BIOLOGIA MOLECULAR EN INMUNOHEMATOLOGIA: PRESENTE Y FUTURO

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Escuela de Tecnología Médica

Universidad Mayor

2014

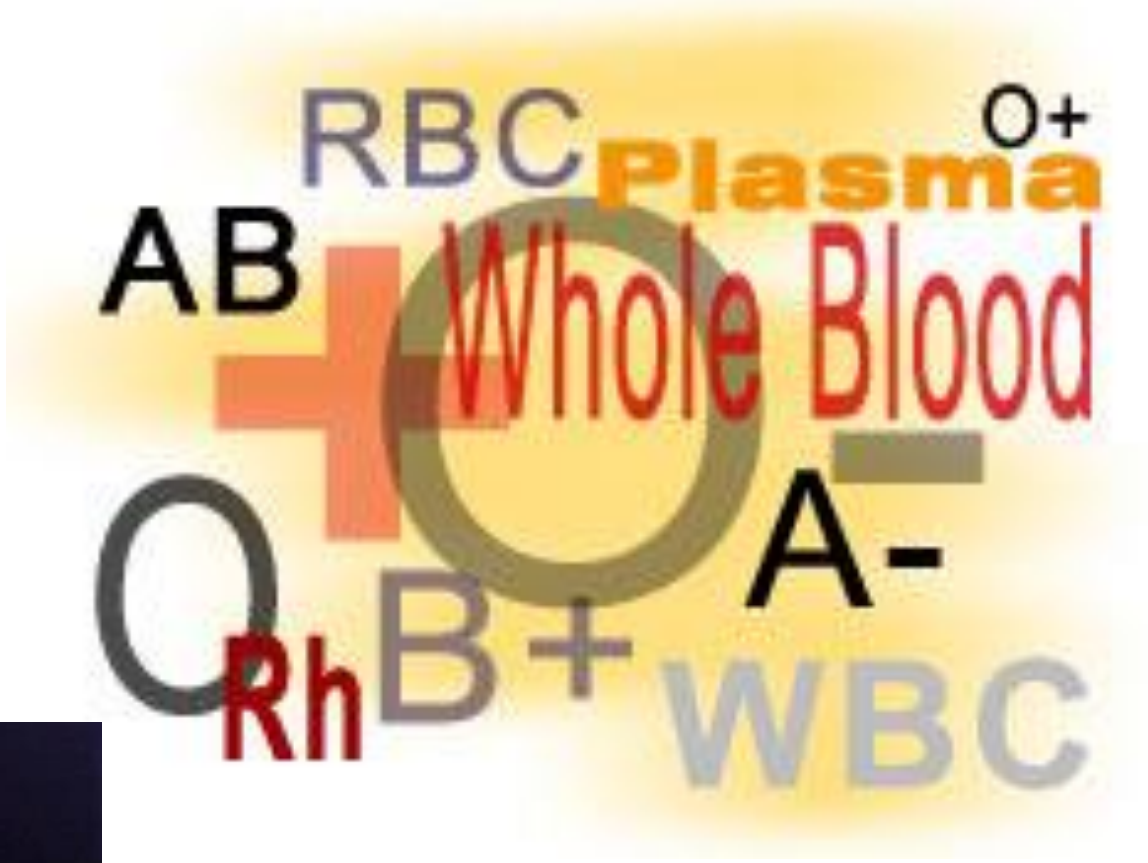

RBC
O+
Plasma
AB
Whole Blood
O
A-
Rh
B+
WBC

Table 1
Blood group systems recognized by the International Society of Blood Transfusion [78].

No.	Name (symbol)	Gene name(s)	No. of antigens
001	ABO	*ABO*	4
002	MNS	*GYPA, GYPB*	46
003	P1PK	*A4GALT*	3
004	Rh (RH)	*RHD, RHCE*	54
005	Lutheran (LU)	*LU*	20
006	Kell (KEL)	*KEL*	35
007	Lewis (LE)	*LE (FUT3)*	6
008	Duffy (FY)	*FY (DARC)*	5
009	Kidd (JK)	*JK (SLC14A1, HUT11A)*	3
010	Diego (DI)	*DI (SLC4A1, AE1, EPB3)*	22
011	Yt (YT)	*YT (ACHE)*	2
012	Xg (XG)	*XG (PBDX)*	2
013	Scianna (SC)	*SC (ERMAP)*	7
014	Dombrock (DO)	*DO (ART4)*	8
015	Colton (CO)	*CO (AQP1)*	4
016	Landsteiner-Wiener (LW)	*LW (ICAM4, CD242)*	3
017	Chido-Rodgers (CH/RG)	*CH (C4B), RG (C4A)*	9
018	H (H)	*H (FUT1)*	1
019	Kx (XK)	*XK*	1
020	Gerbich (GE)	*GE (GYPC)*	11
021	Cromer (CROM)	*CROM (DAF)*	18
022	Knops (KN)	*KN (CR1)*	9
023	Indian (IN)	*IN (CD44)*	4
024	Ok (OK)	*OK (BSG, EMPRIN)*	3
025	Raph (RAPH)	*RAPH (CD151)*	1
026	John Milton Hagen (JMH)	*JMH (SEMA7A, CD108, SEMA-L)*	6
027	I (I)	*I (GCNT2, IGnT)*	1
028	Globoside (GLOB)	*GLOB (B3GALNT1)*	1
029	Gill (GIL)	*GIL (AQP3)*	1
030	Rh-associated glycoprotein (RHAG)	*RHAG*	4
031	FORS (FORS)	*FORS (GBGT1, A3GALNT)*	1
032	JR (JR)	*JR (ABCG2)*	1
033	Lan (LAN)	*LAN (ABCB6)*	1
034[a]	Vel (VEL) [3–5]	*VEL (SMIM1)*	1

[a] The Vel blood group has not been officially approved by the ISBT.

SISTEMA DE GRUPO SANGUÍNEO ABO

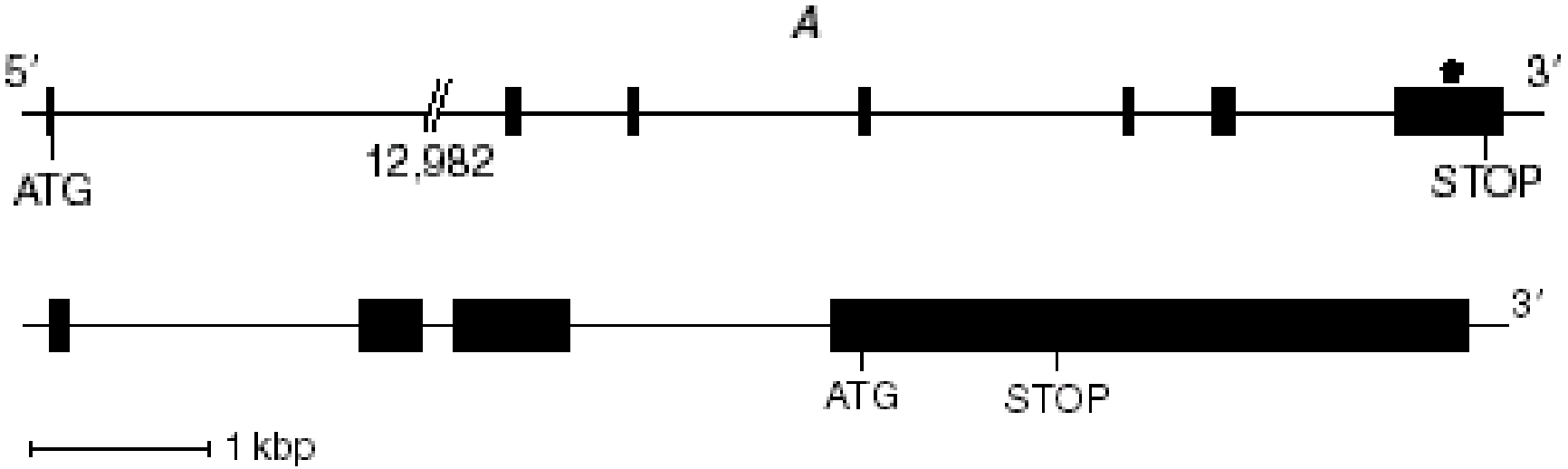
- 1900… Karl Landsteiner
- Primero A y B, luego “O” (Ohne). Finalmente AB.
- 4 antígenos
- Estructura
- Expresión diferencial

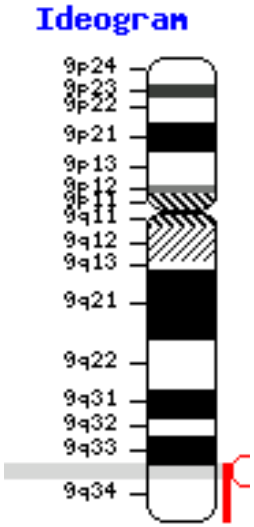
Blood grouping based on RBC agglutination (Landsteiner, 1900)

Serum \ RBC	Dr.St.Dr.	Plee.Dr.	Sturl. Dr.	Erdh. Mr.	Zar. Mr.	Land.
Dr. St.	-	+	+	+	+	-
Dr. Plee.	-	-	+	+	-	-
Dr. Sturl.	-	+	-	-	+	-
Dr. Erdh.	-	+	-	-	+	-
Mr. Zar.	-	-	+	+	-	-
Mr. Land.	-	+	+	+	+	-

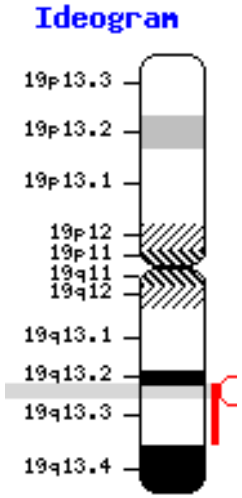
(+ agglutination - no agglutination)

GENÉTICA DEL GRUPO SANGUÍNEO ABO



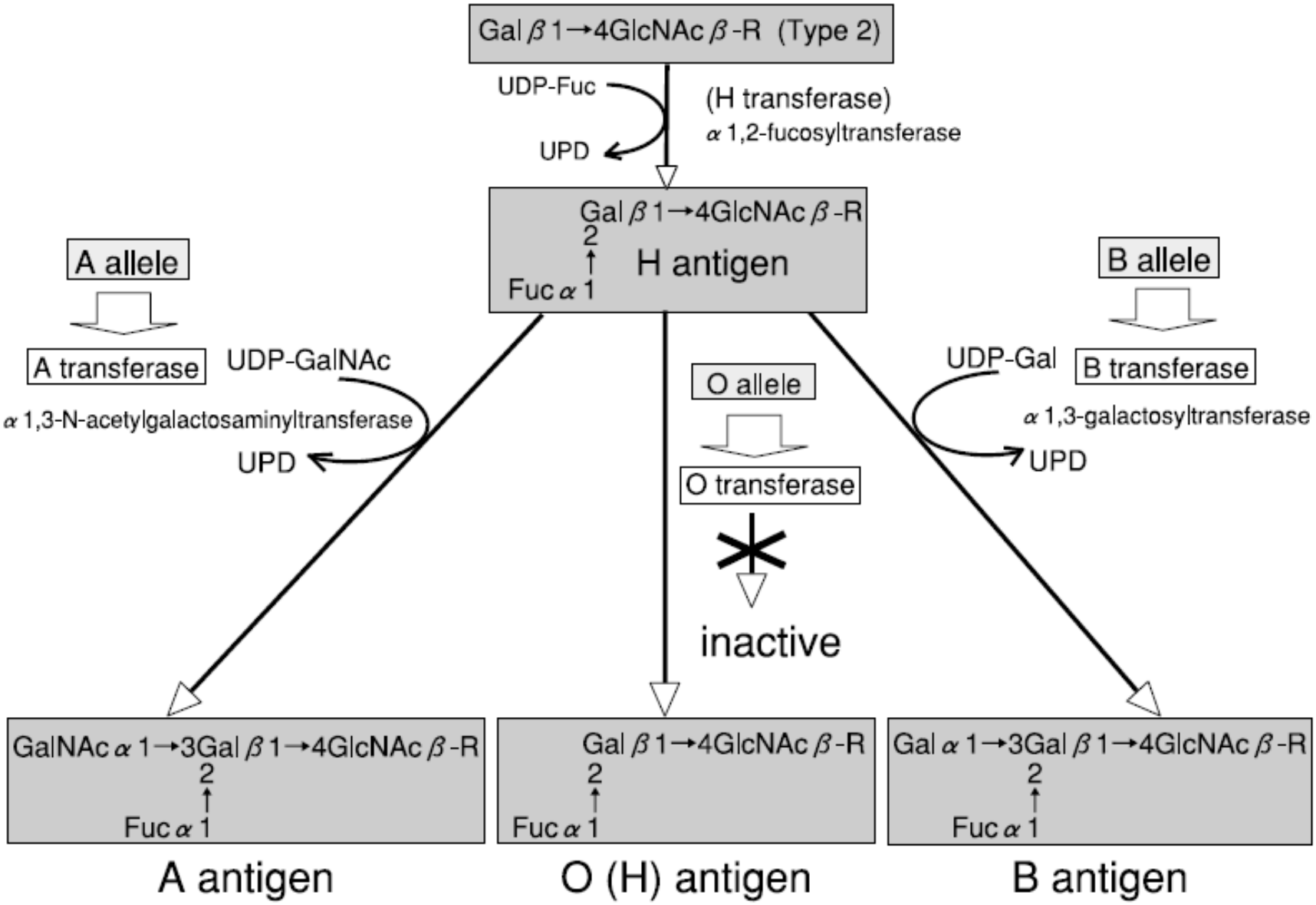


Locus-Gen ABO

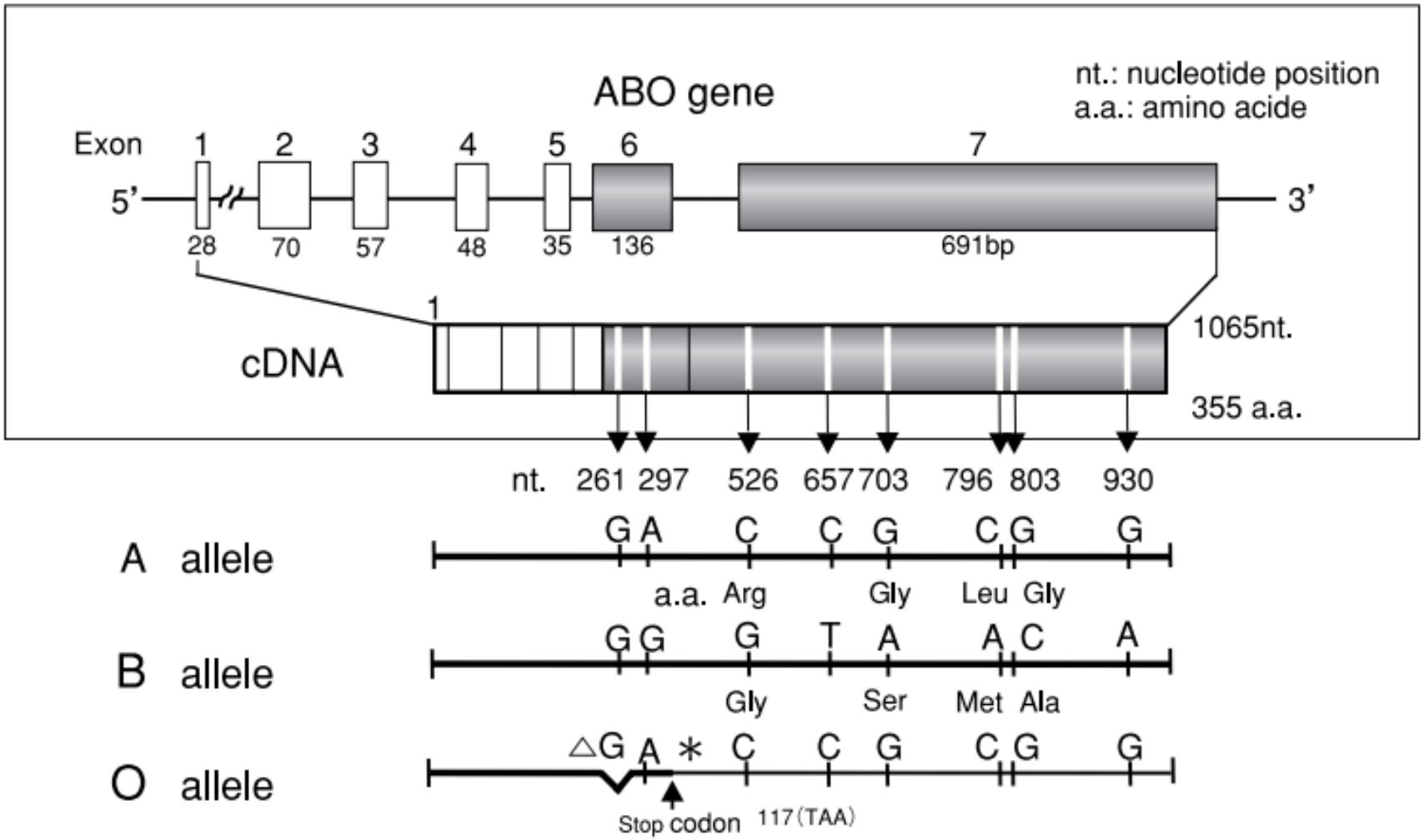


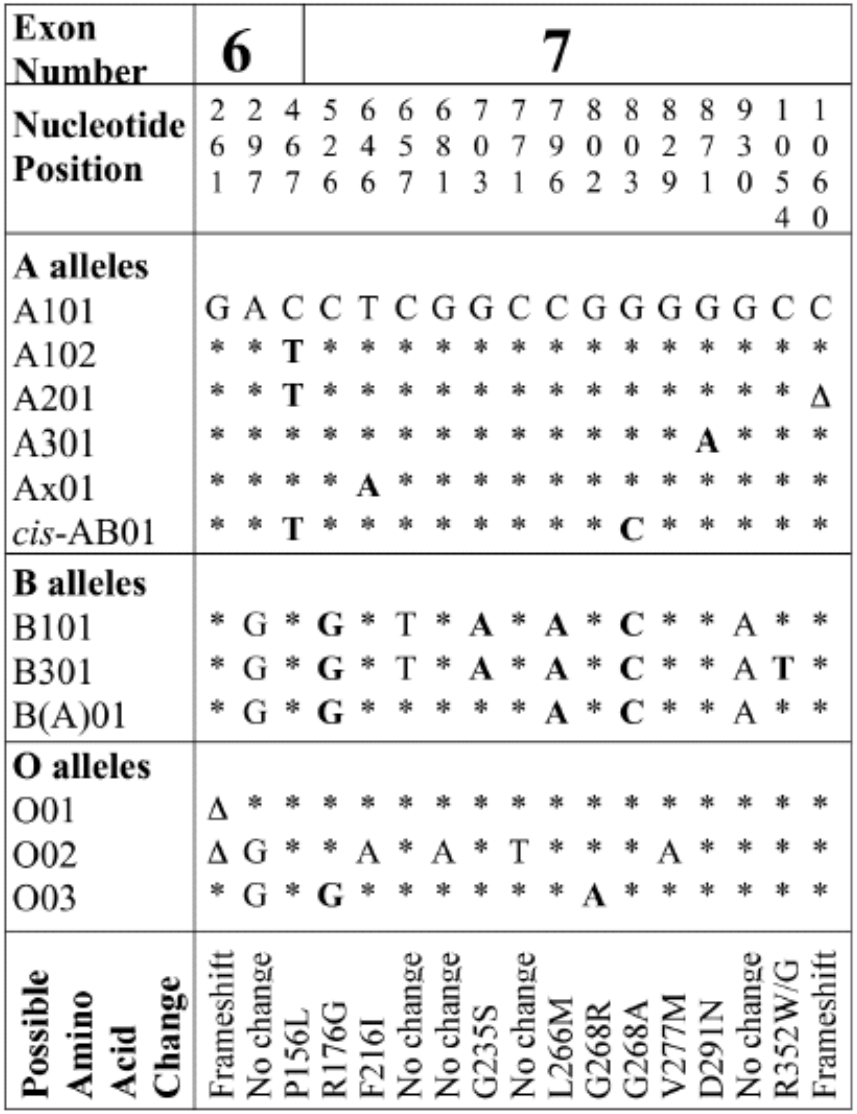
Locus-Gen H (FUT1)

What is the product of ABO blood group gene and H gene ??


Gal β 1→4GlcNAc β -R (Type 2)
UDP-Fuc
UPD
(H transferase)
α 1,2-fucosyltransferase
Gal β 1→4GlcNAc β -R
2
Fuc α 1
H antigen
A allele
A transferase
UDP-GalNAc
α 1,3-N-acetylgalactosaminyltransferase
UPD
O allele
O transferase
inactive
B allele
UDP-Gal
B transferase
α 1,3-galactosyltransferase
UPD
GalNAc α 1→3Gal β 1→4GlcNAc β -R
2
Fuc α 1
A antigen
Gal β 1→4GlcNAc β -R
2
Fuc α 1
O (H) antigen
Gal α 1→3Gal β 1→4GlcNAc β -R
2
Fuc α 1
B antigen

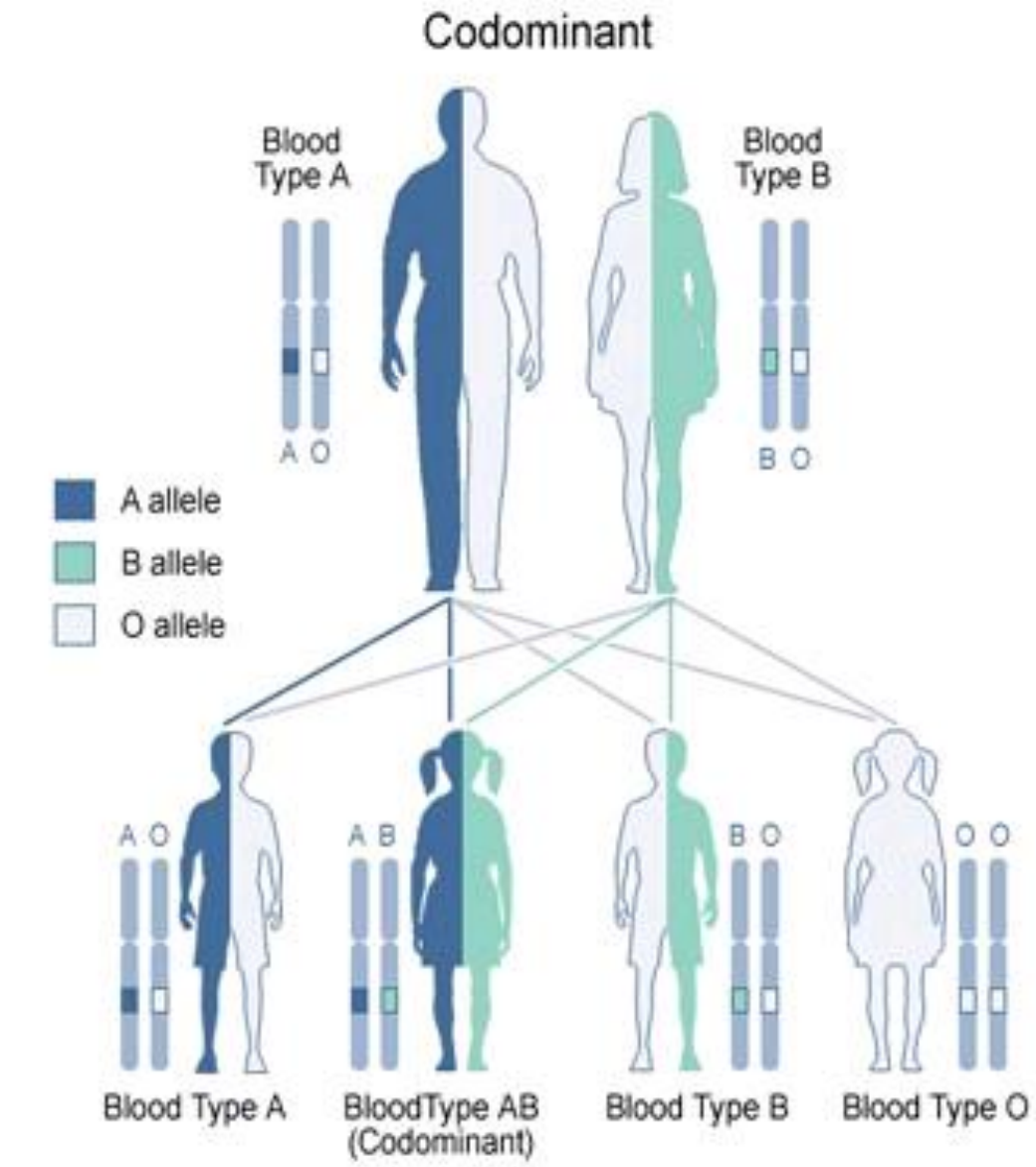
Hosoi E, 2008


ABO gene
nt.: nucleotide position
a.a.: amino acide
Exon 1 2 3 4 5 6 7
5'
3'
28 70 57 48 35 136 691bp
cDNA
1
1065nt.
355 a.a.
nt. 261 297 526 657 703 796 803 930
A allele
G A C C G C G G
a.a. Arg Gly Leu Gly
B allele
G G G T A A C A
Gly Ser Met Ala
O allele
△G A * C C G C G G
Stop codon 117(TAA)



Exon Number	6			7													
Nucleotide Position	261	297	467	526	646	657	681	703	771	796	802	803	829	871	930	1054	1060
A alleles																	
A101	G	A	C	C	T	C	G	G	C	C	G	G	G	G	G	C	C
A102	*	*	**T**	*	*	*	*	*	*	*	*	*	*	*	*	*	*
A201	*	*	**T**	*	*	*	*	*	*	*	*	*	*	*	*	*	Δ
A301	*	*	*	*	*	*	*	*	*	*	*	*	*	**A**	*	*	*
Ax01	*	*	*	*	**A**	*	*	*	*	*	*	*	*	*	*	*	*
cis-AB01	*	*	**T**	*	*	*	*	*	*	*	*	**C**	*	*	*	*	*
B alleles																	
B101	*	G	*	**G**	*	T	*	**A**	*	**A**	*	**C**	*	*	A	*	*
B301	*	G	*	**G**	*	T	*	**A**	*	**A**	*	**C**	*	*	A	**T**	*
B(A)01	*	G	*	**G**	*	*	*	*	*	**A**	*	**C**	*	*	A	*	*
O alleles																	
O01	Δ	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
O02	Δ	G	*	*	A	*	A	*	T	*	*	*	A	*	*	*	*
O03	*	G	*	**G**	*	*	*	*	*	*	**A**	*	*	*	*	*	*
Possible Amino Acid Change	Frameshift	No change	P156L	R176G	F216I	No change	No change	G235S	No change	L266M	G268R	G268A	V277M	D291N	No change	R352W/G	Frameshift

Fig. 2. **Representative alleles at the *ABO* locus.** Nucleotide and deduced amino acid sequences are compared among a dozen *ABO* alleles whose sequences were determined by Yamamoto and colleagues. The *cis-AB* and *B(A)* alleles are included in the *A* and *B* alleles, respectively, because of higher relative sequence homology. The nucleotide substitutions that result in amino acid substitutions are shown in bold type. Δ = deletion of nucleotide.



U.S. National Library of Medicine

Molecular basis associated with variant A transferases[3,4]

(*ABO*A101* taken as the reference allele sequence)

Phenotype	*Nucleotide change*	*Amino acid change*
A_1	467C>T	Pro156Leu
A_2	467C>T; 1059–1061delC	Pro156Leu; fs and 21 extra amino acids
A_2	1054C>T	Arg352Trp
A_2	1054C>G	Arg352Gly
A_2	526C>G; 703G>A; 829G>A	Arg176Gly; Gly235Ser; Val277Met
A_3	871G>A	Asp291Asn
A_x	646T>A	Phe216Ile
A_x	A or B–O^{1v} hybrid	Phe216Ile; Val277Met
A_{el}	798–804insG	fs
A_{el}	467C>T; 646T>A	Pro156Leu; Phe216Ile
A_w	407C>T; 467C>T; 1060delC	Thr136Met; Pro156Leu; Pro354fs
A_w	350C>G; 467C>T; 1060delC	Pro156Leu; Gly177Ala; Pro354fs
A_w	203G>C; 467C>T; 1060delC	Arg68Thr; Pro156Leu; Pro354fs
A_w	965A>G	Glu322Gly
A_w	502C>G	Arg168Gly

Molecular basis associated with variant B transferases[3]

(*ABO*B101* taken as the reference allele sequence)

Phenotype	*Nucleotide change*	*Amino acid change*
B_3	1054C>T	Arg352Trp
B_x	871G>A	Asp291Asn
B_{el}	641T>G	Met214Arg
B_{el}	669G>T	Glu223Asp
B_w	873C>G	Asp291Glu
B_w	721C>T	Arg241Trp
B_w	548A>G	Asp183Gly
B_w	539G>A	Arg180His
B_w	1036A>G	Lys346Glu
B_w	1055G>A	Arg352Gln
B_w	863T>G	Met288Arg

Molecular basis associated with the O phenotype[5]

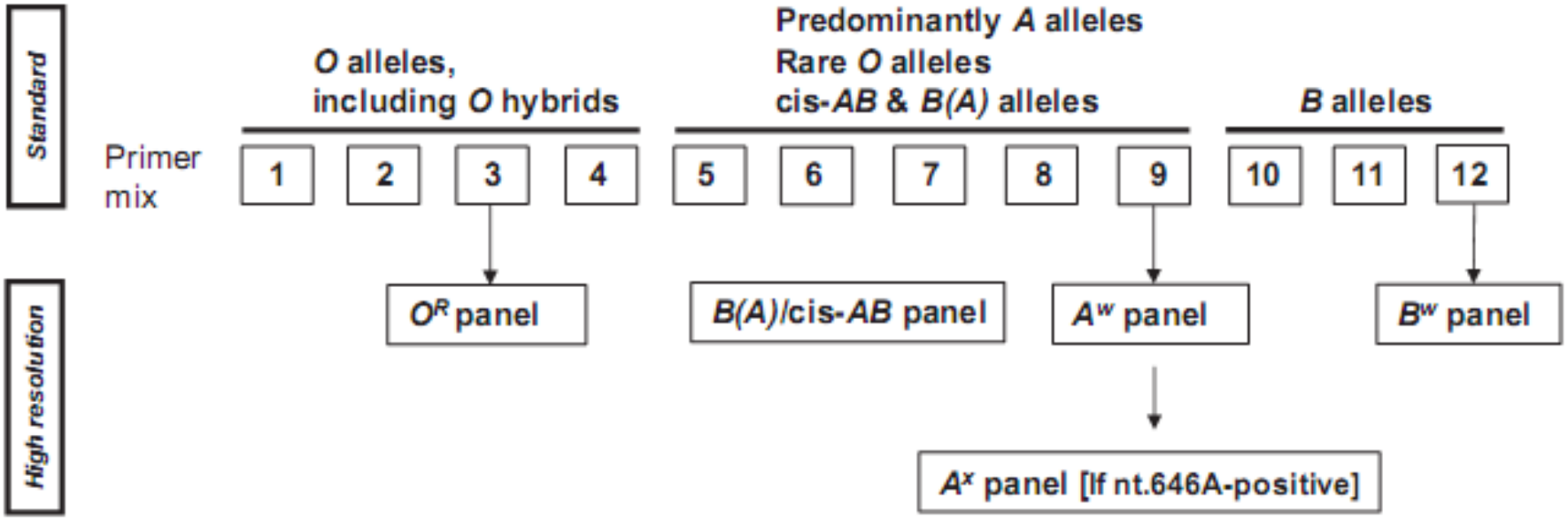
(*ABO*A101* taken as the reference allele sequence)

Allele	*Nucleotide change*	*Amino acid change*
O_1	261delG 88 fs; codon 116Stop	
O_2	526C>G; 802G>A	Arg176Gly; Gly268Arg

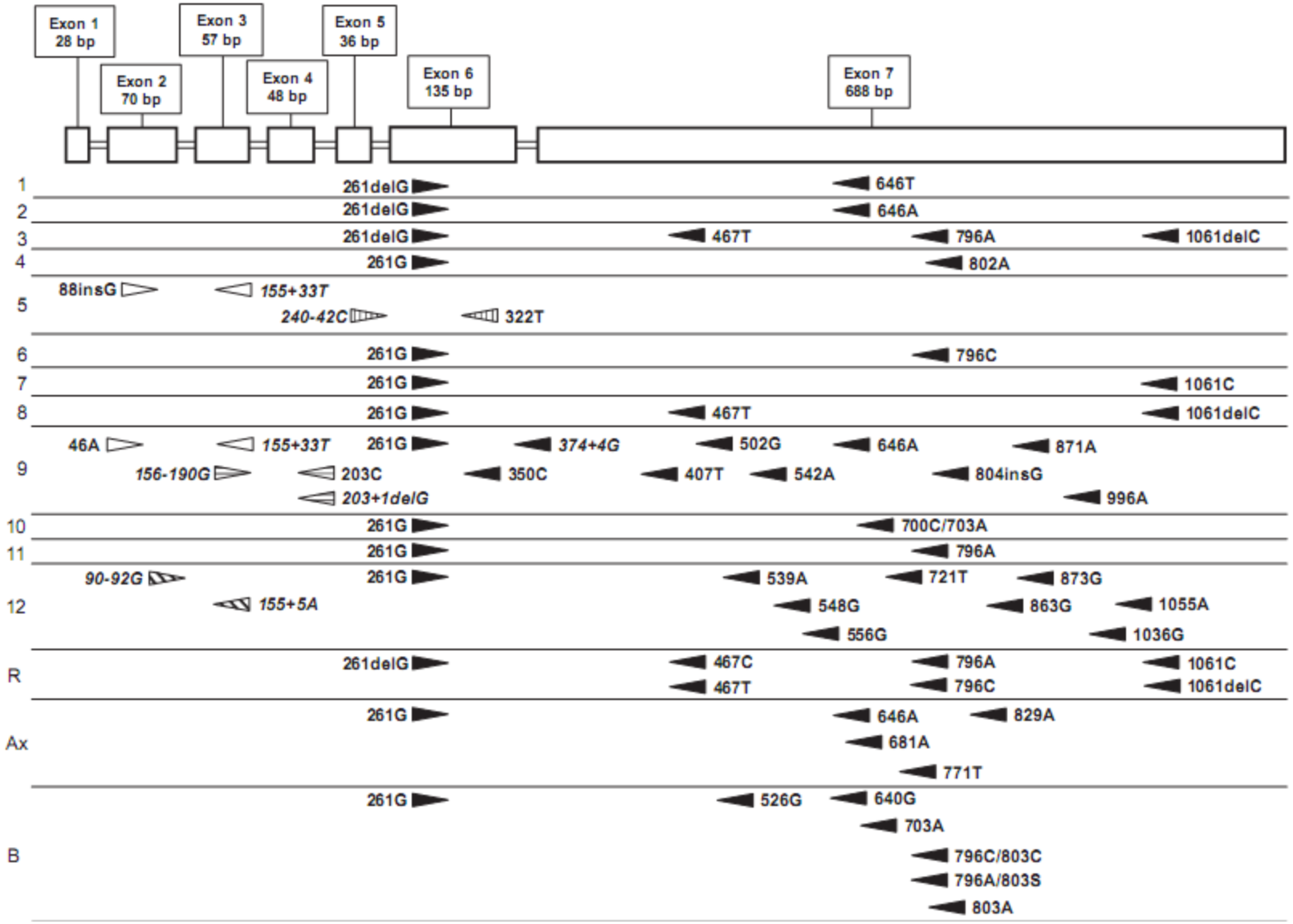
An extensive polymerase chain reaction–allele-specific polymorphism strategy for clinical ABO blood group genotyping that avoids potential errors caused by null, subgroup, and hybrid alleles

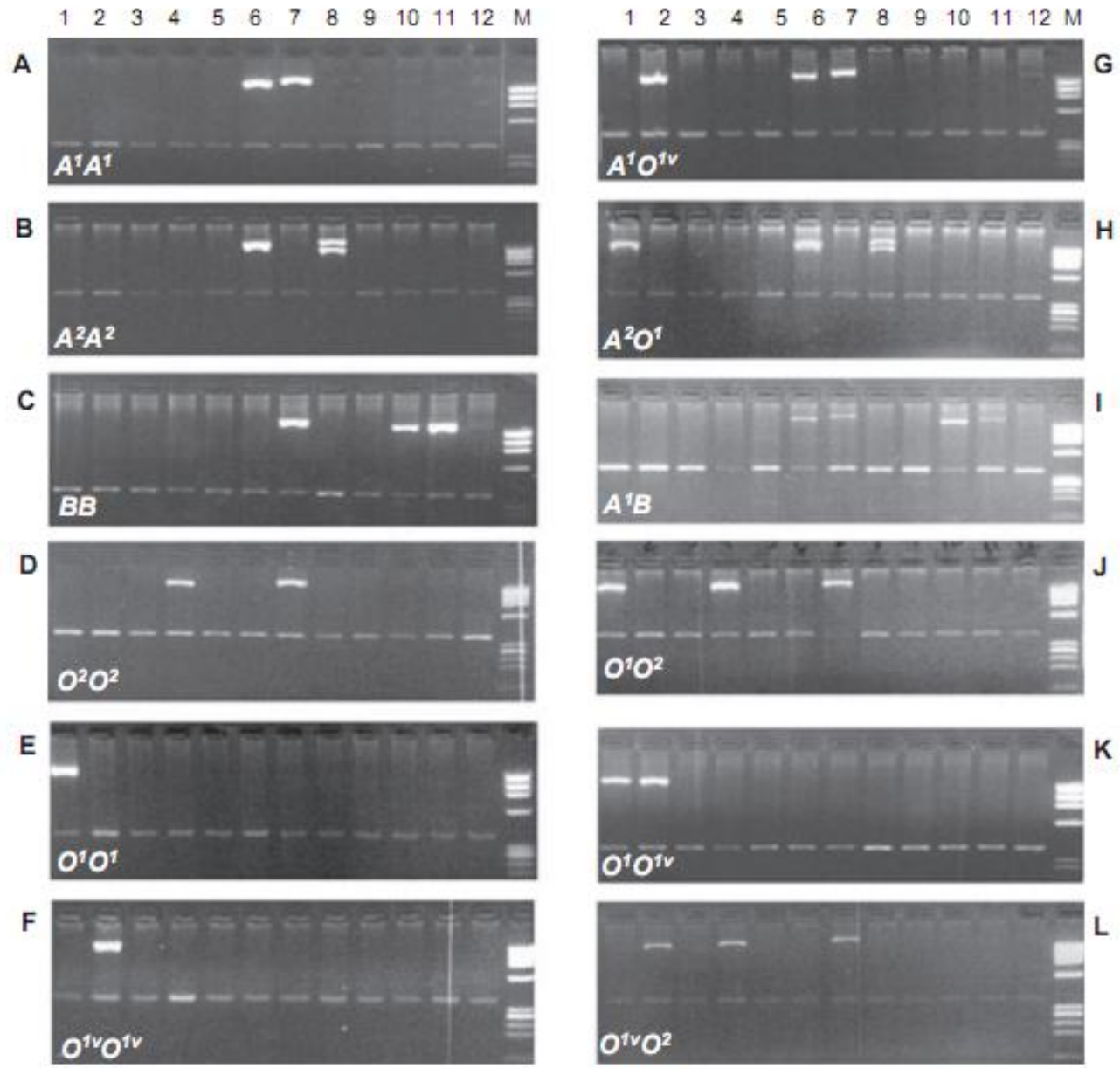
Bahram Hosseini-Maaf, Åsa Hellberg, M. Alan Chester, and Martin L. Olsson

TRANSFUSION 2007;47:2110-2125.



TRANSFUSION 2007;47:2110-2125.


Exon 1
28 bp
Exon 2
70 bp
Exon 3
57 bp
Exon 4
48 bp
Exon 5
36 bp
Exon 6
135 bp
Exon 7
688 bp
1
261delG
646T
2
261delG
646A
3
261delG
467T
796A
1061delC
4
261G
802A
5
88insG
155+33T
240-42C
322T
6
261G
796C
7
261G
1061C
8
261G
467T
1061delC
9
46A
155+33T
261G
374+4G
502G
646A
871A
156-190G
203C
350C
407T
542A
804insG
203+1delG
996A
10
261G
700C/703A
11
261G
796A
90-92G
261G
539A
721T
873G
12
155+5A
548G
863G
1055A
556G
1036G
R
261delG
467C
796A
1061C
467T
796C
1061delC
Ax
261G
646A
829A
681A
771T
B
261G
526G
640G
703A
796C/803C
796A/803S
803A


1 2 3 4 5 6 7 8 9 10 11 12 M
A
A1A1
B
A2A2
C
BB
D
O2O2
E
O1O1
F
O1vO1v
1 2 3 4 5 6 7 8 9 10 11 12 M
G
A1O1v
H
A2O1
I
A1B
J
O1O2
K
O1O1v
L
O1vO2

Brief Communication: Molecular Characterization of O Alleles at the ABO Locus in Chilean Aymara and Huilliche Indians

Elena Llop,[1] Hugo Henríquez,[1,5] Mauricio Moraga,[1] Mario Castro,[2,3] and Francisco Rothhammer[1,4*]

[1]*Human Genetics Program, ICBM, Faculty of Medicine, University of Chile, Chile*
[2]*Morphology Program, ICBM, Faculty of Medicine, University of Chile, Chile*
[3]*Department of Anthropology, Faculty of Social Sciences, University of Chile, Chile*
[4]*University of Tarapaca, CIHDE, Arica, Chile*
[5]*Faculty of Health Sciences, Diego Portales University, Chile*

AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY 131:535–538 (2006)

TABLE 3. Gene frequencies of O alleles of the ABO blood group system defined molecularly in South American Amerinds

Population	O^1	O^{1v}	Other O alleles	n[a]	References
Arara (Brazil)	0.03	0.97	–	15	Olsson et al. (1998)
Aymara (Bolivia)	0.27	0.60	0.13	252	Roubinet et al. (2001)
Aymara (Chile)	0.35	0.65	–	168	This study
Cayapa (Ecuador)	0.50	0.39	0.11	148	Roubinet et al. (2001)
Chango (Chile	0.39	0.61	–	80	Henríquez et al. (2004)
Huilliche (Chile)	0.19	0.81	–	150	This study
Kayapo (Brazil)	0.16	0.84	–	16	Olsson et al. (1998)
Parakaña (Brazil)	0.35	0.65	–	124	Barjas-Castro et al. (2003)
Yanomama (Brazil	0.09	0.91	–	17	Olsson et al. (1998)

[a] n indicates the number of chromosomes analyzed.

TABLE 4. Association between mutation G542A and alleles O^1 and O^{1v}

	Number of individuals according to mutation G542A, to the homozygote or heterozygote state		
Genotype	GG	GA	AA
Aymara			
O^1/O^1	8	0	0
O^1/O^{1v}	35	8	0
O^{1v}/O^{1v}	21	12	0
Huilliche			
O^1/O^1	4	0	0
O^1/O^{1v}	15	5	0
O^{1v}/O^{1v}	39	12	0
Santiago			
O^1/O^1	18	0	0
O^1/O^{1v}	28	3	0
O^{1v}/O^{1v}	23	10	0

TABLE 5. Percentages of $O^{1v(G542A)}$ allele in various Amerindian populations

Population	Country	$O^{1v(G542A)}$ allele frequencies	References
Arara	Brazil	0.456	Olsson et al. (1998)
Aymara	Chile	0.119	This study
Cayapa	Ecuador	0.041	Roubinet et al. (2001)
Huilliche	Chile	0.113	This study
Kayapo	Brazil	0.395	Olsson et al. (1998)
Parakaña	Brazil	0.221	Barjas-Castro et al. (2003)
Yanomama	Brazil	0.428	Olsson et al. (1998)

■ Rev Méd Chile 2004; 132: 663-672

Caracterización genético molecular de habitantes de Caleta Paposo, último reducto Chango en Chile

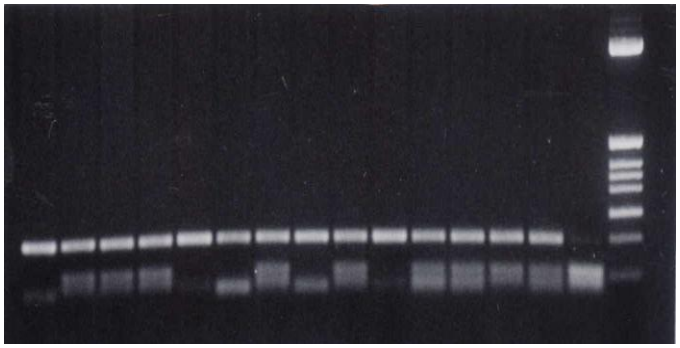
Hugo Henríquez B, Mauricio Moraga V, Elena Llop R, Francisco Rothhammer E.

Molecular and genetic characterization of Changos descendants living in Paposo Cove

Tabla 2. Resultados de la caracterización molecular vía PCR-RFLP (Grupo sanguíneo ABO) y PCR-ASP (Grupo sanguíneo Duffy) para la población de caleta Paposo

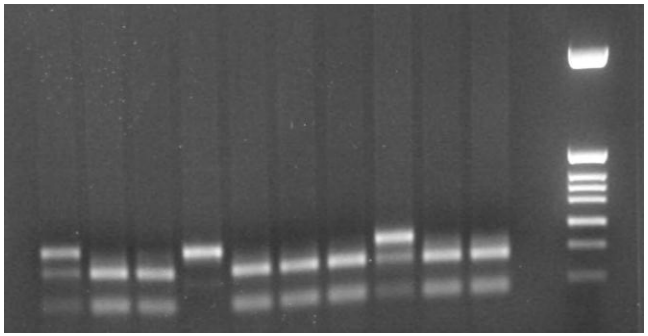
Sistema		Frecuencia
ABO	ABO*O101	0,39
	ABO*O201	0,53
	ABO*A	0,08
	ABO*B	0
Duffy	FY*A	0,58
	FY*B	0,42
	FY- (nulo)	0

CLASIFICACIÓN ABO MOLECULAR



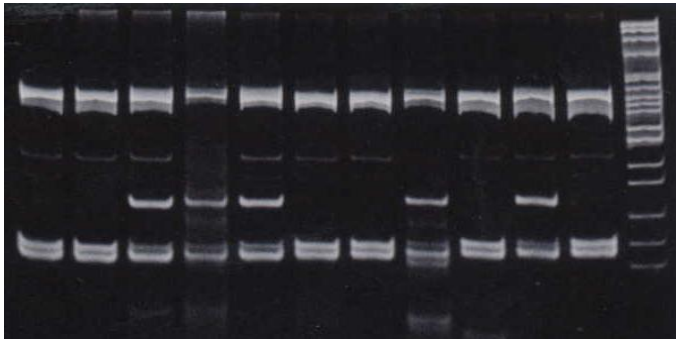
Ex 4: PCR-RFLP *BstUI*

148pb 65/83



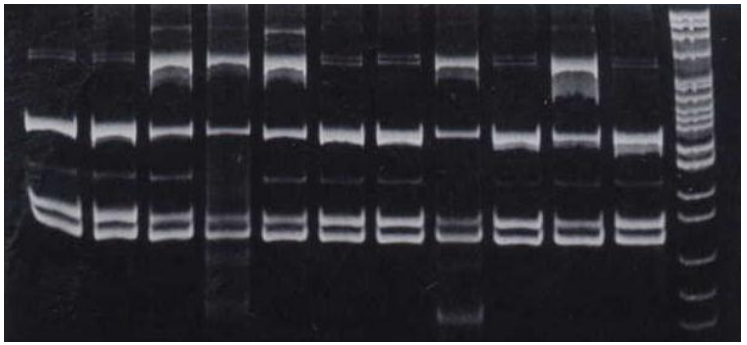
Ex 6: PCR-RFLP *KpnI*

187pb 54/133



Ex 7: PCR-RFLP *MboI*

373pb 68/(**72/24**)96/209



Ex 7: PCR-RFLP *DdeI*

373pb 97/(**169/107**)276

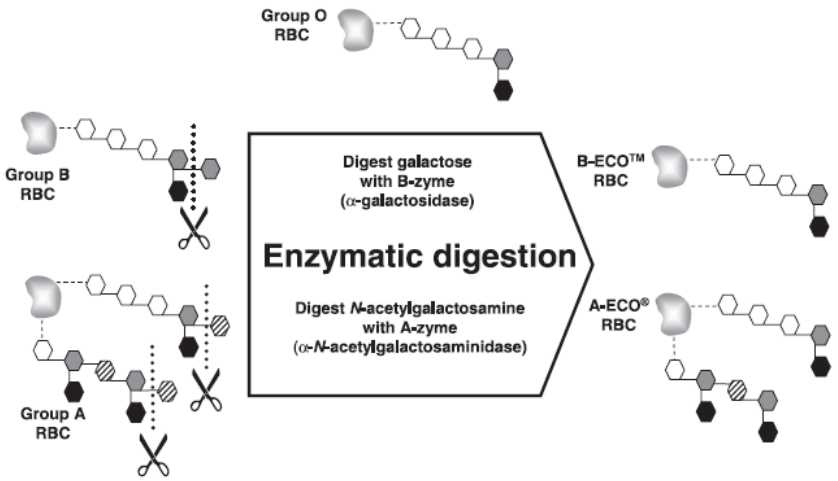
bjh review

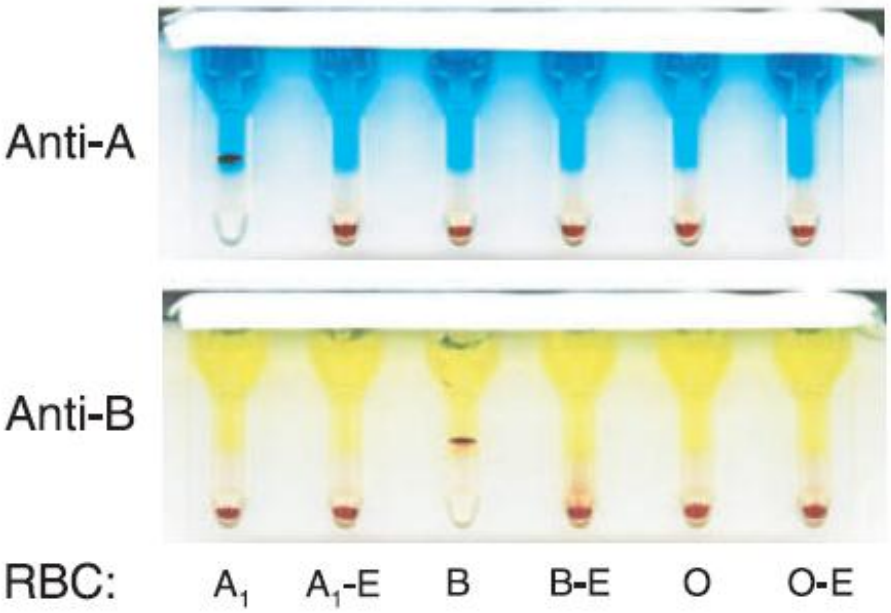
Modifying the red cell surface: towards an ABO-universal blood supply

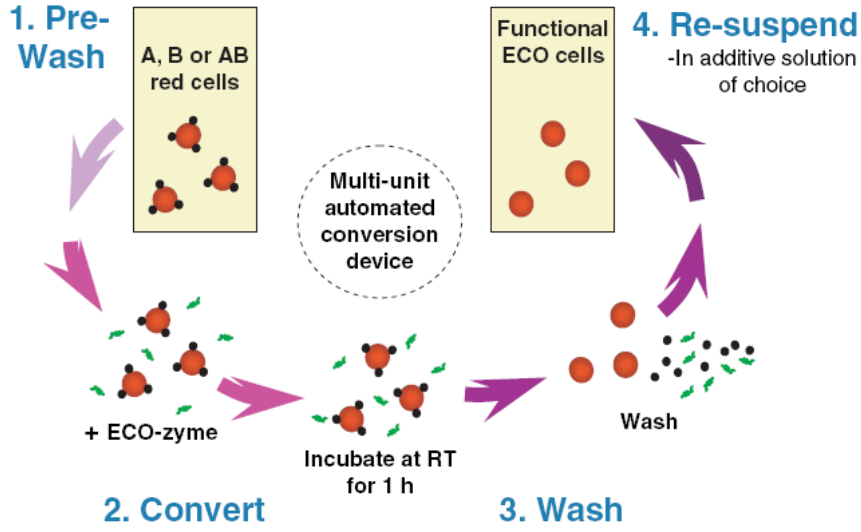
Martin L. Olsson[1,2] and Henrik Clausen[3]

[1]Division of Haematology and Transfusion Medicine, Department of Laboratory Medicine, Lund University and University Hospital Blood Centre, Lund, Sweden, [2]Department of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA, and [3]Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen N, Denmark

 British Journal of Haematology, **140**, 3–12


Group O RBC
Group B RBC
Digest galactose with B-zyme (α-galactosidase)
Enzymatic digestion
Digest N-acetylgalactosamine with A-zyme (α-N-acetylgalactosaminidase)
B-ECO™ RBC
A-ECO® RBC
Group A RBC


Anti-A
Anti-B
RBC:
A1
A1-E
B
B-E
O
O-E


1. Pre-Wash
A, B or AB red cells
Multi-unit automated conversion device
Functional ECO cells
4. Re-suspend
-In additive solution of choice
+ ECO-zyme
Incubate at RT for 1 h
Wash
2. Convert
3. Wash

SISTEMA DE GRUPO SANGUÍNEO Rh o RHESUS

Table 1. Rh antigens listed in sequence of initial discovery*

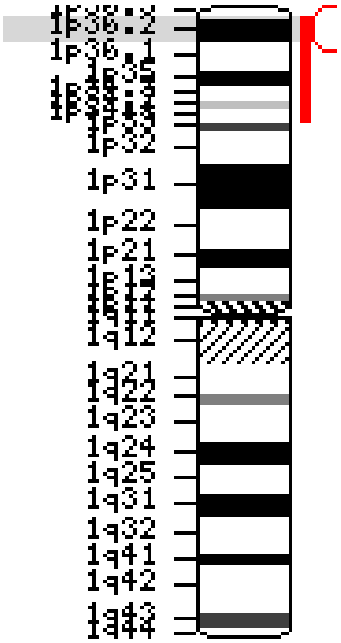
Antigen	Year of discovery	% incidence in a Caucasian population
D	1939/1940	85
C	1941	70
c	1941	80
E	1943	30
e	1945	98
C^w	1946	1
Hr_0	1950	> 99
f	1953	64
Be^a	1953	< 1
C^x	1954	< 1
E^w	1955	< 1
V	1955	< 1
Go^a	1958	< 1
G	1958	85
$Ce(rh_i)$	1958	70
Hr	1960	> 99
hr^s	1960	98
VS	1960	< 1
cE	1961	30
CE	1961	< 1
C^G	1962	70
D^w	1962	< 1
Rh26	1964	80
hr^H	1964	< 1
Rh29	1967	> 99
Evans	1968	< 1
Rh32	1971	< 1
Rh33	1971	< 1
Rh35	1971	< 1
Hr^B	1972	> 99
hr^B	1972	98
Tar	1975	< 1
Rh39	1979	> 99
Rh41	1980	70
Rh42	1980	< 1
Crawford	1980	< 1
Nou	1981	> 99
Dav	1982	> 99
Riv	1983	< 1
FPTT	1988	< 1
Sec	1989	> 99
BARC	1989	< 1
JAL	1990	< 1
STEM	1993	< 1
LOCR	1994	< 1
MAR	1994	> 99
JAHK	1995	< 1
DAK	2003	< 1
CENR	2004	< 1

*From the 2004 report of the ISBT Committee on Terminology for Red Cell Surface Antigens. Vox Sang 2004;87:304-6.

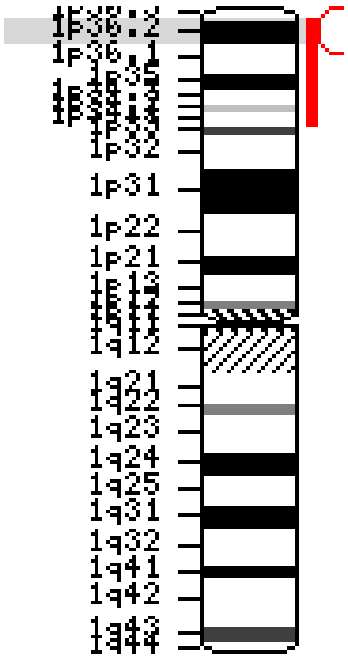
SISTEMA DE GRUPO SANGUÍNEO Rh o RHESUS: GENÉTICA

- 2 genes homólogos: RHD y RHCE en el cromosoma 1. 69 Kb y enfrentados por sus extremos 3'.
- SMP1
- Cajas Rh
- 1 gen "accesorio": RHAG, codificado en el cromosoma 6(homología:40%)
- Homología de más de 90% en su secuencia (D y CE)
- Codifican para proteínas de transmembrana de 12 dominios(417 aa)
- Antígenos C y c sólo difieren en 4 aa, uno de ellos crítico.
- Prolina 226, crítico para polimorfismo E/e.
- Herencia: Mendeliana Dominante.

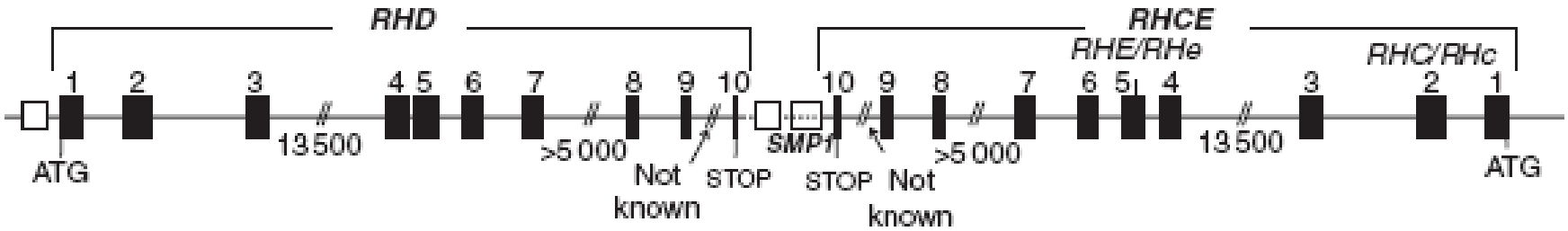
Ideogram



Ideogram



Gene map

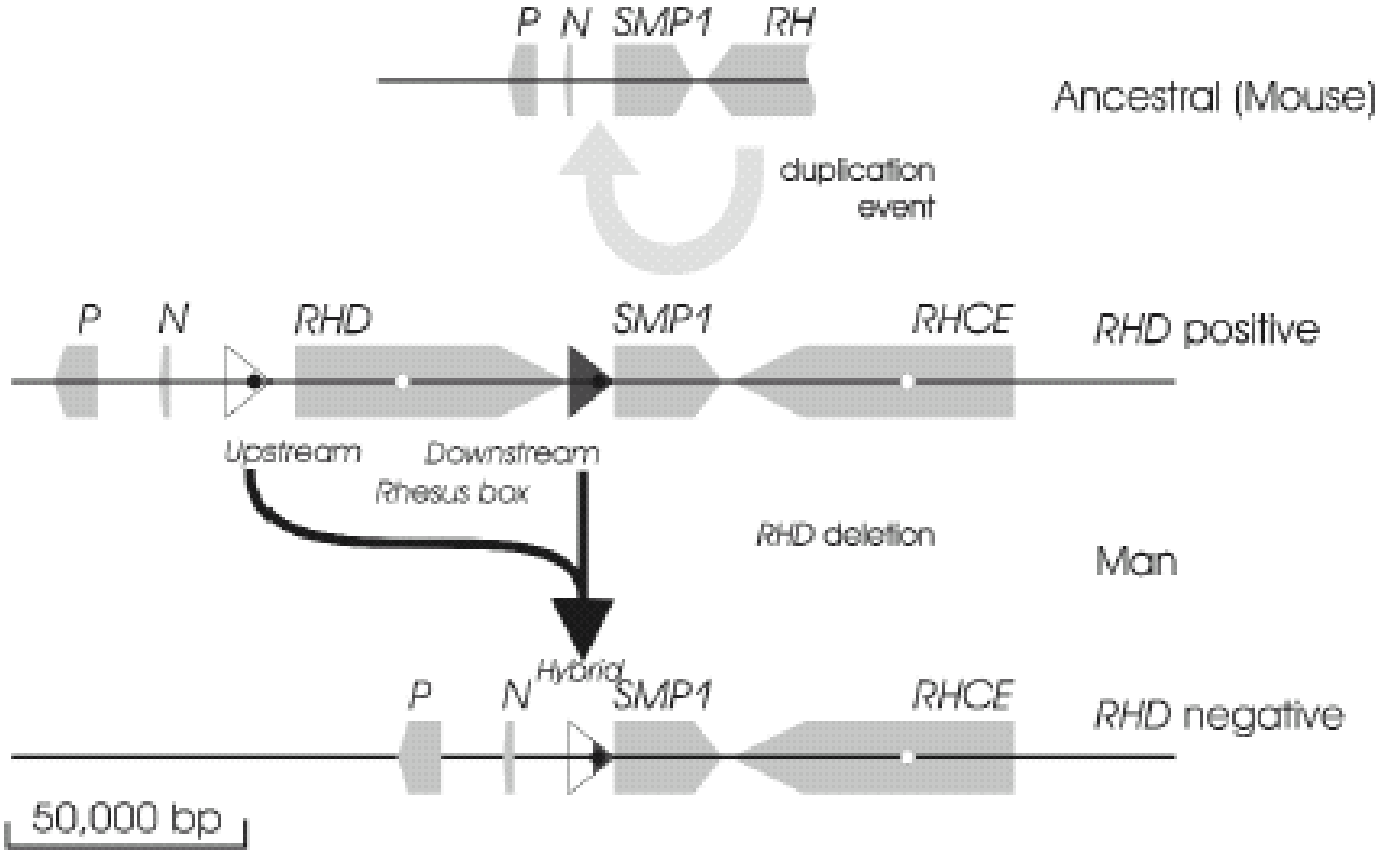


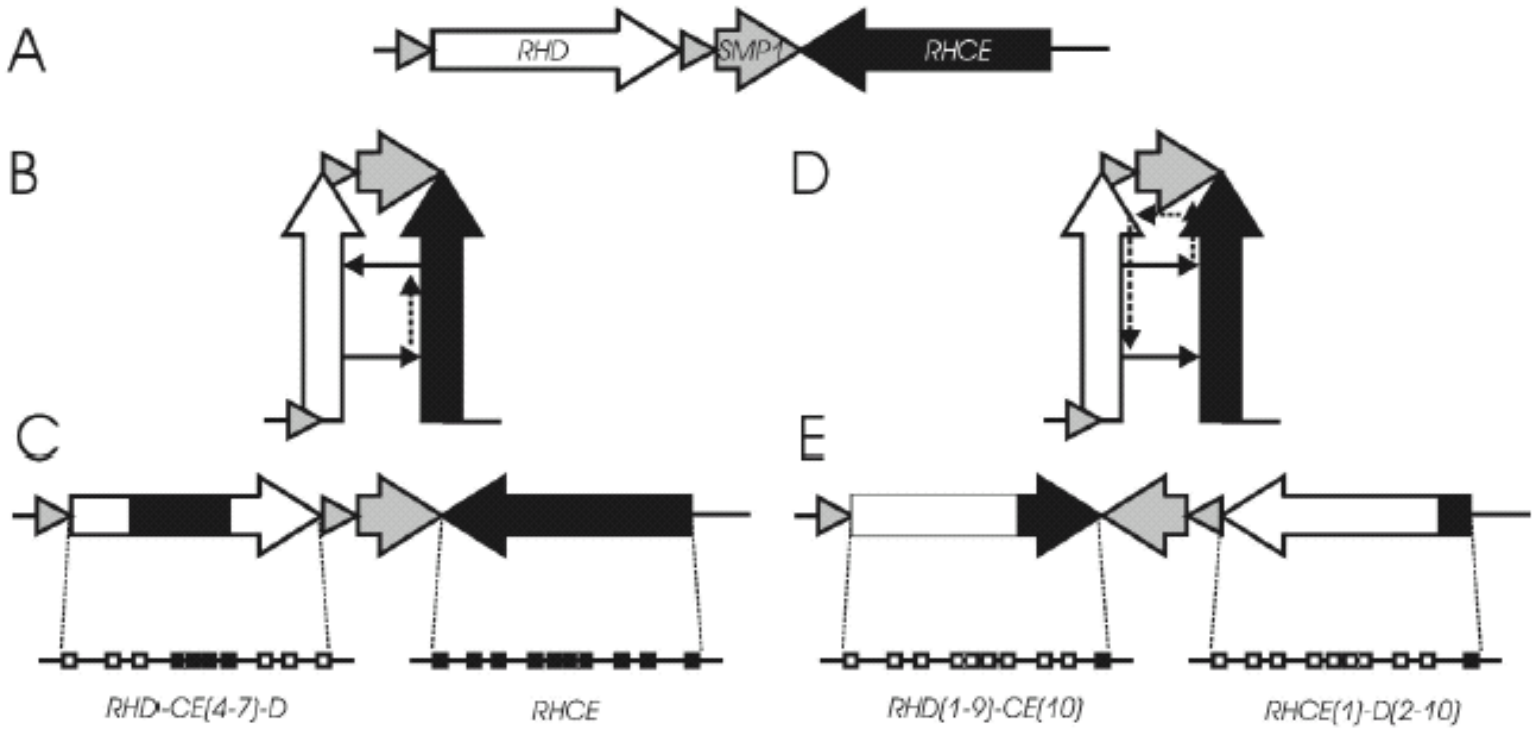
RHC/RHc (178A>C; 203G→A; 307T>C) encode C/c (I60L, S68N, S103P)

RHE/RHe (676C>G) encode E/e (P226A)

Rhesus box

1 kbp


P N SMP1 RH
Ancestral (Mouse)
duplication event
P N RHD SMP1 RHCE
RHD positive
Upstream
Downstream
Rhesus box
RHD deletion
Man
Hybrid
P N SMP1 RHCE
RHD negative
50,000 bp


A
RHD
SMP1
RHCE
B
D
C
E
RHD-CE(4-7)-D
RHCE
RHD(1-9)-CE(10)
RHCE(1)-D(2-10)

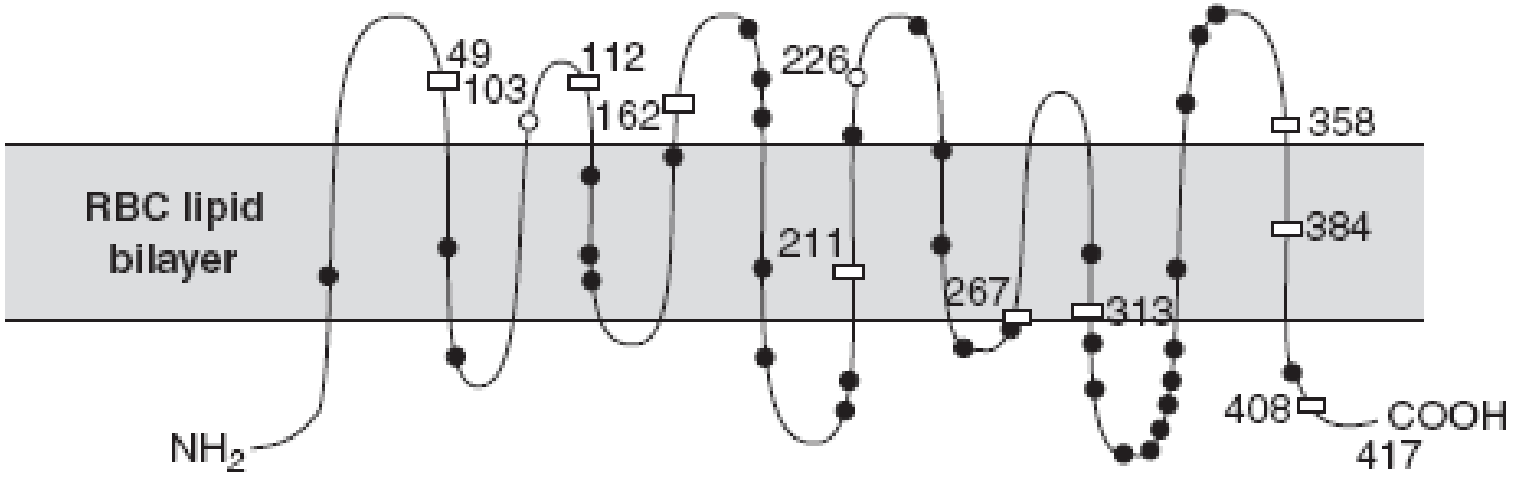
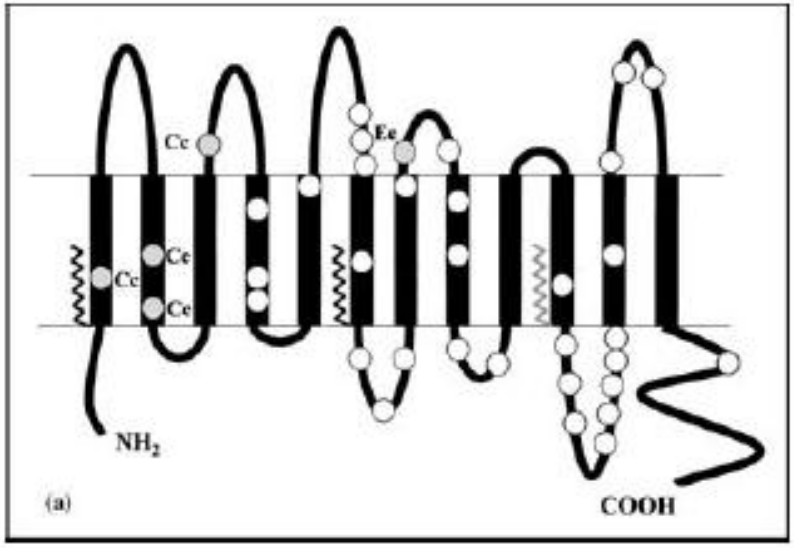

RBC lipid bilayer
NH2
49
103
112
162
226
211
267
313
358
384
408
COOH
417

Table 5.7 Locations of D epitopes, according to the model of Liu *et al.* [260].

D epitope	Extracellular loop required
epD2 (some)	3+4+6
epD3 (most)	6 + other RhD-specific residues*
epD3 (some)	6
epD4	6 + other RhD-specific residues*
epD5 (some)	3+4
epD5 (some)	3+4+6
epD5 (one)	4+6
epD6/7 (some)	3+4
epD6/7 (some)	3+4+6
epD8	1+2+3+5
epD9 (some)	6
epD9 (some)	6 + other RhD-specific residues*

*Some epitopes also appear to require the presence of RhD-specific cytoplasmic and/or transmembrane residues to stabilize the configuration.

Daniels, 2002. Human Blood Groups



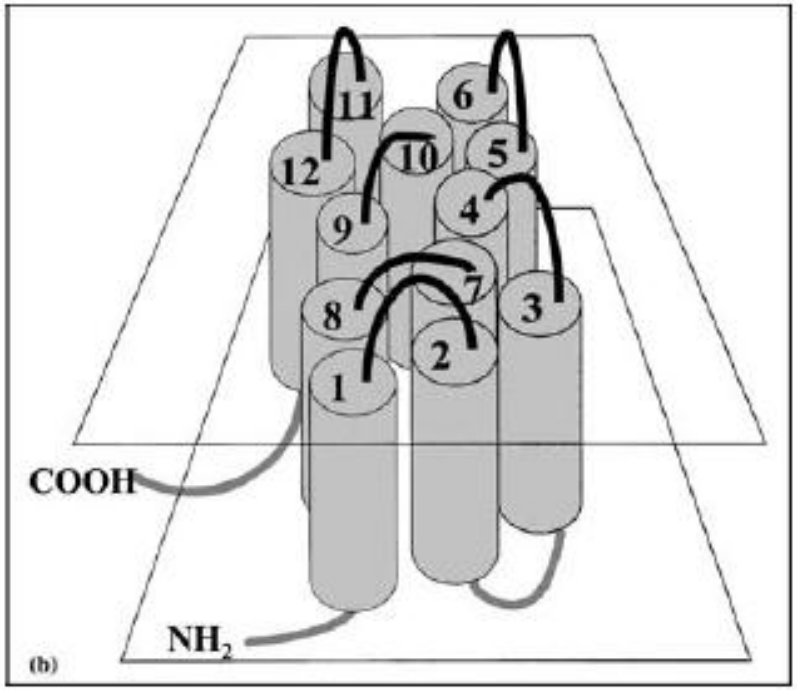
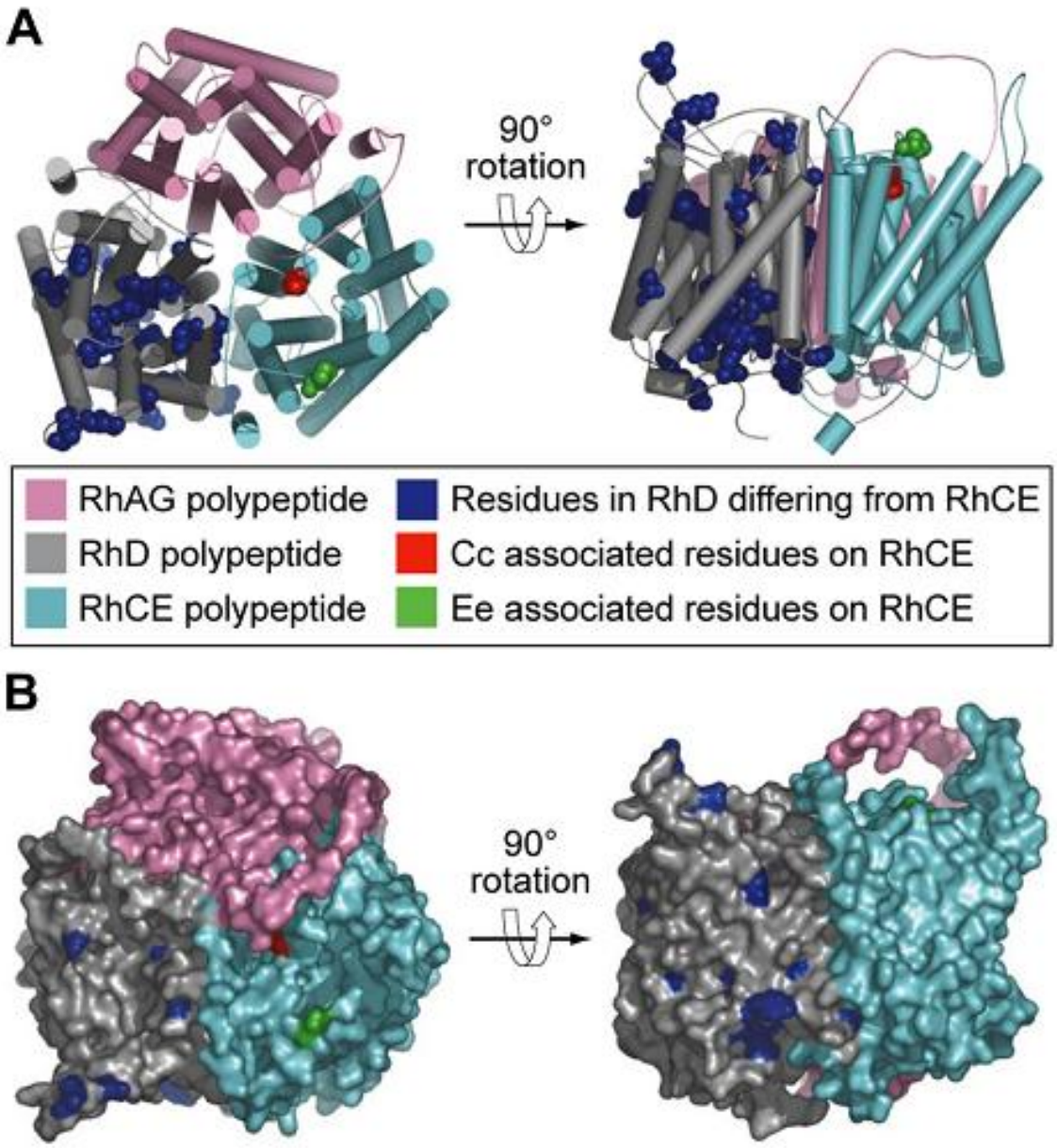


Table 5.8 Estimated number of D antigen sites per red cell for various Rh phenotypes [167,208,266,268].

Phenotype	D sites per cell (range)
DCe/dce	9 900–14 600
DcE/cde	12 000–19 700
Dce/dce	12 000–23 200
DCe/DCe	14 500–22 800
DCe/DcE	23 000–31 000
DcE/DcE	15 800–33 300
D−−/D−−	110 000–202 000

For numbers of C, c, and e sites, see Table 5.9.

Daniels, 2002. Human Blood Groups


A
90°
rotation
RhAG polypeptide
RhD polypeptide
RhCE polypeptide
Residues in RhD differing from RhCE
Cc associated residues on RhCE
Ee associated residues on RhCE
B
90°
rotation

Vox Sanguinis
The International Journal of Transfusion Medicine
Vox Sanguinis

Vox Sanguinis (2009)

ORIGINAL PAPER

DOI: 10.1111/j.1423-0410.2009.01243.x

A new blood group system, RHAG: three antigens resulting from amino acid substitutions in the Rh-associated glycoprotein

L. Tilley,[1] C. Green,[1] J. Poole,[1] A. Gaskell,[1] K. Ridgwell,[1] N. M. Burton,[2] M. Uchikawa,[3] H. Tsuneyama,[3] K. Ogasawara,[3] Ç. A. Akkøk[4] & G. Daniels[1]

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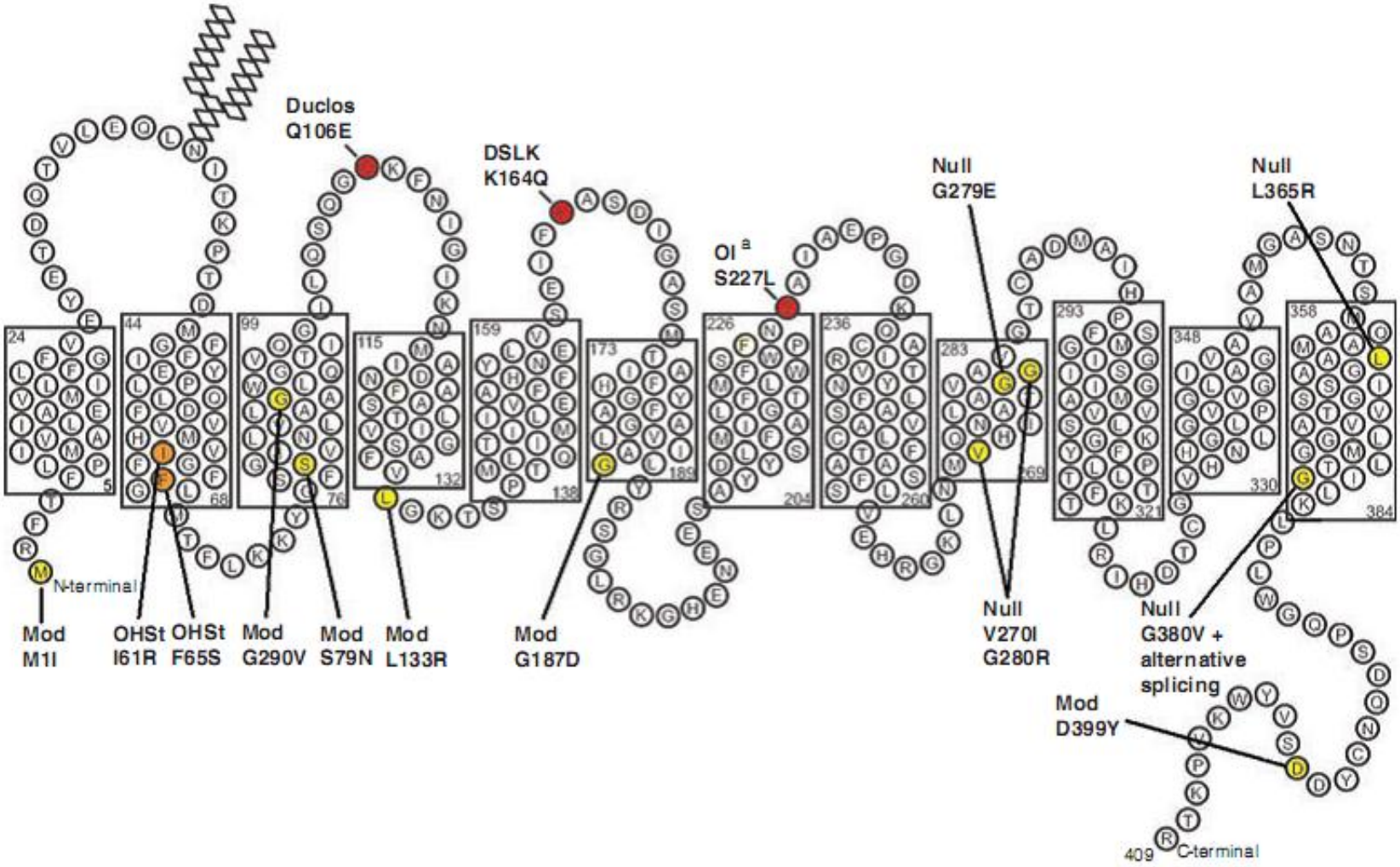
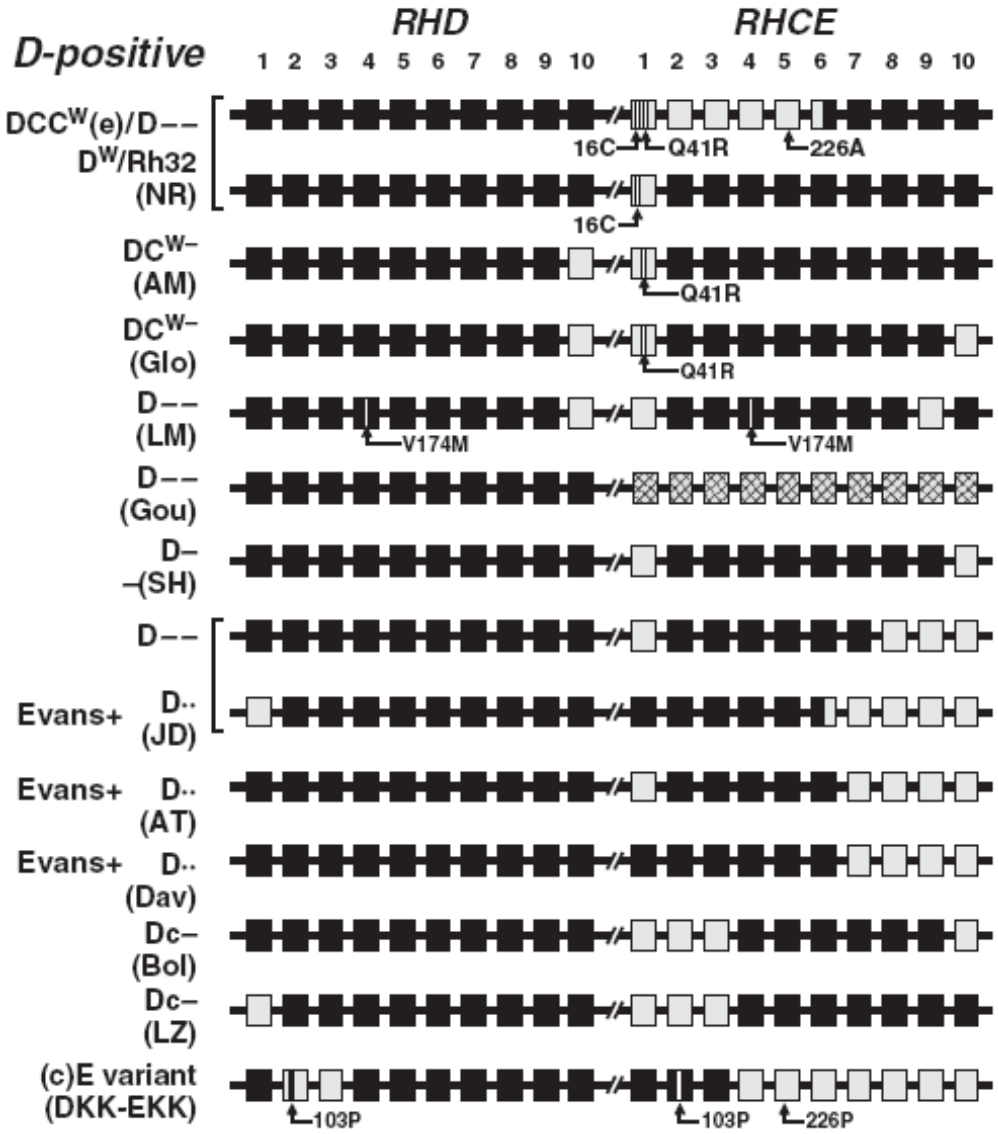
[2] *Department of Biochemistry, University of Bristol, Bristol, UK*

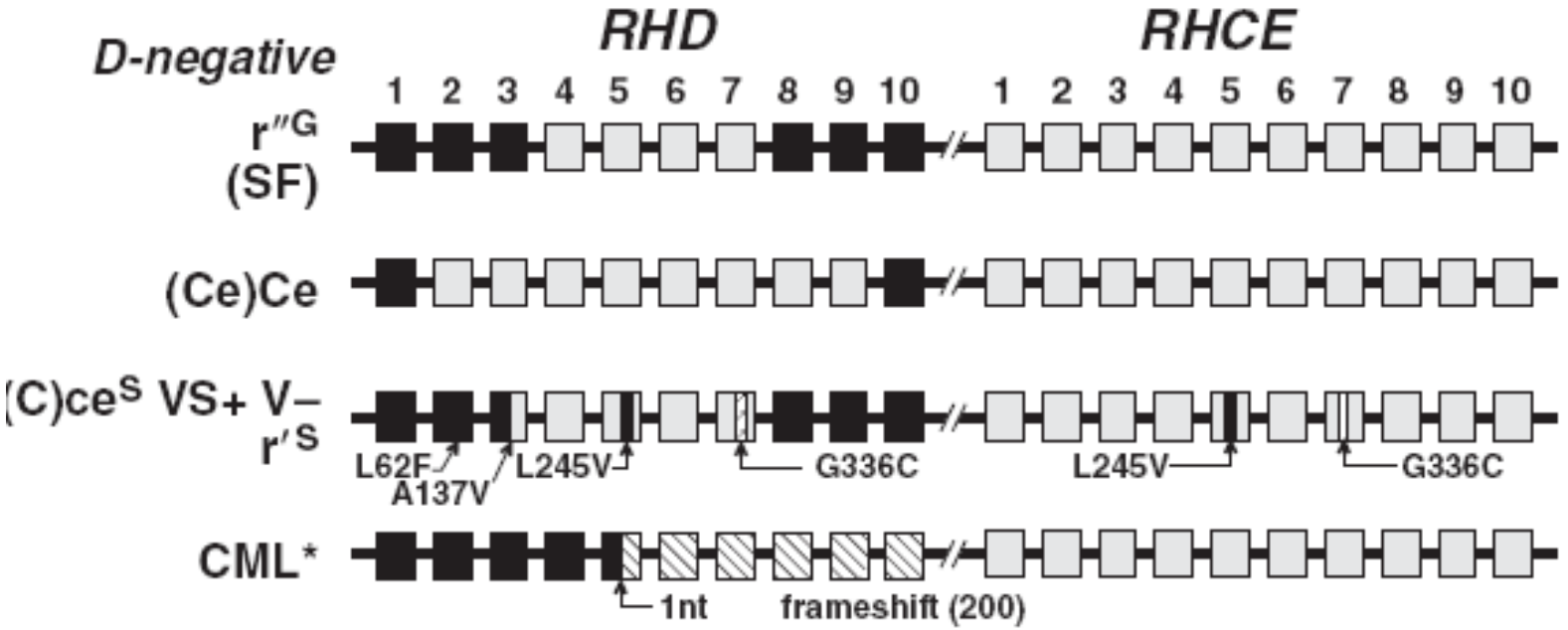
[3] *Japanese Red Cross Tokyo Blood Center and Japanese Red Cross Central Blood Institute, Tokyo, Japan*

[4] *Department of Immunology and Transfusion Medicine, Oslo, Norway*


Duclos
Q106E
DSLK
K164Q
OI a
S227L
Null
G279E
Null
L365R
N-terminal
Mod
M1I
OHSt
I61R
OHSt
F65S
Mod
G290V
Mod
S79N
Mod
L133R
Mod
G187D
Null
V270I
G280R
Null
G380V +
alternative
splicing
Mod
D399Y
C-terminal
409

SISTEMA DE GRUPO SANGUÍNEO Rh o RHESUS: VARIABILIDAD Y REARREGLOS

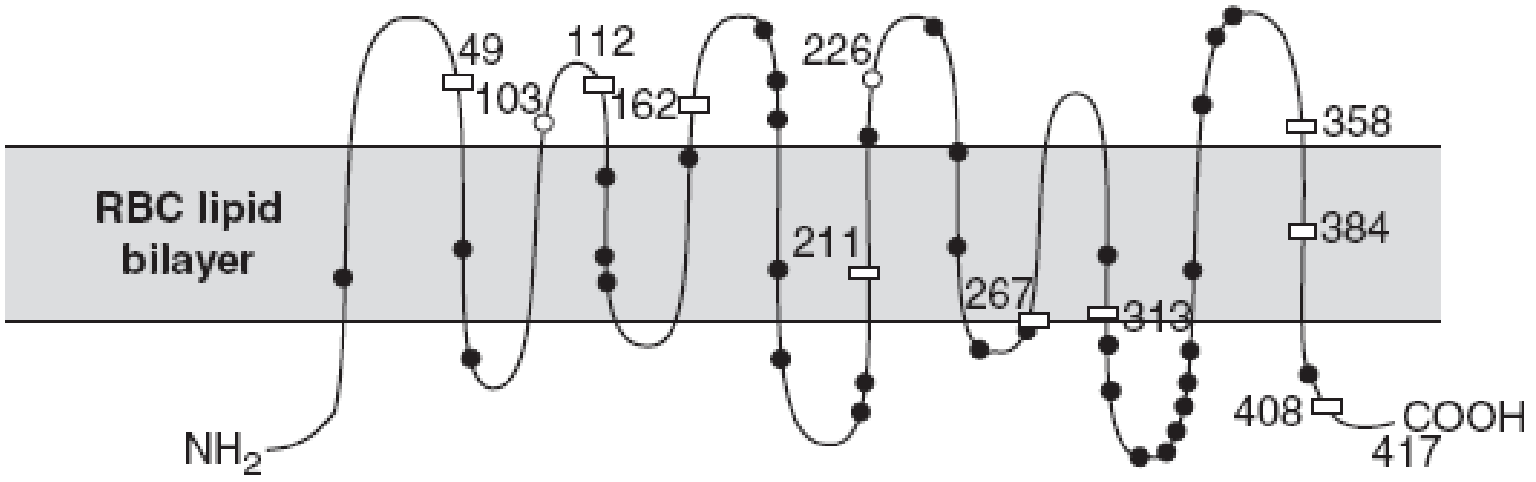




* *RHD* and *RHCE* identified in a D-positive patient, with chronic myeloid leukemia, who became D-negative

SISTEMA DE GRUPO SANGUÍNEO Rh o RHESUS: ANTÍGENO D

- Difiere de RhCE por 32 a 35 aa.
- Aprox. 35000 por célula.
- Resistente a tratamientos enzimáticos.
- Expresiones alteradas: Du, D mod, D null, D parcial



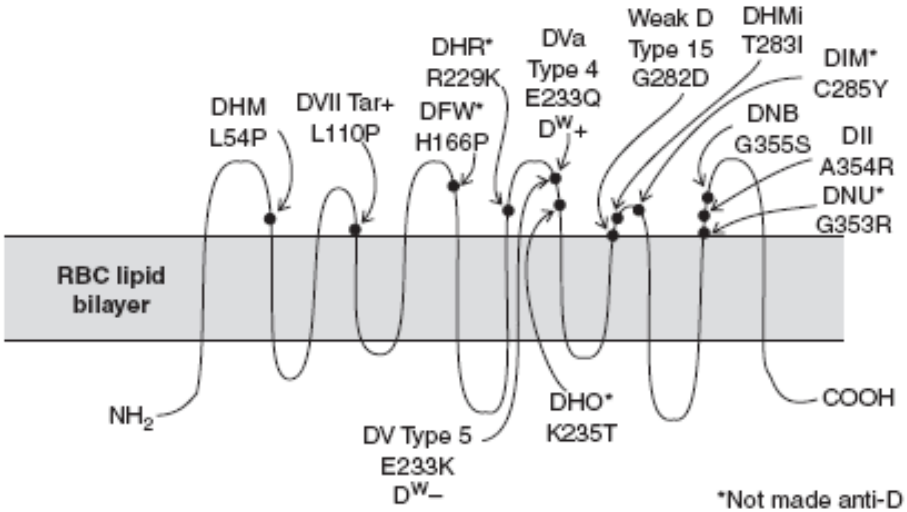
- Más importantes: Du y D parcial.

D categories

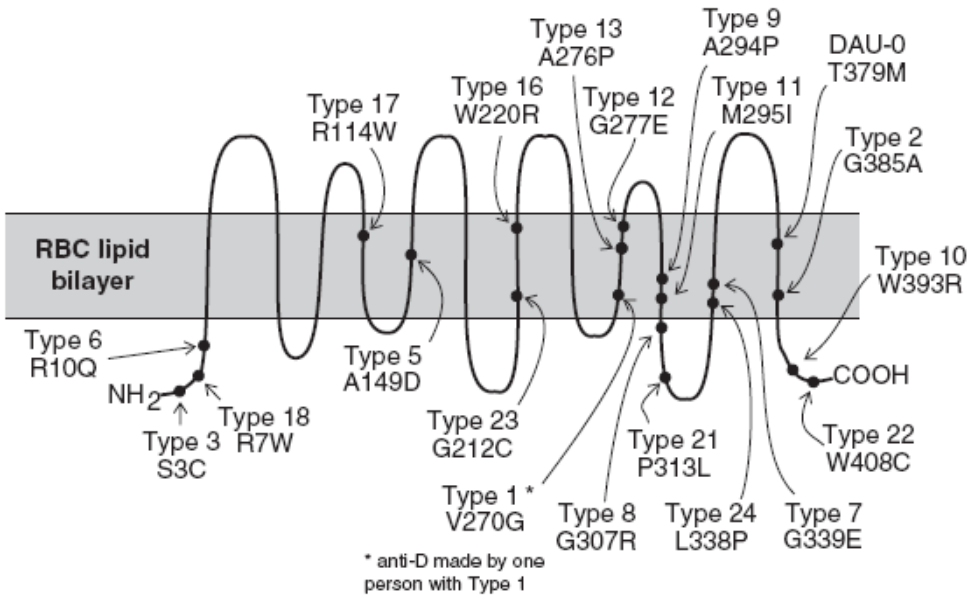
	Anti-D from						
Cells	*II*	*IIIa*	*IIIc*	*IVa*	*IVb*	*Va*	*VI*
II	0	+	+	+	V	+	+
IIIa	+	0	0	+	+	+	+
IIIb	+	0	0	+	+	V	+
IIIc	+	0	0	+	+	+	+
IVa	0	V	0	0	0	+	+
IVb	0	V	V	0	0	0	+/0
Va	+	0	0	+	+	0	+/0
VI	0	0	0	+/0	+/0	0	0
VII	+	+/0	+/0	+	+	+/0	+
DFR	+	0	+/0	+	+	0	0
DBT	0	NT	V	0	0	+/0	+/0

+ = positive; +/0 = positive with some sera and negative with other sera; 0 = negative; V = variable strength of positive reaction and some sera negative; NT = not tested.

Molecular basis of partial D phenotypes[3–16]



Molecular basis of weak D[7,11,16,18]



Definitions of weak D and partial D

The terms weak D and partial D have often been used to determine how a patient is transfused or whether anti-D immunoglobulin prophylaxis should be given. These terms, however, are not adequately defined and, therefore, not suitable for this purpose. Three types of differentiating definitions have been used, but none is satisfactory.

1 Weak D antigens have all D epitopes; partial D antigens lack one or more D epitopes. This is difficult to define serologically, because a negative reaction with a particular monoclonal antibody or by a specific method could result from weak expression of the epitope, rather than its absence. DIIIa appears to have all epitopes, but individuals with DIIIa red cells often make anti-D and so their red cells must be lacking at least one D epitope.

2 Individuals with partial D antigens can make anti-D; those with weak D antigens cannot. This is the usual interpretation of the dichotomy, but it is dependent on an immune response. If anti-D has not been found in any person with a particular D variant, this does not mean that another patient with the same variant will not make anti-D following immunization with D+ red cells. For example, weak D types 4·2 and 15 have been classed as weak D, yet all have subsequently been found in numerous patients who have made alloanti-D (Wagner *et al*, 2000).

3 In partial D, the RhD proteins have amino acid changes outside of the membrane, whereas in weak D the RhD proteins have one or more amino acid substitutions within either the membrane-spanning domains or the cytoplasmic loops of the protein, but not externally

British Journal of Haematology, 2013, **161,** 461–470

Mutations in *RHD* encoding the D-negative phenotype

Mechanism	*Associated haplotype*	*Ethnicity*
Gene deletion	ce	Caucasians
Duplication of 37 bp at intron 3/exon 4 junction; missense mutations; stop codon in exon 6 (*RHD* pseudogene)	ce	Blacks
48G>A in exon 1; Trp16Stop	Ce	
121C>T in exon 1; Gln41Stop	Ce	
270G>A in exon 2; Trp90Stop	cE	Chinese
990C>G in exon 7; Tyr330Stop	Ce	
711del C in exon 5; fs; Val245Stop	cE	Chinese
906insGGCT in exon 6; fs; IVS6 + 2t>a	Ce	Chinese
5′ end of exon 4 delACAG; fs; Met167Stop	Ce	Caucasians
600 delG; Leu228Stop		
635G>T in exon 5; Gly212Val	Ce	
IVS8 + 1 g>a	Ce	Caucasians

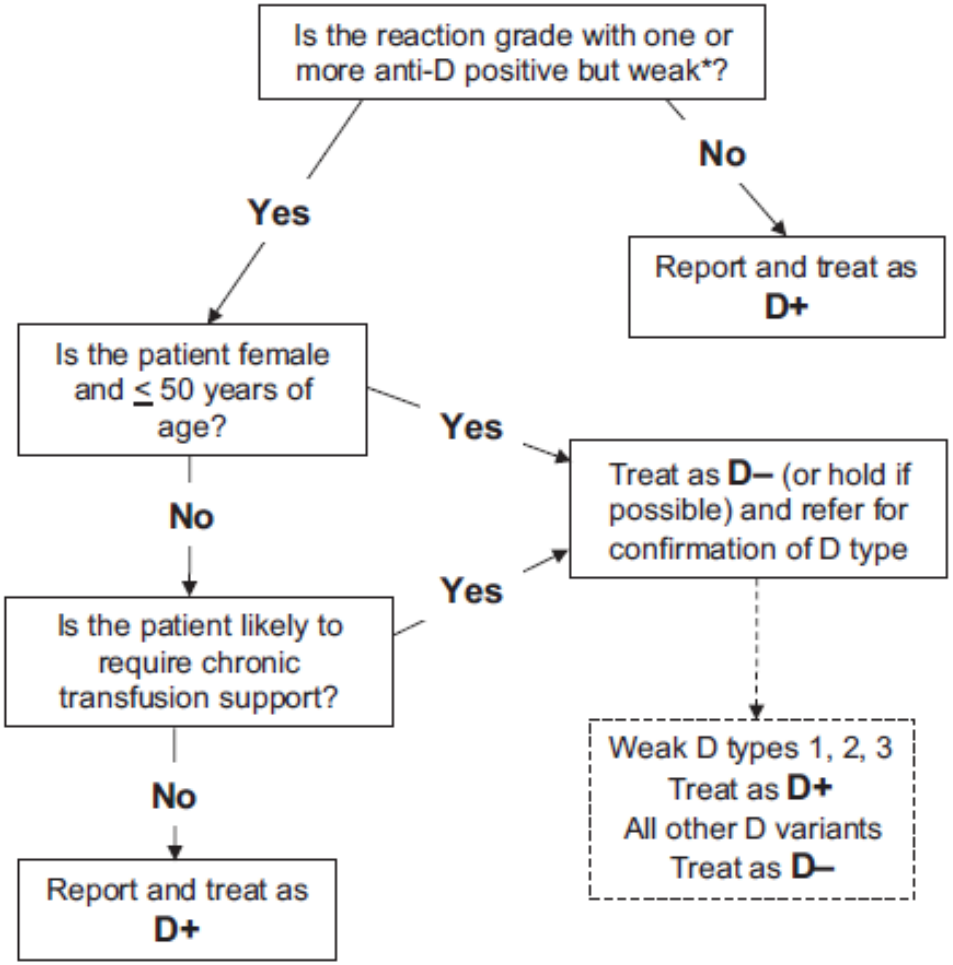


Fig. 3. Reporting of D typing anomalies and selection of red cells. Adapted from: (Milkins *et al*, 2013) Guidelines for pre-transfusion compatibility procedures in blood transfusion laboratories. *Transfusion Medicine*, **23**, 3-35. With permission from John Wiley & Sons. *Weak reaction is defined by local policy and in line with manufacturers' instructions – likely to be <3+ or <2+ depending on system used. Recommendation in dashed box is not in the UK guidelines.

TABLE 1. Frequency of D variants found in a population of 360 Brazilian blood donors, *RHCE* allele association, and flow chart used for identification of the variant

Associated *RHCE* allele	Flow chart	D variant	Number	Percent
ce	3	Weak D Type 4.2.2	110	30.50
ce	3	Weak D Type 4.0	81	22.50
Ce	1	Weak D Type 3	59	16.40
cE	2	Weak D Type 2	25	6.95
Ce	1	Weak D Type 38	21	5.83
Ce	1	Weak D Type 1	19	5.28
ce	3	DAR1	14	3.90
Ce	1	D^{VII}	6	1.66
ce	1	DAU6	6	1.66
ce	1	DMH	4	1.11
ce	1	DAU0	2	0.56
ce	4	DOL2	2	0.56
ce	4	DAU5	1	0.27
ce	1	DAU1	1	0.27
Ce	1	DNB	1	0.27
Ce	1	Weak D Type 18	1	0.27
ce	4	D^{V} Type 2	1	0.27
ce	5	D^{III} Type 6	1	0.27
ce	6	D^{IVa} Type 2	1	0.27
ce	3	Weak D Type 4.1	1	0.27
ce/Ce	4	DV Type 1/*RHDψ*	1	0.27
ce/ce	3	DAR1/Weak D Type 4.2.2	1	0.27
ce/ce	3	Weak D Type 4.2.2/Weak D Type 4.0	1	0.27
		Total	360	100.00

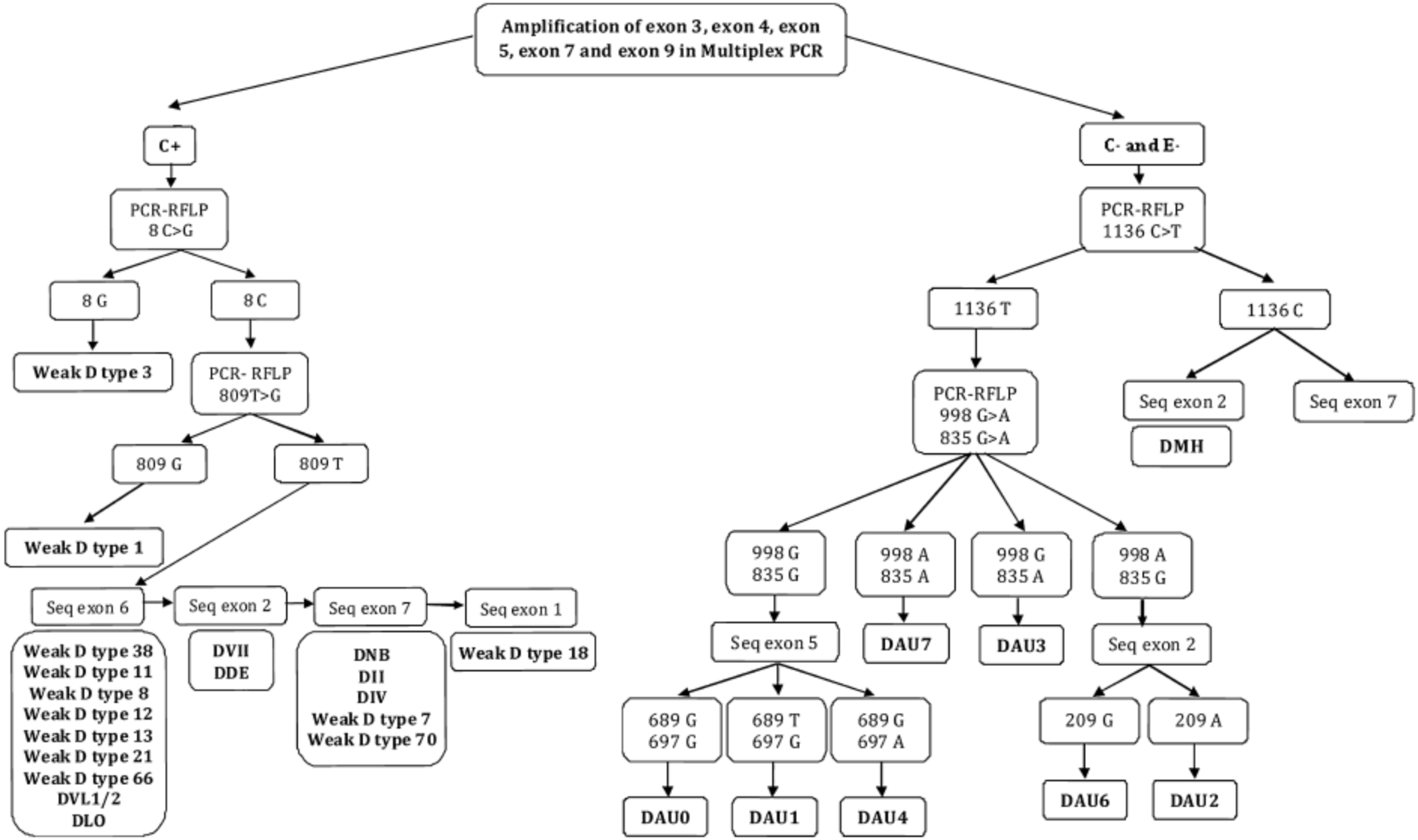


Fig. 1. Flow Chart 1 applied when all exons of the multiplex PCR procedures are amplified.

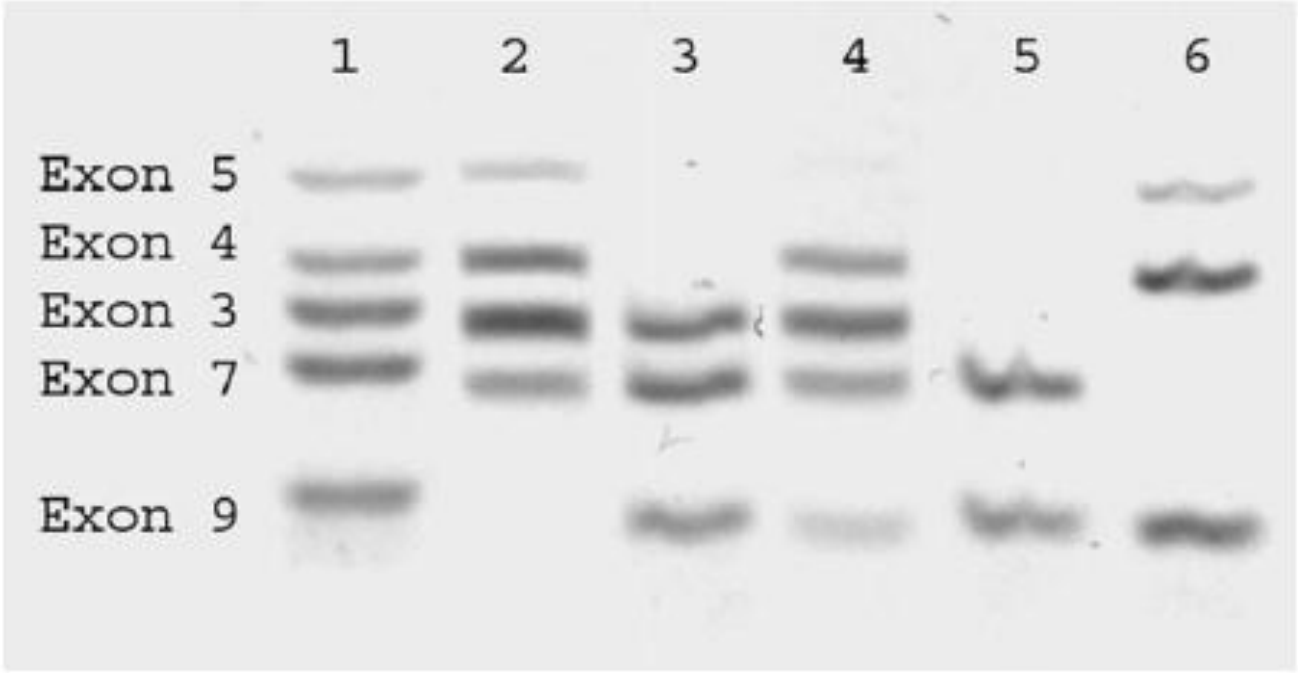


Fig. 7. Multiplex PCR analysis on 4% agarose gel. Lanes 1 through 6 correspond to Flow Charts 1 through 6, respectively.

How we incorporate molecular typing of donors and patients into our hospital transfusion service

Hedyeh Shafi, Ihab Abumuhor, and Ellen Klapper

TRANSFUSION 2014;54:1212-1219.

			Rh				Kell						Kidd		Duffy		MNS				Lutheran		Diego		Colton		Dombrock				LW		Scianna		Hemoglobin S		
Chip	Sample	Status	c	C	e	E	K	k	Kpa	Kpb	Jsa	Jsb	Jka	Jkb	Fya	Fyb	M	N	S	s	Lua	Lub	Dia	Dib	Coa	Cob	Doa	Dob	Joa	Hy	LWa	LWb	Sc1	Sc2	HbS		
HEA85546_1	W05051200 5911		0	+	+	0	0	+	0	+	0	+	+	+	0	+	+	+	0	+	0	+	0	+	+	0	0	+	+	+	+	0	+	0	0		
HEA85546_2	W05051200 5912		+	+	+	+	0	+	0	+	0	+	+	+	+	+	+	0	+	+	0	+	0	+	+	0	+	+	+	+	+	0	+	0	0		
HEA85546_3	W05051200 5913		+	0	+	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	0	+	0	+	+	0	+	0	+	+	+	0	+	0	0		
HEA85546_4	W05051200 5917		+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	+	+	+	0	+	+	+	+	0	+	0	0		
HEA85546_5	W05051200 5923		+	0	+	0	0	+	0	+	0	+	+	0	+	0	+	0	+	0	0	+	0	+	+	0	+	0	+	+	+	0	+	0	0		
HEA85546_6	W05051200 5925		0	+	+	0	0	+	0	+	0	+	+	+	+	0	+	+	0	+	0	+	0	+	+	0	0	+	+	+	+	0	+	0	0		
HEA85546_7	W05051200 5926		+	+	+	+	0	+	0	+	0	+	+	+	0	+	+	0	+	+	0	+	0	+	+	0	+	0	+	+	+	0	+	0	0		
HEA85546_8	W05051200 5932		0	+	+	0	0	+	0	+	0	+	+	+	+	0	+	+	0	+	0	+	0	+	+	0	+	+	+	+	+	0	+	0	0		

Transfusion and Apheresis Science 50 (2014) 183–188

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Review

Is Next Generation Sequencing the future of blood group testing?

Louise Tilley *, Shane Grimsley

International Blood Group Reference Laboratory, NHS Blood and Transplant, Bristol, UK

Table 4
Genotyping tools available.

Platform	Company	Analysis	Note
Research developed			
GenomeLab™ SNPstream®	Beckman Coulter, USA	19 Ags/22 Ags	[13,30–32]
MALDI-TOF MS	Sequenom Bioscience, USA	13 Ags	[17]
OpenArray®	Life Technologies, USA	17 Ags	[35,36]
SNaPshot®	Life Technologies, USA	35 Ags/16 Ags	[33,34]
Commercially available			
BLOODchip Reference	Progenika Biopharma SA, Spain (Grifols/Novartis)	128 SNPs or 33 RBC and 12 PLT Ags	CE marking [19,40,41]
IDCore	Progenika Biopharma SA, Spain (Grifols/Novartis)	23 RBC Ags	[41]
IDCore+	Progenika Biopharma SA, Spain (Grifols/Novartis)	33 RBC Ags	[41]
IDCore^{XT}	Progenika Biopharma SA, Spain (Grifols/Novartis)	37 RBC Ags	[41]
IDHPA	Progenika Biopharma SA, Spain (Grifols/Novartis)	12 PLT Ags	[41]
BioArray™ HEA Beadchip™	Immucor, USA	24 SNPs or 38 RBC Ags	CE marking [48–50]
BioArray™ HPA	Immucor, USA	22 PLT Ags	CE marking [50]
BioArray™ RHCE Beadchip™	Immucor, USA	35 variants	[50]
BioArray™ RHD Beadchip™	Immucor, USA	75 variants	[50]
HIFI BLOOD 96™	AXO Science, France	29 RBC Ags	CE marking [50]

Table 1
Capabilities of selected sequencing platforms from Illumina and Life Technologies.

	System	Sequence output	Read length	Time
Illumina	MiSeq (v3 chemistry)	Up to 15 Gb	2 × 300 bp	65 h
	HiSeq 2500 (v4 chemistry)	Up to 1 Tb	2 × 125 bp	6 days
Life Technologies	Ion PGM (Ion 318 chip)	Up to 2 Gb	35–400 bp	4.4–7.3 h
	Ion Proton (PI chip)	Up to 10 Gb	200 bp	2–4 h

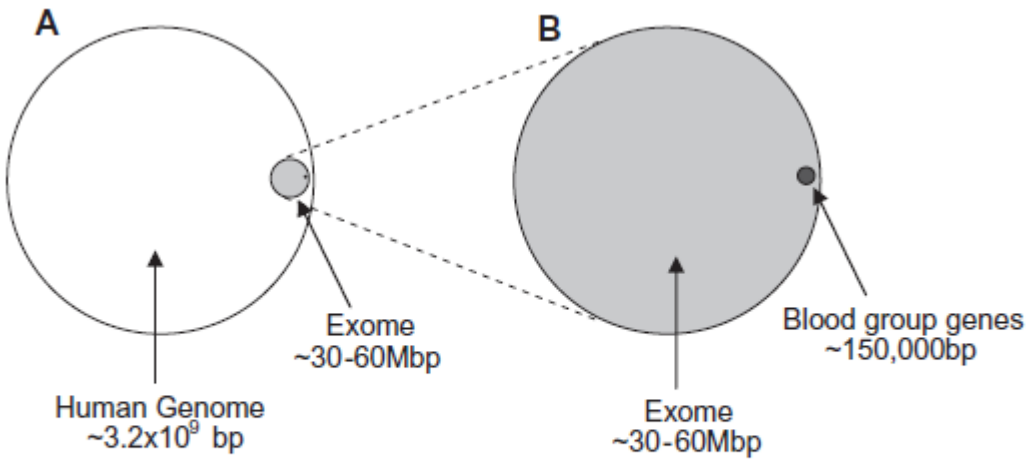

Fig. 1. A representation of the approximate relative proportions of the human exome as compared to the human genome (A) and the proportion of the exome encoding blood group related genes (B).