoph Gassner

Blutspende Zürich, Schlieren, Switzerland

Tuesday, September 11th 2012 in Graz, DGTI, Östriiich

Geno- meets Phenotype in blood group typing in ~ 1990

Nature. 1990 May 17;345(6272):229-33.

Molecular genetic basis of the histo-blood group ABO system.

Yamamoto F, Clausen H, White T, Marken J, Hakomori S.

Biomembrane Institute, Seattle, Washington.

Abstract

The histo-blood group ABO, the major human alloantigen system, involves three carbohydrate antigens (ABH). A, B and AB individuals express glycosyltransferase activities converting the H antigen into A or B antigens, whereas O (H) individuals lack such activity. Here we present a molecular basis for the ABO genotypes. The A and B genes differ in a few single-base substitutions, changing four amino-acid residues that may cause differences in A and B transferase specificity. A critical single-base deletion was found in the O gene, which results in an entirely different, inactive protein incapable of modifying the H antigen.

Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 10925–10929, November 1992
Medical Sciences

Molecular cloning and primary structure of the human blood group RhD polypeptide

(erythrocyte membrane/RhD antigen/cDNA/gene analysis/PCR)

CAROLINE LE VAN KIM, ISABELLE MOURO, BAYA CHÉRIF-ZAHAR, VIRGINIE RAYNAL, CATHERINE CHERRIER, JEAN-PIERRE CARTRON*, AND YVES COLIN

Unité Institut National de la Santé et de la Recherche Médicale U76, Institut National de Transfusion Sanguine, 6 rue Alexandre Cabanel, 75015 Paris, France

Reasoning for molecular blood group typing

blood

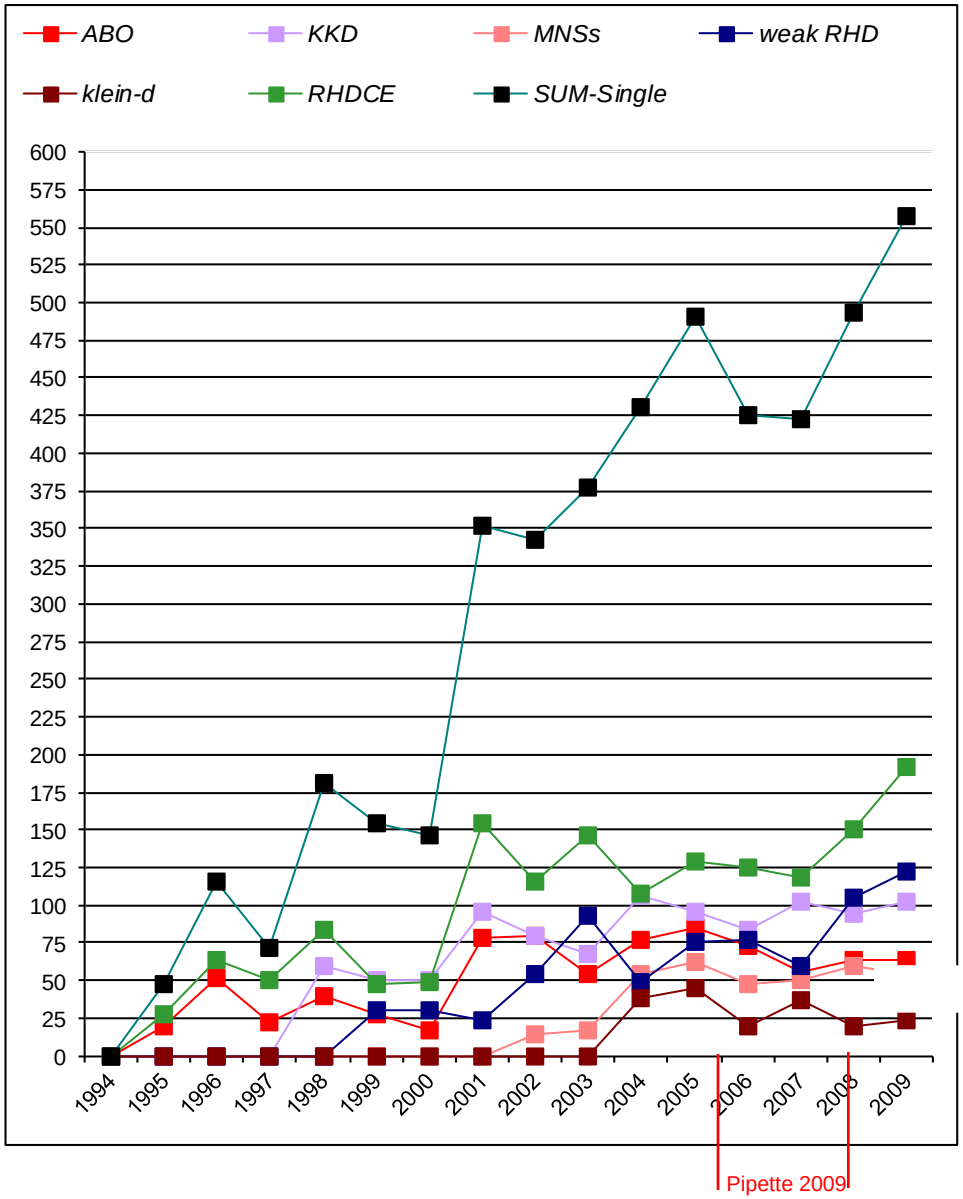
2009 114: 248-256
Prepublished online May 1, 2009;
doi:10.1182/blood-2008-11-146860

Red cell genotyping and the future of pretransfusion testing

David J. Anstee

Table 1: Useful applications of red cell genotyping in transfusion medicine

- Fetal DNA typing
- Extensive blood group typing of donors for alloimmunized patients
- Determining the blood group of a recently transfused patient
- Screening blood donors to find rare blood group phenotypes
- Determining the frequency of blood group polymorphisms in a population
- Determining *RHD* zygosity for fathers of fetuses at risk for HDFN
- Blood group typing of patients with autoimmune hemolytic anemia



Arten und Anzahl Untersuchungen Mol BG Innsbruck 1994-2009 (ca. 50‘000 Ery-Conc. / Jahr)

Blutgruppendiagnostik auf molekularer Ebene

Christoph Gassner

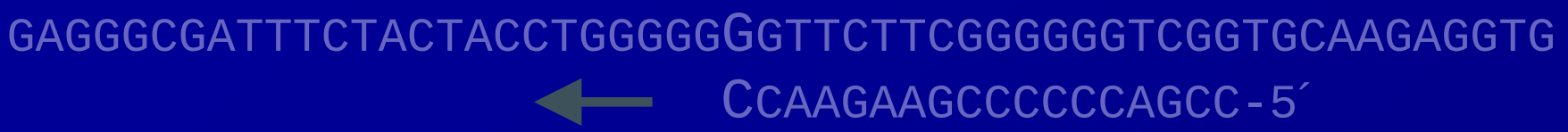
Sonderheft
Transfusionsmedizin
der

pipette
SWISS LABORATORY MEDICINE
Nr. 4, August 2009

Tabelle 1
Exemplarische Übersicht der Begründungen für die Untersuchung molekularer Blutgruppen in 1162 Fällen.

Haupt-Begründung	n	%	Detail-Begründung	n	%
Abklärung ABO, fehlende Isoaggl.	77	6,6%	hiervon Abklärung weak A	28	36,4%
Abklärung KEL	23	2,0%			
Abklärung FY	8	0,7%			
Abklärung RARE	20	1,7%	hiervon Abklärung LU	8	40,0%
Abklärung RHCE	25	2,2%	hiervon Abkl. weak RHC	18	72,0%
Abklärung RHD	314	27,0%	hiervon Abkl. weak RHD	233	74,2%
Bestimmung RHD-Zygotie	9	0,8%			
Bestimmung HPA	28	2,4%			
«Ery-Beladung mit AK (pos. DCT)»	151	13,0%			
«vortransfundiert»	183	15,7%			
Ringversuche	64	5,5%			
Sicherung Referenz-DNA	118	10,2%			
Andere Gründe als oben	110	9,5%			
Befund fehlt	32	2,8%			
	1162	100,0%			

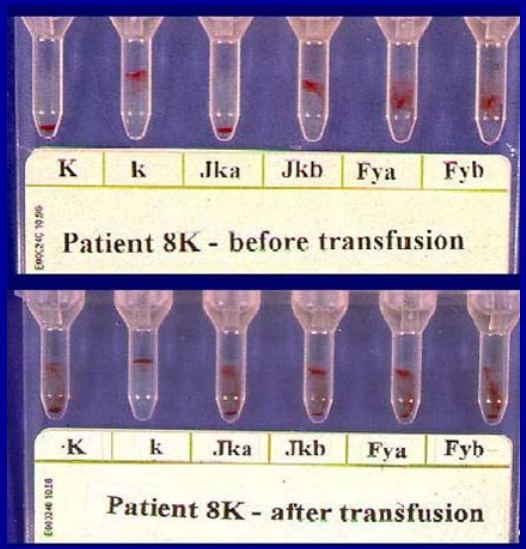
Detection of SNPs by Polymerase Chain Reaction using Sequence Specific Priming (PCR-SSP)

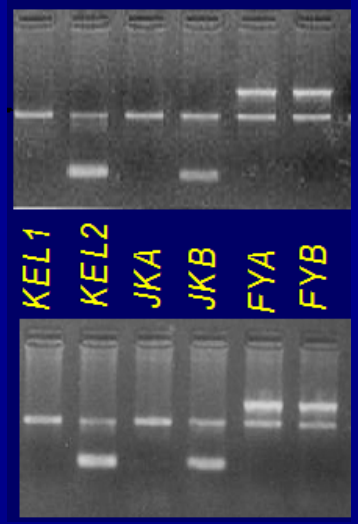


Specificity of the PCR-SSP relies on the relative inability of the TAQ-Polymerase to start polymerisation from 3´ mismatched primers...

Every mutation (SNPs) needs to be detected in an individual PCR-SSP.

PHENO- and GENOtyping before and after massive transfusion





IMMUNOHEMATOLOGY

Differentiation of autologous *ABO, RHD, RHCE, KEL, JK,* and *FY* blood group genotypes by analysis of peripheral blood samples of patients who have recently received multiple transfusions

P. Rožman, T. Dovč, and C. Gassner

936 **TRANSFUSION** Volume 40, August 2000

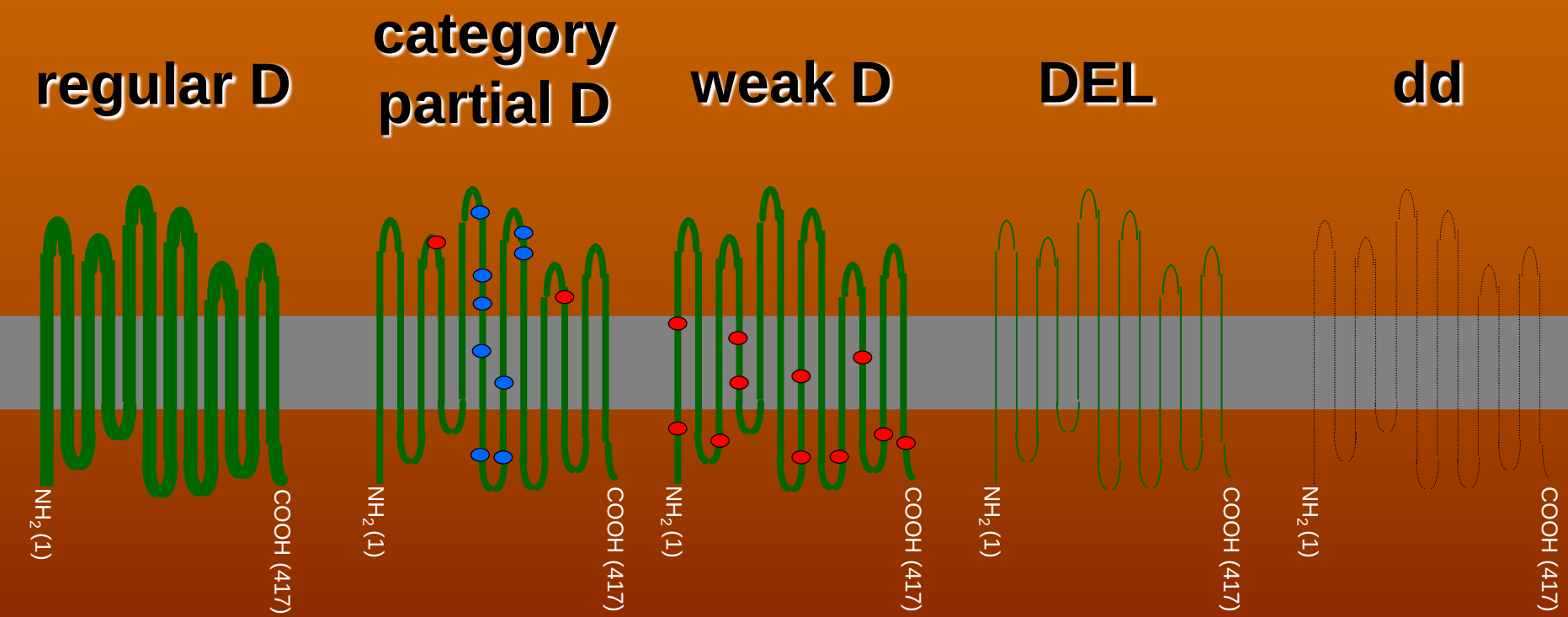
Case	Patient	Units (22)
	kk	Kk (2)
	Jk(a-b+)	Jk^a pos (17)
	Fy(a+b+)	all compatible

General **NON**-white blood cells reduced blood units

	range	average
Transfusion events	2 - 6	3.0
Units per 1 transfusion	1 - 36	7.9
Age of units	1 - 25d	5.2d
Sampling after transfusion	0 - 24d	4.7d

"The posttransfusion genotyping results were not influenced by either the amount of blood transfused in a transfusion event (range, 1-36 units) or the sampling period after a transfusion event (range, several hours-24 days)."

D in the erythrocyte membrane ...



inner side

Blutgruppendiagnostik auf molekularer Ebene

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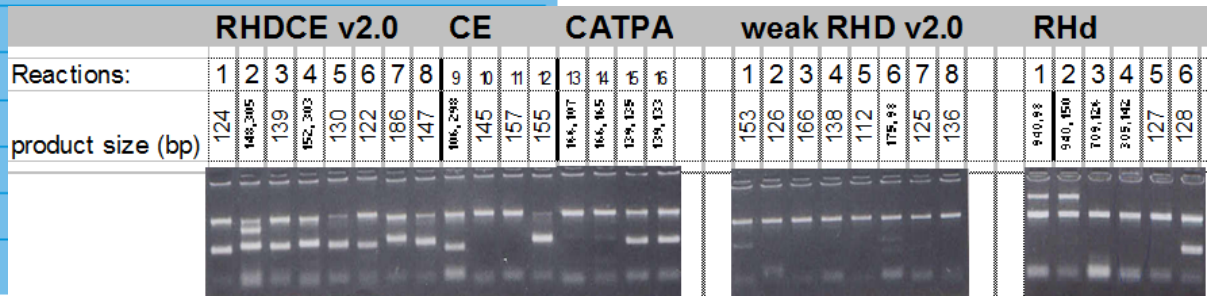
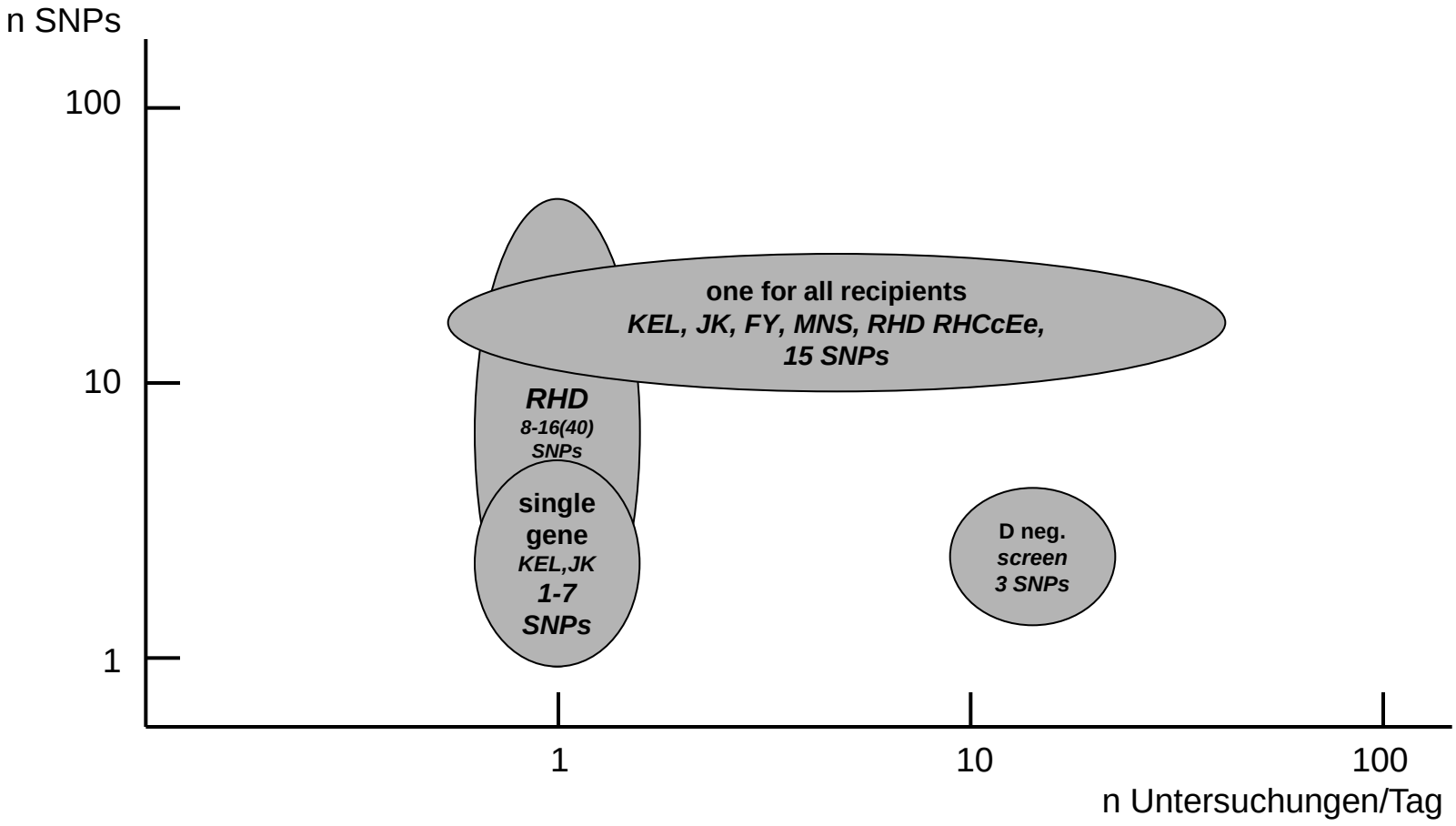
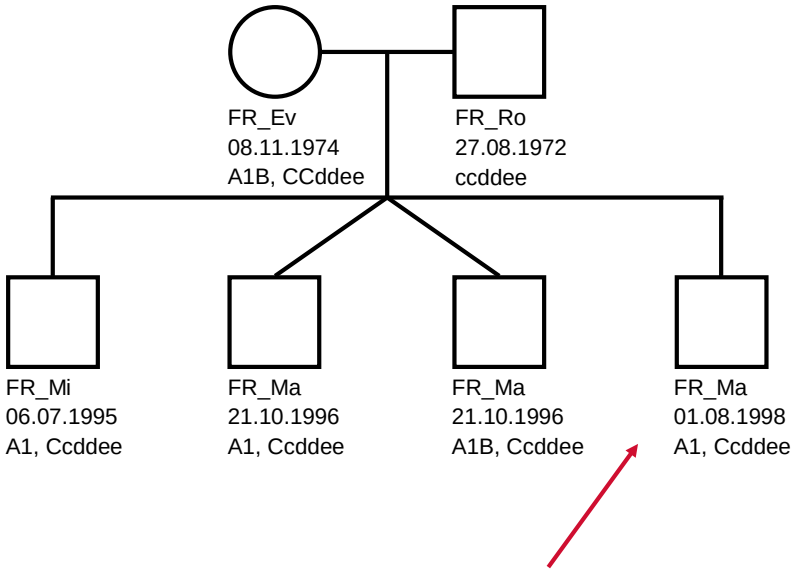



n SNPs
100
10
1
one for all recipients
KEL, JK, FY, MNS, RHD RHCcEe,
15 SNPs
RHD
8-16(40)
SNPs
single
gene
KEL,JK
1-7
SNPs
D neg.
screen
3 SNPs
1
10
100
n Untersuchungen/Tag

Do not transfuse D pos blood to D neg recipients!

CASE REPORT



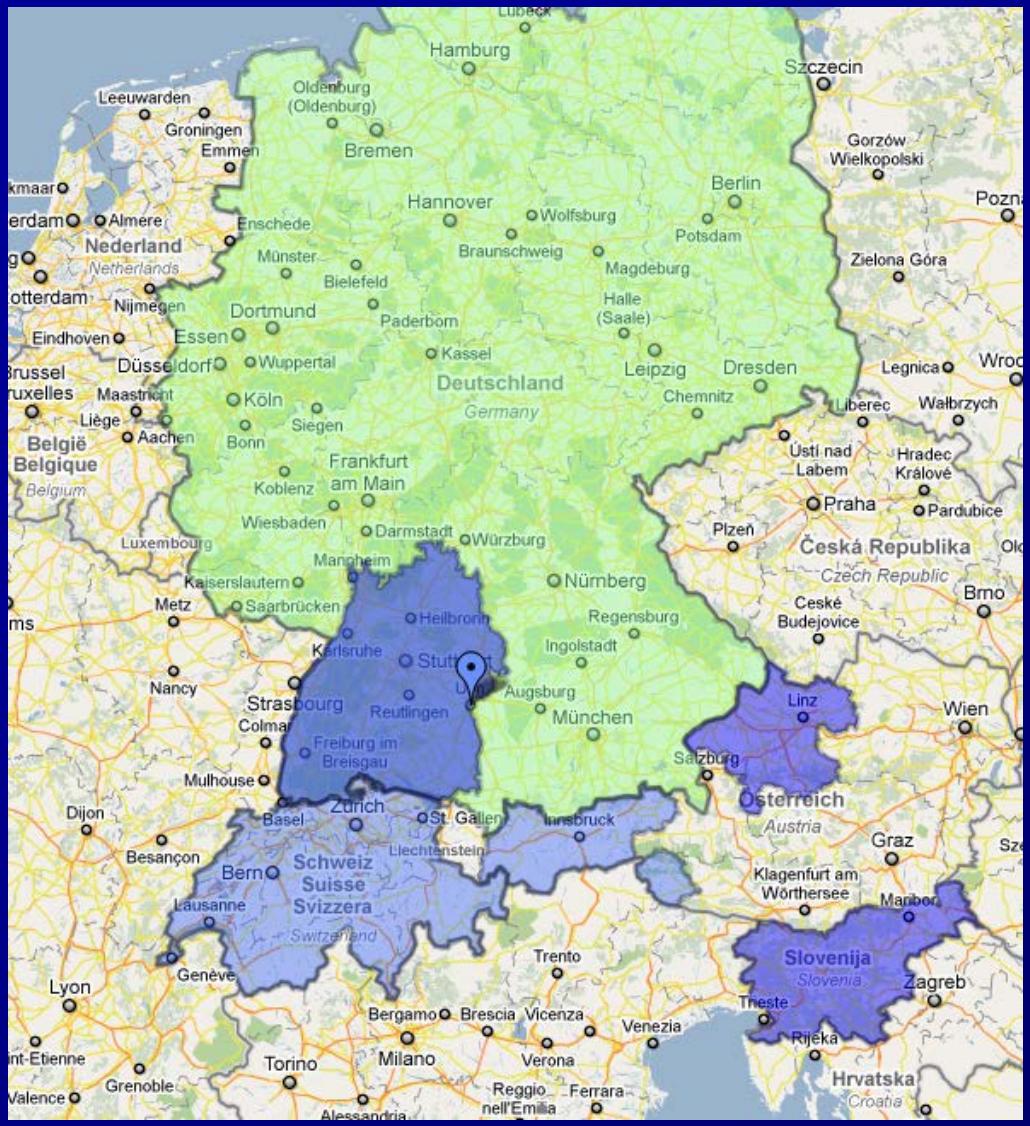
During 3rd pregnancy mother FR_Ev 08.11.1974 received 3 RBC units of CCddee phenotype (01.08.1998):
LU-Id 29.12.1935
SO-He 13.05.1952
TS-He 02.05.1945

During CCddee retesting in March 2002, LU-Id 29.12.1935
showed a weak D in ICT.
DNA testing showed a RHD gene of unknown specificity.

Consequently, serum of mother FR-Ev 08.11.1974 was investigated and showed allo-anti D:
++, standard LISS COOMBS (37°)
++++, papainised COOMBS

Towards routine mass-application: **SCREENING** of D negative donors.

Screening for *RHD* among D negative Caucasians and Asians



Transfusion. 2009 Apr;49(4):676-81.
Polin H et al
Upper Austria AT

Transfusion. 2009 Mar;49(3):465-71.
Flegel WA et al
Baden-Württemberg

Transfusion. 2005 Apr;45(4):527-38.
Gassner C et al
Switzerland, Tyrol AT, Slovenia, Northern Germany, Braunschweig, Kirov RU

BMC Genet. 2001;2:10.
Wagner FF et al
Baden-Württemberg

Results of Bern & Zurich Investigations, 2005 to 2012, (alleles complete)

	SAMPLES WITH RHD GENE	2001 ULM	2005 IBK	2009 IBK	2009 ULM	2009 LINZ	SUM	2002 Guang-dong	2004 Tainan Taiwan	2005 Seoul Korea	2005 Shan-dong	2006 Gwan g-ju	2008 Fujian	SUM	2005 BE Stu I	2007 BE Stu II	2012-BE	2009 ZH	2012-ZH	ex	SUM	
	SUM: with big letters	753	1700	960	2999	1934	8346	52	132	107	25	51	46	413	652							
	SUM: ccddee	7688	0	0	43038	21396	72122	50	162	157	49	72	58	548								
	SUM: all	**8441**	**1700**	**960**	46037	23330	**80468**	**102**	**294**	**264**	**74**	**123**	**104**	**961**	**652**	17391	11565	**8200**	**3610**		**41418**	
Hybrid genes	C c dd e e RHD-CE(2-7)-D1	2					2										1				1	
	C c dd e e RHD-CE(2-9)-D1	3	39	16		11	69			1				1	2	5	16		2	2 LU	25	
	C c dd e e RHD-CE(2-9)-D2	8				1	9	8	15	22	5	12	9	71								
	C c dd e e RHD-CE(3-9)-D					1	1								2		3				5	
	C c dd e e RHD-CE(2-8)-D		4				4										1				1	
	C c dd e e RHD-CE(3-7)-D															3					3	
	c c dd E e RHD-CE(3-7)-D, (3-8)																		2	1 LU	2	
	C c dd e e RHD-CE(3-7)-D, Cde^5	1					1								1		1				2	
	C c dd e e RHD-CE(4-9)-D					2	2								2						2	
	c c dd E e RHD-CE(4-9)-D															1					1	
	c c dd E e RHD-CE(4-7)-D1	3	1				4												1		1	
	C c dd e e RHD-CE(4-7)-D	1					1											1			1	
	c c dd E e RHD-CE(8-9)-D	3			17		20															
RHD Null	c c dd e e D neg: RHD psi	1			14	3	18									4	6	5			15	
	c c dd E e D neg: RHD(W16X)	2			2		4										4	1	1		6	
	C c dd e e D neg: RHD(343delC)				2		2									1					1	
	? ? dd ? ? D neg: RHD(489delAGC)																2				2	
	c c dd e e D neg: RHD 545(delCTGT)															1					1	
	C c dd e e D neg: RHD(R318X)				2		2										1				1	
RHD DEL	C c dd e e DEL: RHD(M295I)	7	8	4	14	2	35								4		7	5	2	2 LU	18	
	? ? dd ? ? DEL: RHD(M295T)																3				3	
	C c dd e e DEL: RHD(IVS3+1g>a)	3	6		16	24	49								3	1	3	4			11	
	C c dd e e DEL: RHD(IVS3+5g>a)															1	1				2	
	C c dd e e DEL: RHD(IVS5-38del4)														2						2	
	C c dd e e DEL: RHD(K409K)	5			4	1	10	35	94	42	22	16	27	236			2	1			3	
	C c dd e e DEL: RHD-CE(10), or del ex 10																	1			1	40
RHD weak, parti	C c dd e e RHD partial DVL-2														2			4	1	1 LU	7	
	c c dd e e weak D type 4.3					31	31															
	c c dd E e weak D type 5		2			1	3															
	C c dd e e weak D type 31														1		2				3	
	C c dd e e weak D type 32					2	2															
	C c dd e e weak D type 38															1	1	1			3	13
	C c dd e e regular RHD ?		9	1	1		11								3						3	
	? ? dd ? ? RHD-CE(2)-D																1				1	
	? ? dd ? ? RHD(P291R)														1						1	
	? ? dd ? ? not yet specified																2				2	
	SUM	45	89	21	96	94	345	47	109	68	28	29	38	319	23	18	57	23	9		130	

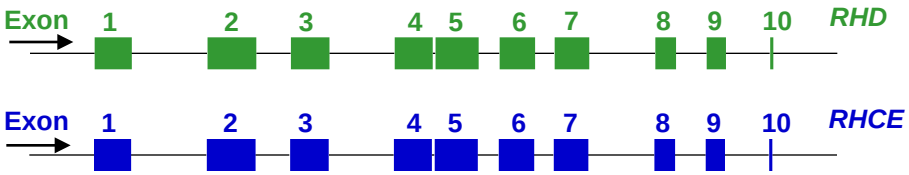
RhD-Screening at the ZHBSD

Pooled blood sample screening (Wagner et al., 2001)

★ Single blood sample screening

III 2009 - 2011

2012 on …



★ 1. Preparation of 95 single DNAs (microtiter plate)

2. PCR-Test
Exon 5 & 10

3. PCR-Test Exon
7 & B-Allel