Partial D & Weak D

Picking Up the Rhesus Pieces

Heart of America Association of Blood Banks
April 24, 2012

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BloodCenter of Wisconsin

HAABB
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Objectives

- List the reasons for RhD typing discrepancies
- Discuss the biochemical and molecular characteristics of RhD & *RHD*
- Understand the differences among partial, weak, D_{el} variants and D epitopes on RhCe protein
- Describe the advantage of a molecular resolution of Rh discrepancies

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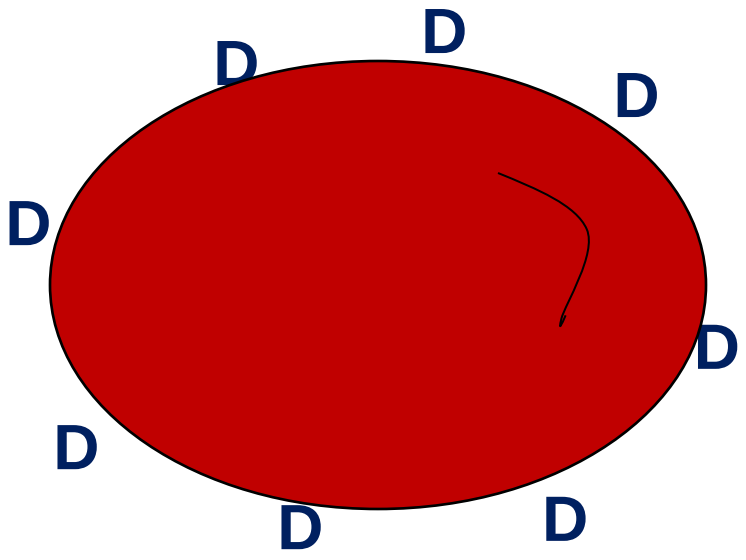
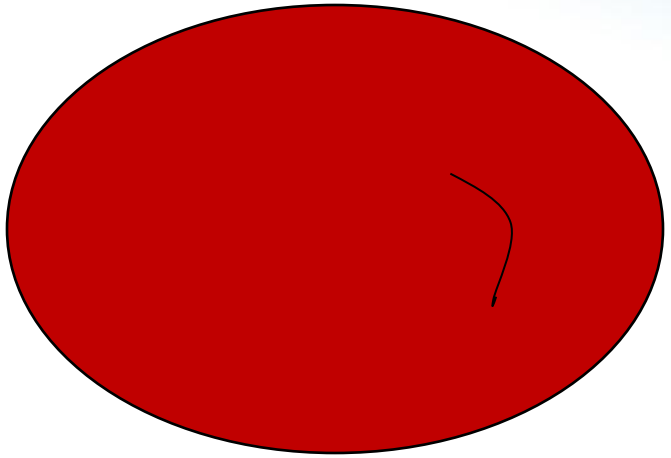
Rh DESIGNATION



Rh Positive
85%



Rh Negative
15%

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RhD Typing Discrepancies

- RhD antigen expression
  - *RHD* gene mutations
- Reagent differences
- Method variability

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Variables Impacting Rh Typing

CONTRIBUTORS OF VARIABILITY	VARIABLES				
***RHD* Gene**	Weak D	C in Trans to *RHD*	Partial D	D_{el}	
D epitopes on RhCE Protein	ceCF	R_0^{Har} or DHAR			
Anti-D Reagents	Polyspecific Slide and Modified Tube Human IgG	Monoclonal IgG	Monoclonal IgM	Monoclonal IgM Human IgG	Monoclonal Blends
Testing Platform	Test Tubes IS & IAT	Column Agglutination	Solid Phase	Liquid Microtiter	
Individual being Rh Typed	Transfusion Recipient	Obstetrical Patient	Cord Blood	Donor Blood	

Transfusion Technology Report Vol. #013 Immucor, Inc.

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What is D?

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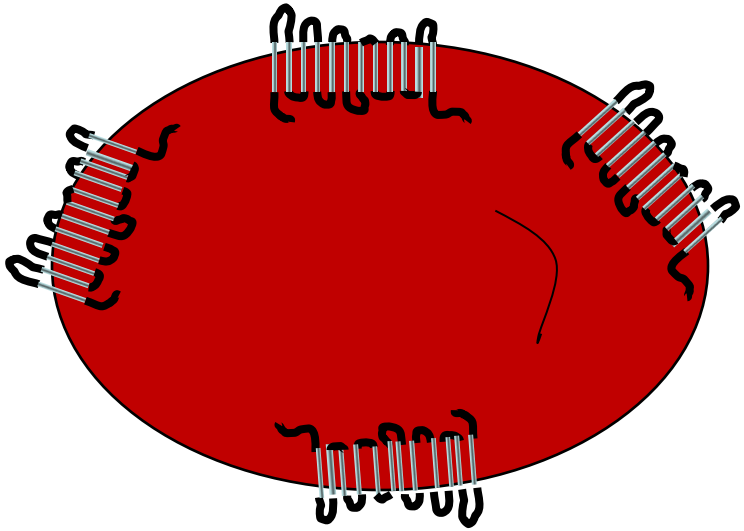
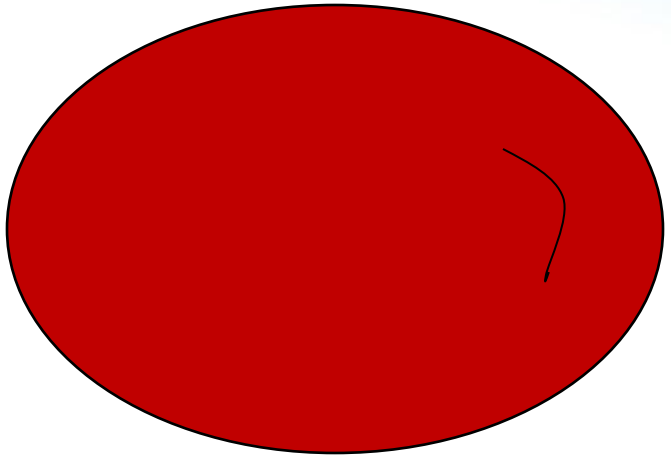
Rh DESIGNATION



Rh Positive
85%



Rh Negative
15%

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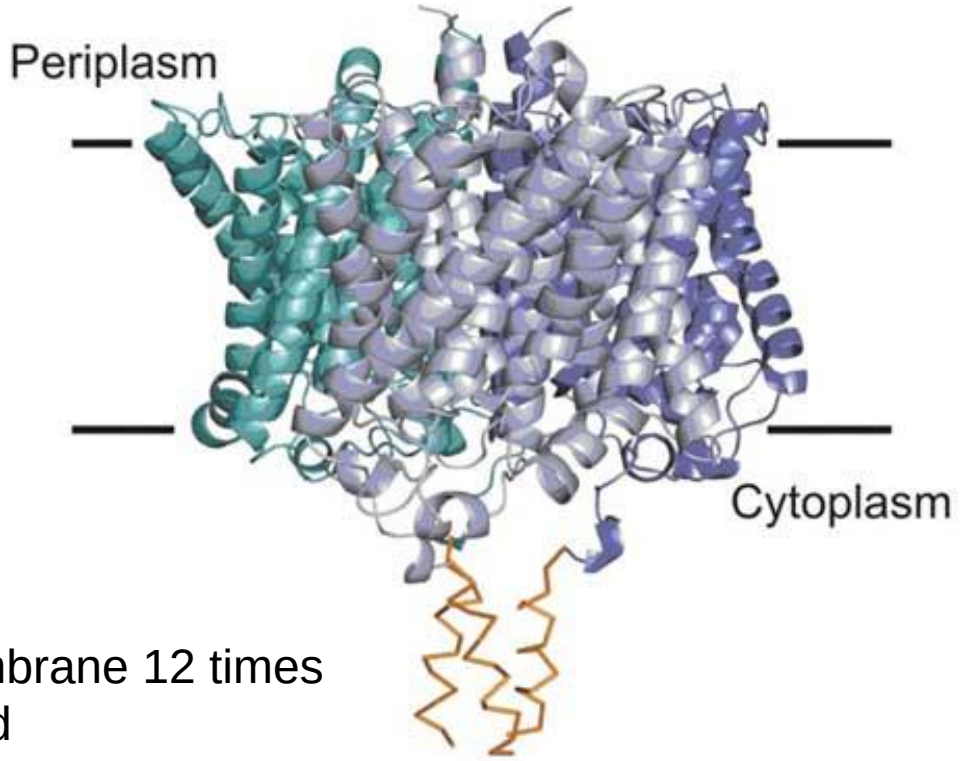
Rh Protein

Multi-pass membrane protein



- Crosses RBC membrane 12 times
- No sugars attached

http://www.jic.ac.uk/corporate/about/publications/advances/images_10/protein.jpg

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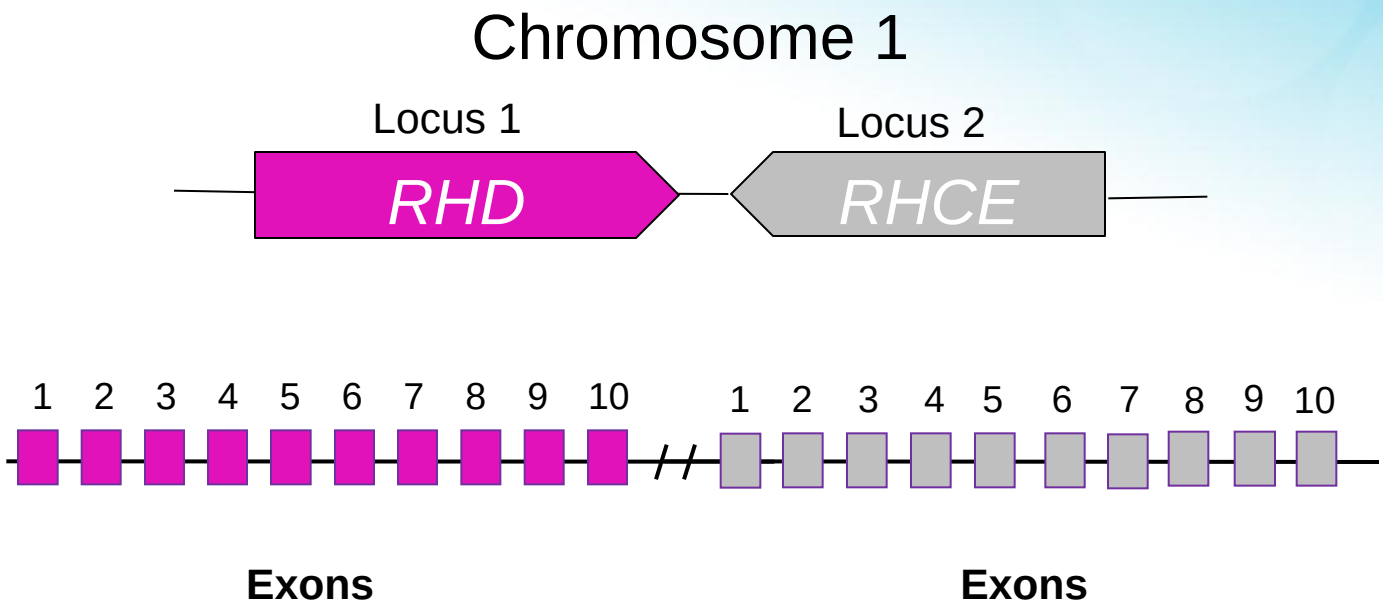
RH Genes – Rh Positive



Locus 1 - presence of *RHD* codes for the presence of D or no D. Differs from *RhCE* by 34 to 37 amino acids (C or c)

Locus 2 - presence of *RHCE* codes for Ce, CE, cE, ce.

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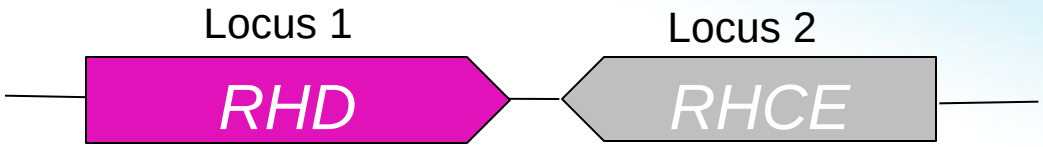
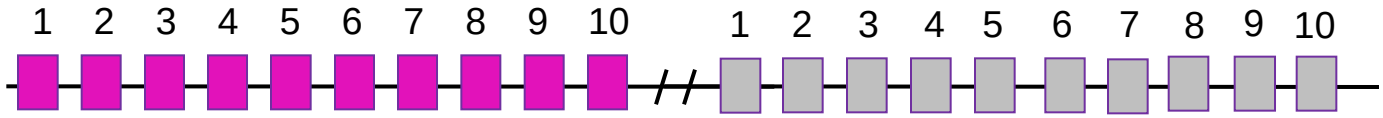
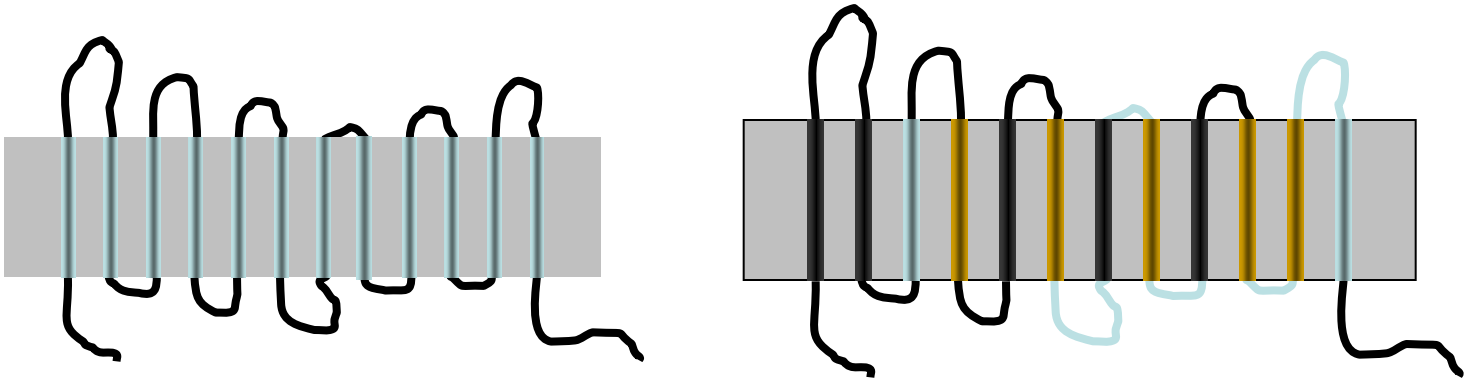
RH Genes – Rh Positive

Chromosome 1







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Rh (D) Negative

- Deletion of *RHD* – in European ancestry
- Inactivating mutations of *RHD*
  - *RHDψ* in African Americans
- Hybrid *RHD-CE-D* in African backgrounds

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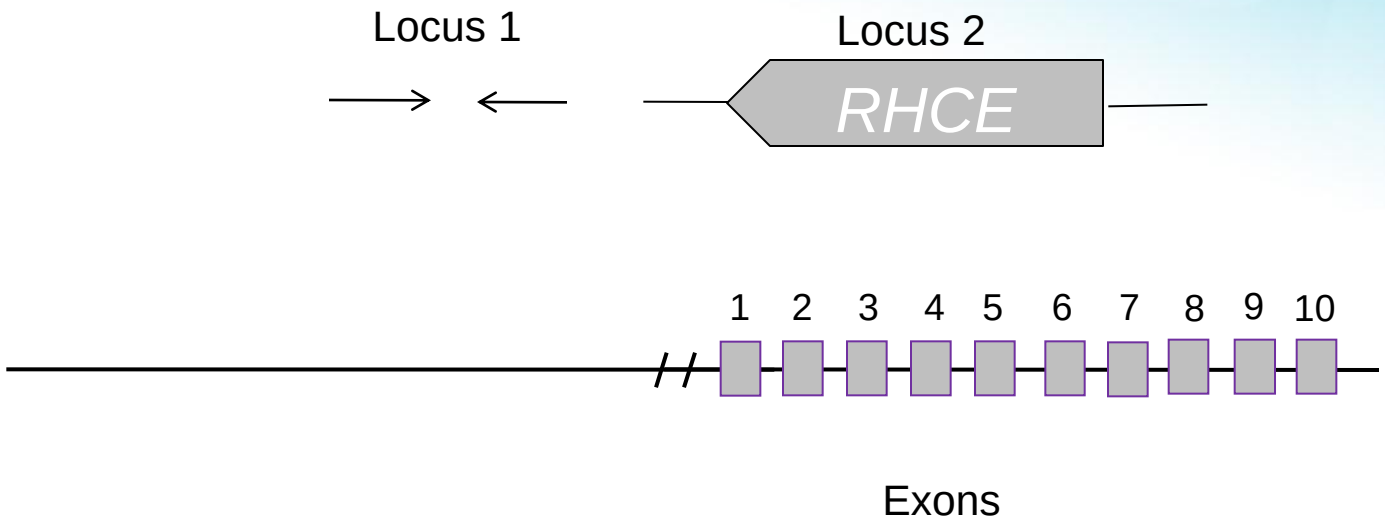
RH Genes in Rh Negative Caucasians

Chromosome 1



Locus 1 **deletion of *RHD*** therefore, no D antigen.

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Rh (D) Negative – African Background

19%	*RHD* deletion
66%	*RHDψ*
19%	Hybrid *RHD-CE-D*

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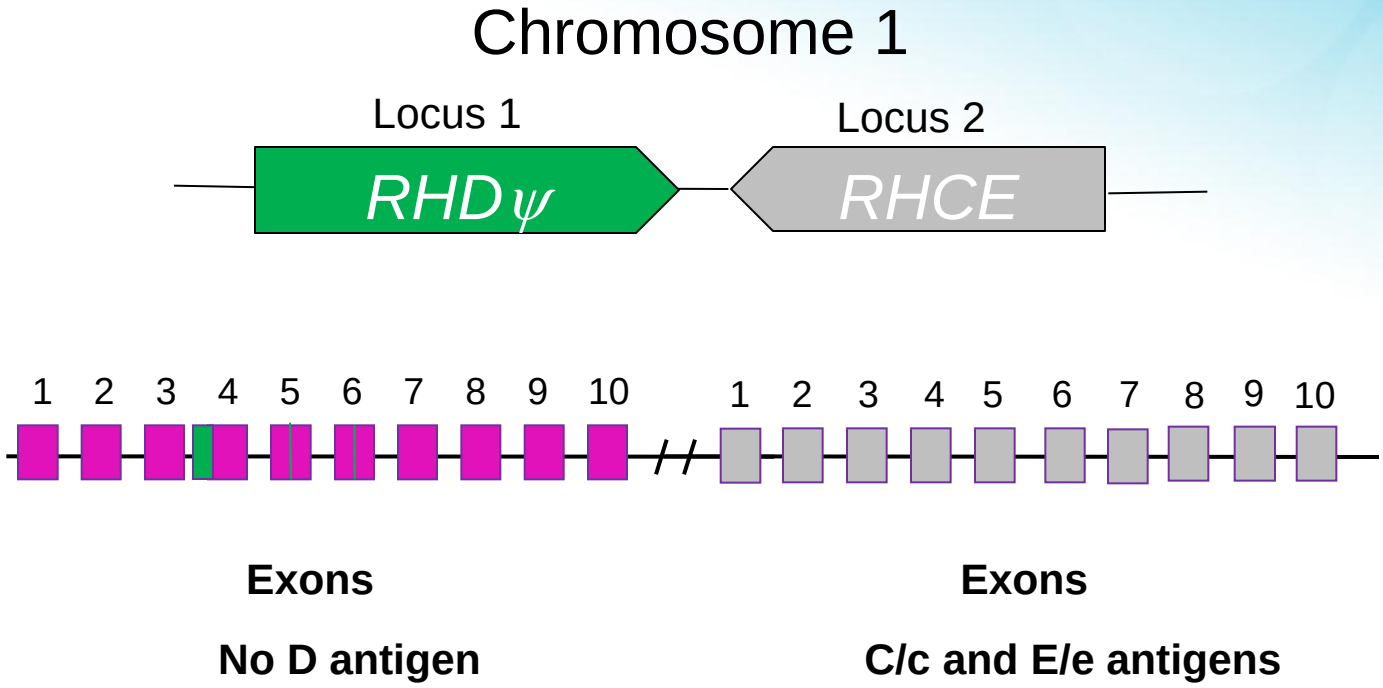
RH Genes in Rh Negative - African Background



Locus 1 – 37 bp insertion & several mutations in *RHD* results in no product

66% of AAs have *RHDψ*

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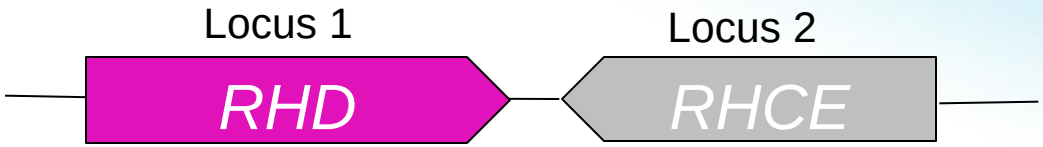
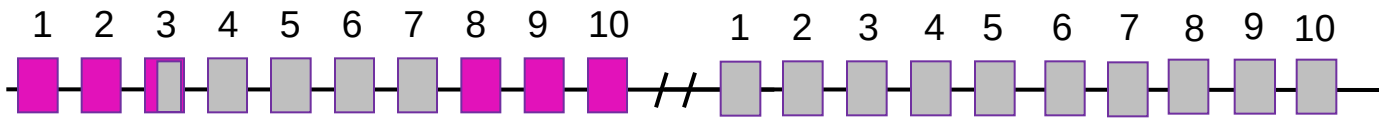
Rh (D) Negative – African Background

Chromosome 1





No D antigen **C/c and E/e antigens**

Locus 1 – *RHCE* inserted in *RHD* results in no D antigen and weak C.

15% of AAs have *hybrid RHD-CE-D*

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What About Weak Expression of D?

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WEAK EXPRESSION OF RhD HISTORY

- D^u
- D mosaics
- Weak D – general term used
- Partial D
- *Weak D*
  - Specific group of RhD variants
- D-elution alleles

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WEAK D HISTORY

- Described by Stratton (1946)
- D antigen not detected by all anti-D
- Mistakenly called the D^u antigen
  - D^u+ blood to a D- person causes production of ***anti-D not anti-D^u***

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WEAK D
Reactivity with Anti-D

- Agglutinated with some anti-D on direct agglutination (IS)

- Negative on direct agglutination (IS)
  - D antigen detected by IAT only

Frequency of Weak Expression

Hopkins	Scotland	1967	0.56%
Garretta	France	1974	0.66%
Beck	USA	1990	0.2%
Jenkins	USA	2004	0.4%
Flegel	Germany	2006	0.4%

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WEAK D
Variation in RhD Expression

- Do not make anti-D
- Able to make anti-D

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Weak Expression of D
Do Not Make Anti-D

- C in *trans* with *RHD* (Ceppellini effect)
  - r’ haplotype
- Weak D “Types”: single amino acid changes
- Weak D Type 2
  - Very weak(+) when in *trans* with r’

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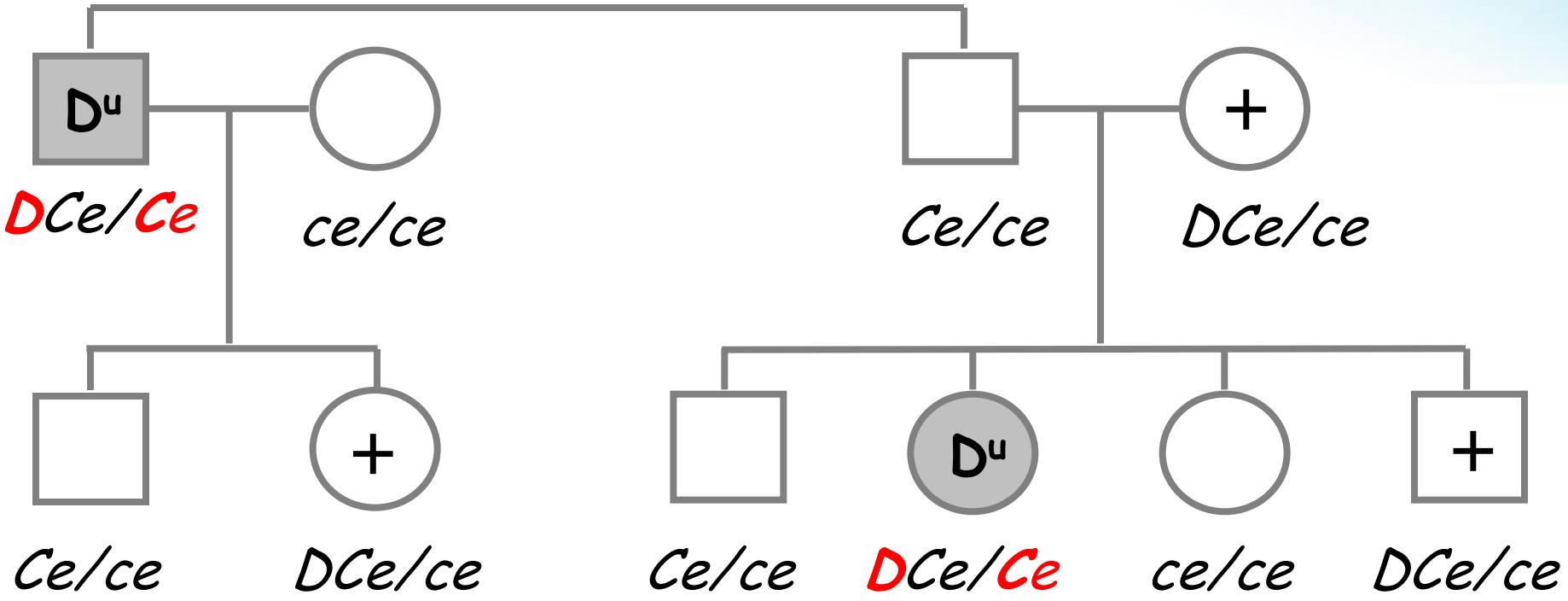
Ceppelini Effect



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Weak D Types
Do Not Make Anti-D

- Missense mutations in regions of *RHD* encoding **transmembrane/cytoplasmic portion of D**
- Less protein inserted into RBC membrane
- Can type as Rh-positive or Rh-negative by direct agglutination with monoclonal (IgM) anti-D reagents

	IS
Anti-D	**3+**

or

	IS	D IAT	Ct. IAT
Anti-D	**0**	**3**	**0**

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Some Weak D Types

- Type 1
- Type 2
- Type 3
- Type 4.0

Account for 90% of Weak D;
Do not produce Anti-D

- Type 4.2
- Type 5
- Type 11
- Type 15
- Type 19
- Type 20

Known to form Anti-D when exposed to D+ RBCs

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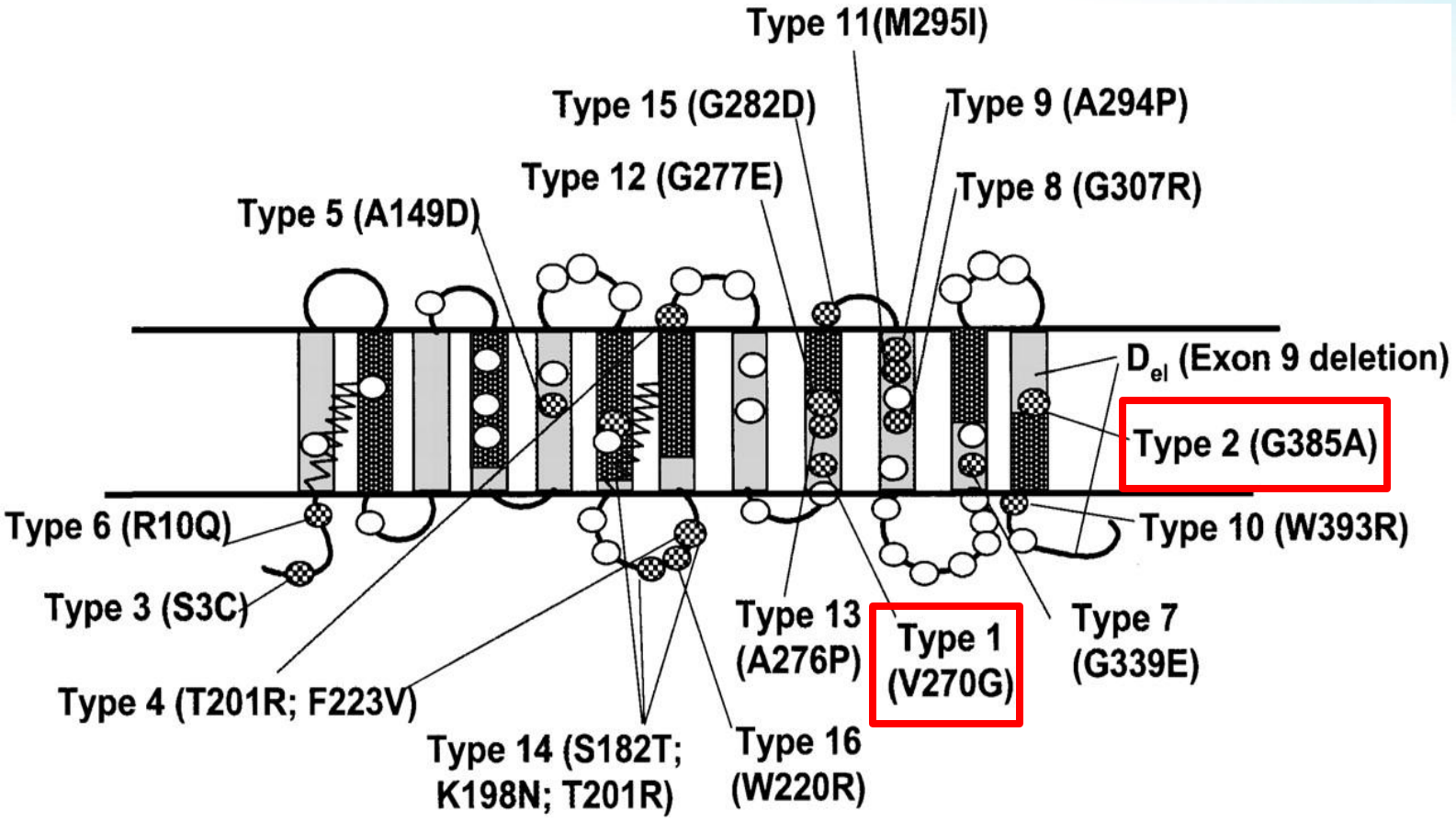
Molecular Basis of Weak D



Avent and Reid
Blood (2000) 95:375

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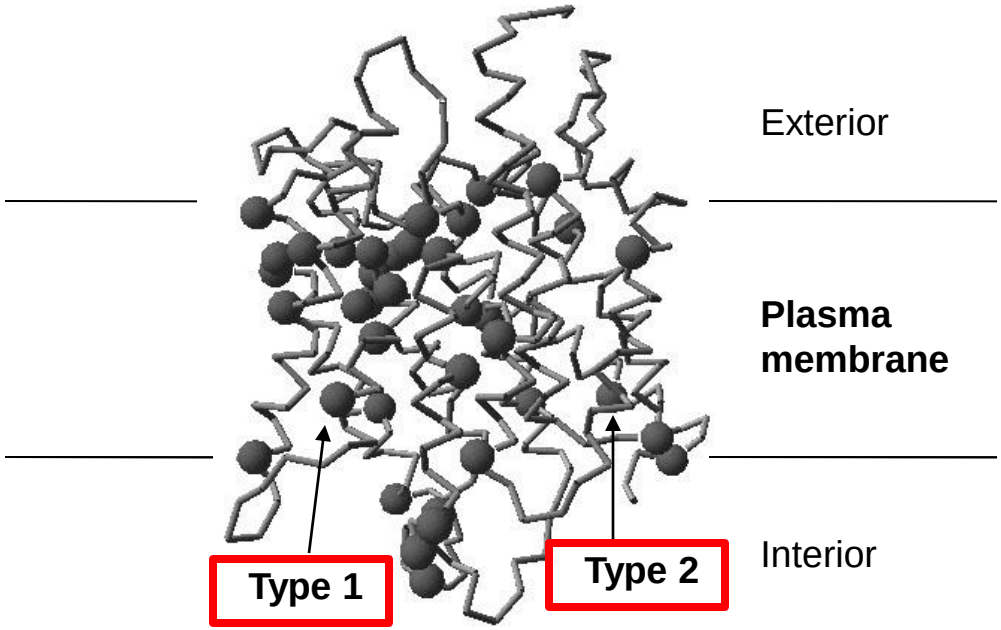
Weak D



CM Westhoff

	IS	D IAT	Ct. IAT
Anti-D	**0**	**3**	**0**

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Weak D Types 1 and 2

- Most common weak D types
- Weak D Type 1
  - R_1r (D+C+E-c+e+)
- Weak D Type 2
  - R_2r (D+C-E+c+e+)

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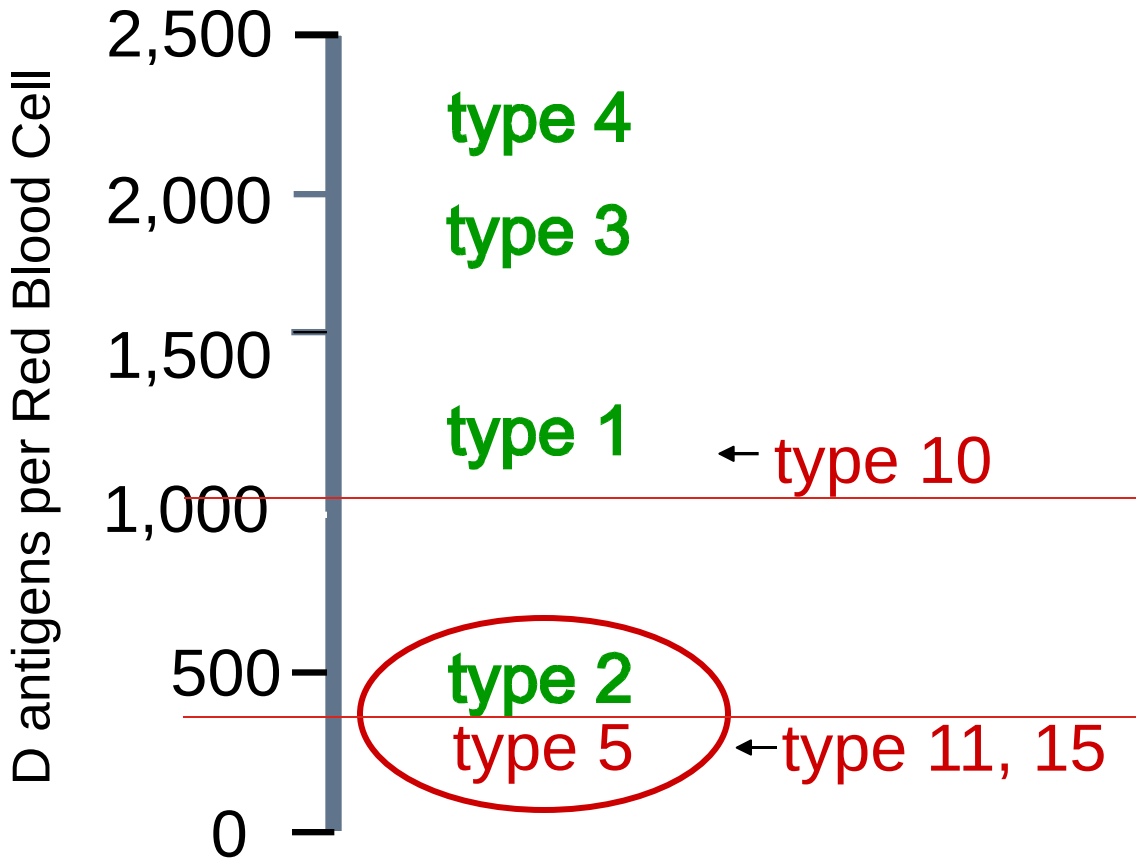
D Antigen Copy Number



G Denomme

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Weak Expression of D
Able to Make Anti-D

- Partial Ds: hybrid *RHD* alleles
  - DVI
  - DIIIa
  - DIVa, DIVb, others
- D_{el}: detection by adsorption/elution
- D epitopes on *RHCE* gene

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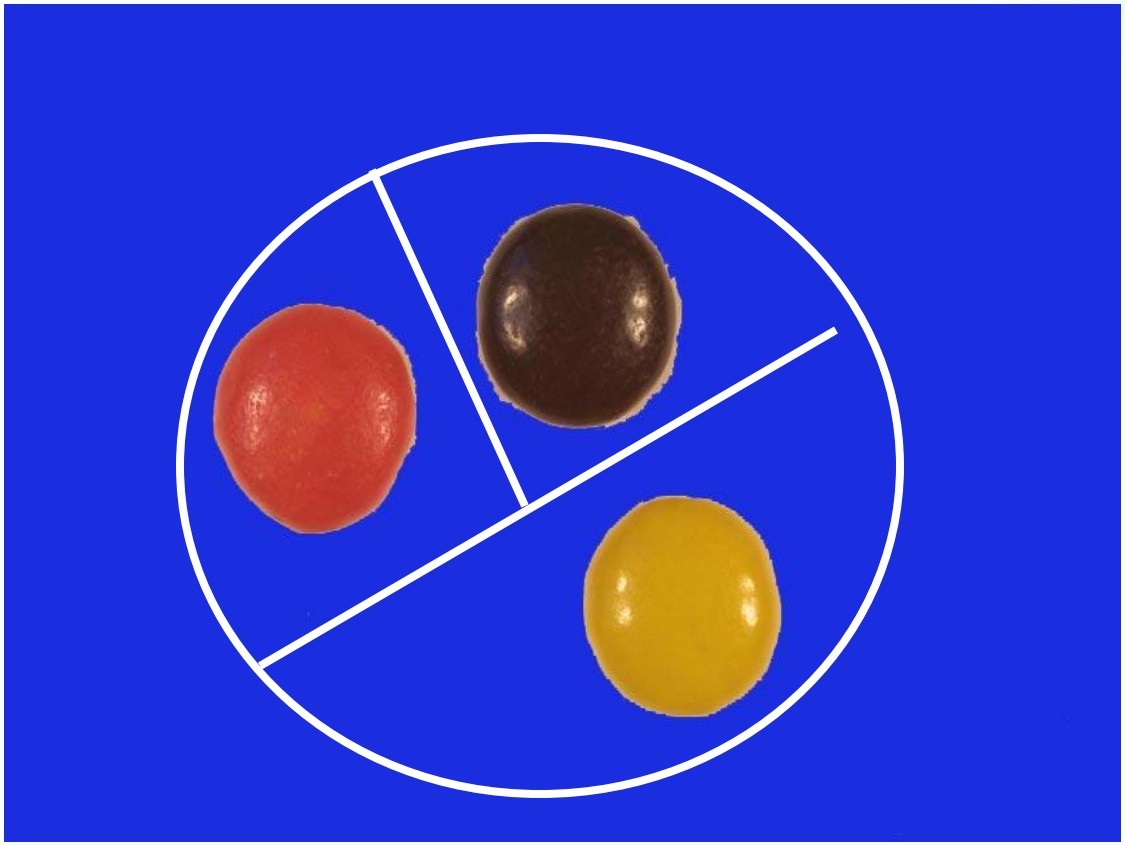
RHESUS PIECES



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PARTIAL D

- Partial D
  - Lack exofacial epitopes
    - Hybrid proteins
    - Missense mutations affecting **exofacial protein**

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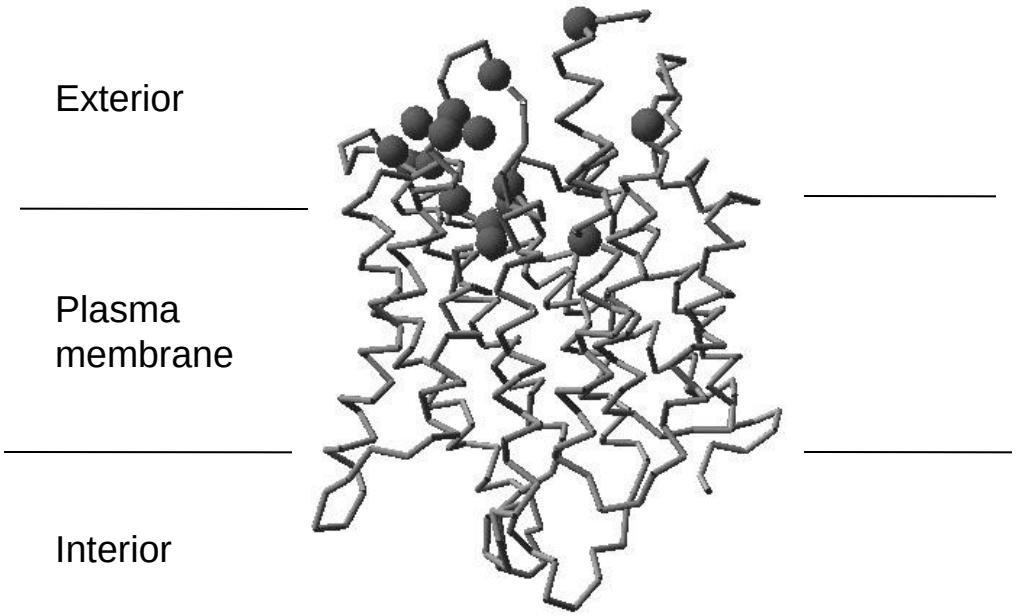
PARTIAL D



CM Westhoff

	IS	D IAT	Ct. IAT
Anti-D	**0**	**3**	**0**

or

	IS
Anti-D	**3+**

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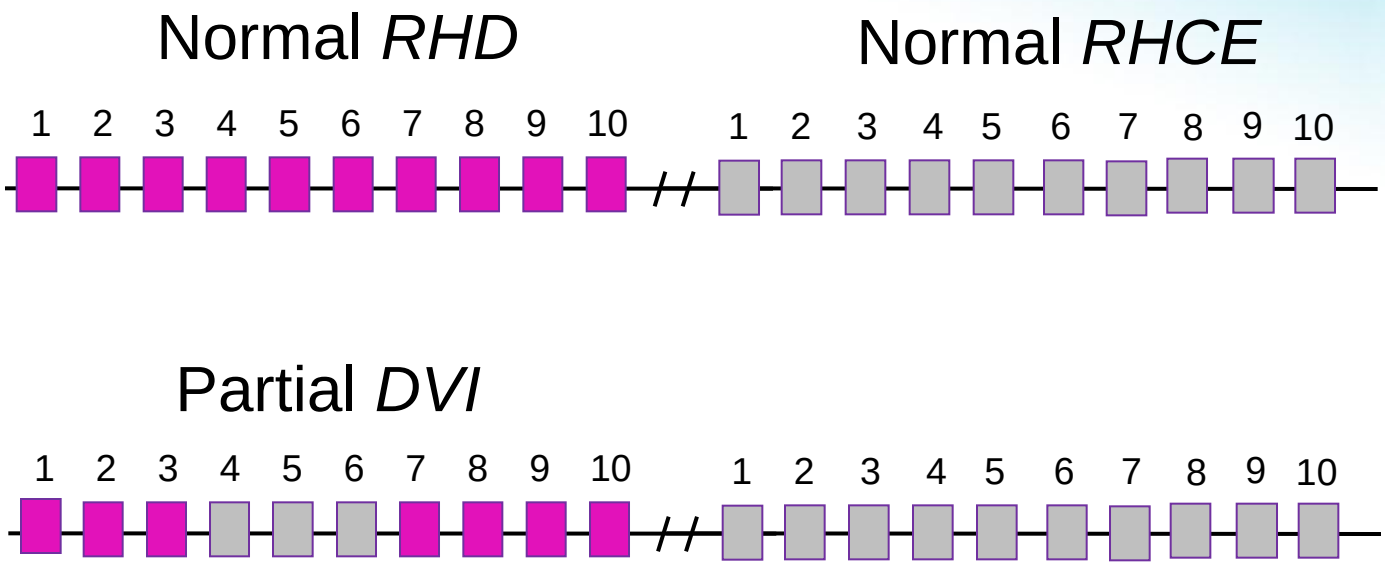
PARTIAL DVI



One example of Partial *DVI* gene where 3 exons of *RHCE* gene are inserted into *RHD* gene.

	IS	D IAT	Ct. IAT
Anti-D	**0**	**3**	**0**

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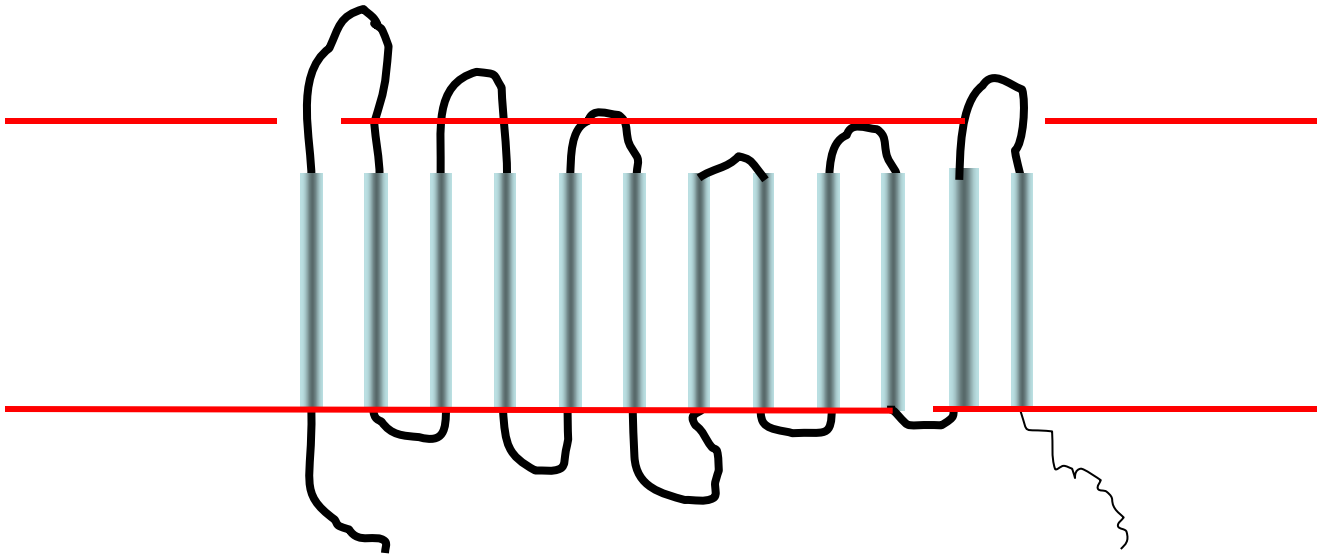
D_{el}

- Type as D-negative (IS & IAT), only adsorb & elute anti-D
- Severely reduced protein
- 2 individuals have made anti-D after receiving D+ blood



Deletion of exon 9 in Asians occurs in 10-30%

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D Epitope on *RHCE* Genes

- Crawford (ceCF) phenotype
- R_0^{Har}, also known as D^{HAR}

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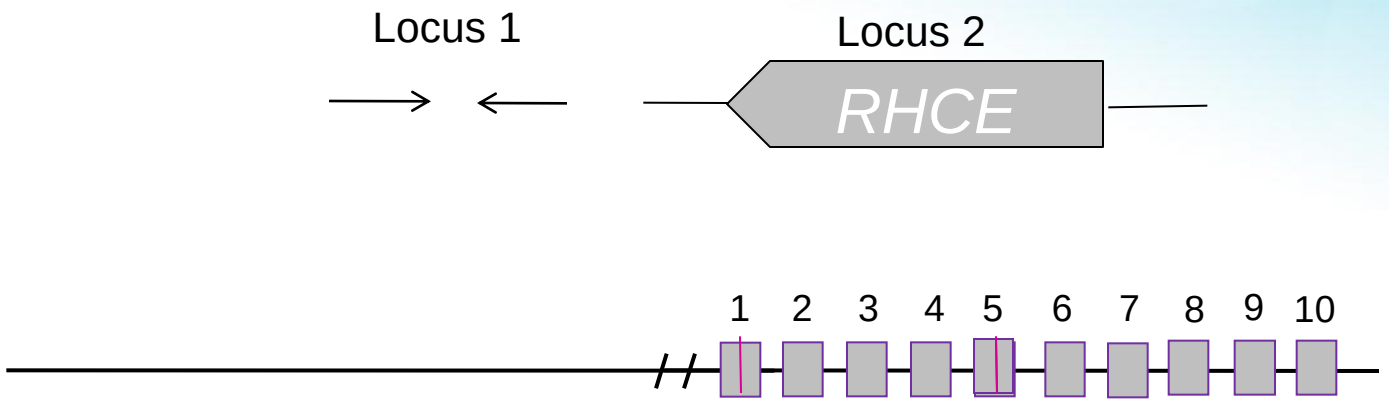
D Epitope on *RHce* Gene - D^{CF}



D^{CF} results from 3 nucleotide changes, **48G>C**, **697C>G**, **733C>G** in *RHce* gene.

	IS
Anti-D	**3+**

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Anti-D Reagents: Reactions with Crawford Phenotype RBCs

	Anti-D		RBCs
Reagent	**IgM**	**IgG**	**Crawford**
GammaClone	GAMA401	F8D8	Pos
Immucor-4	MS201	MS26	Neg
Immucor-5	TH28	MS26	Neg
Ortho Bioclone	MAD2	Human polyclonal	Neg
Ortho (ID-MTS)	MS201		Neg

Reactive clones in some European reagents: RUM-1, D175-2, F5S, H2D5D2F5, MCAD-6

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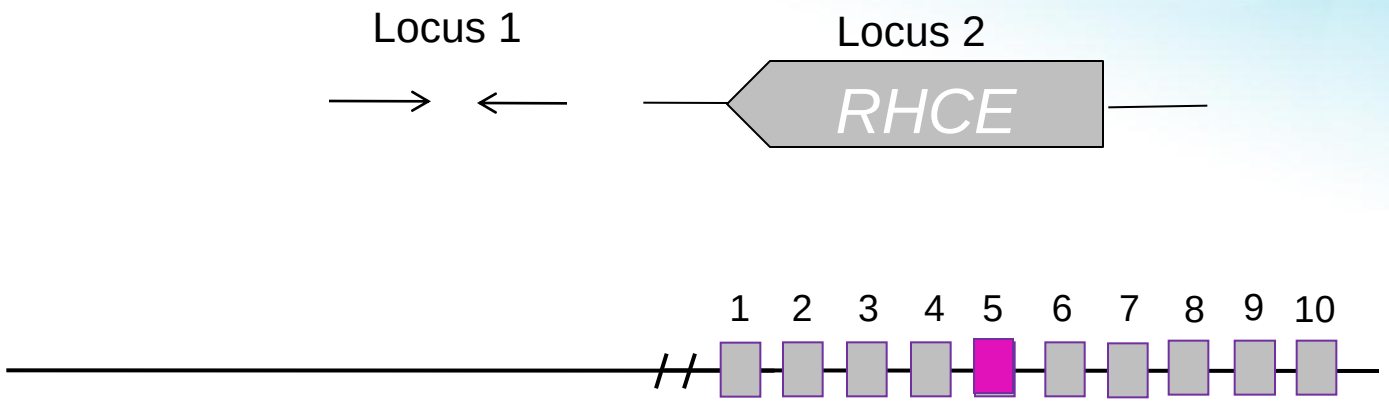
D Epitope on *RHCE* Gene - D^{HAR}



D^{HAR} results from one *RHD* exon inserted into the *RHCE* gene.

	IS
Anti-D	**3+**

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R_0^{Har} Phenotype: Reactivity with Reagent Anti-D

	Anti-D		RBCs
Reagent	**IgM**	**IgG**	**R_0^{Har}**
Gamma-Clone	GAMA401	F8D8	Pos*
Immucor-4	MS201	MS26	Pos*
Immucor-5	TH28	MS26	Pos*
Ortho Bioclone	MAD2	Human polyclonal	Neg
Ortho (ID-MTS)	MS201		Pos
Biotest (Bio-Rad)	BS232	BS221 H41 11B7	Pos
Quotient - Alpha	LDM1		Pos
Quotient - Delta	LDM1	ESD1M	Pos

*Positive reactions often weaker at IAT

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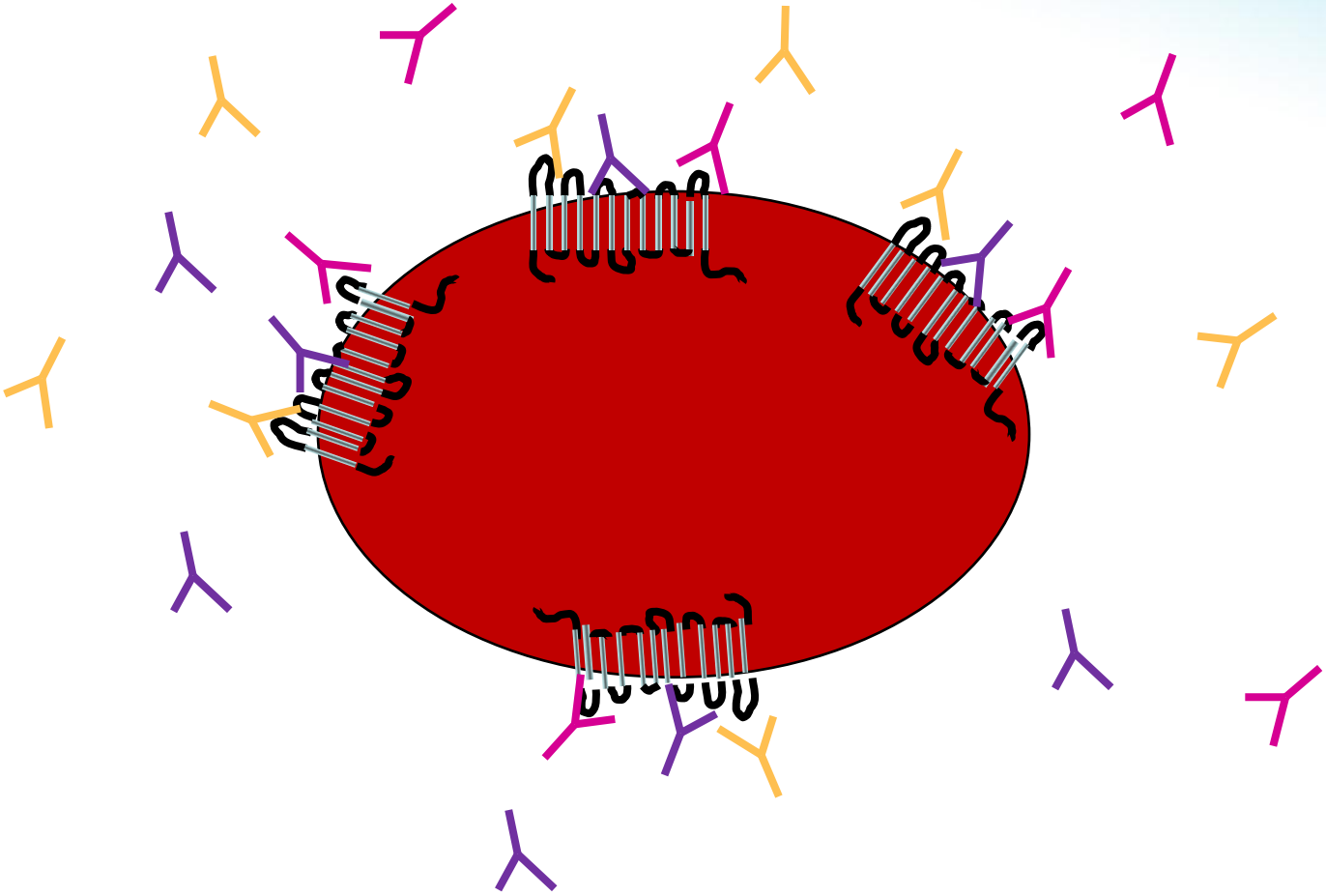
MoAb Anti-D's

Method	Manufacturer	IgM	IgG
Tube	Ortho	MAD2	Human
Tube	Gamma	GAMA 401	F8D8
Tube	Immucor-4	MS201	MS26
Tube	Immucor-5	Th28	MS26
Tube	Alba(Quotient BD) alpha	LDM1	
Tube	Alba (Quotient BD) delta	LDM1	ESD1M
Tube	Biotest (Bio-Rad)	BS232	BS221 H41 11B7
Gel	ID-MTS	MS201	

Human IgG Anti-D



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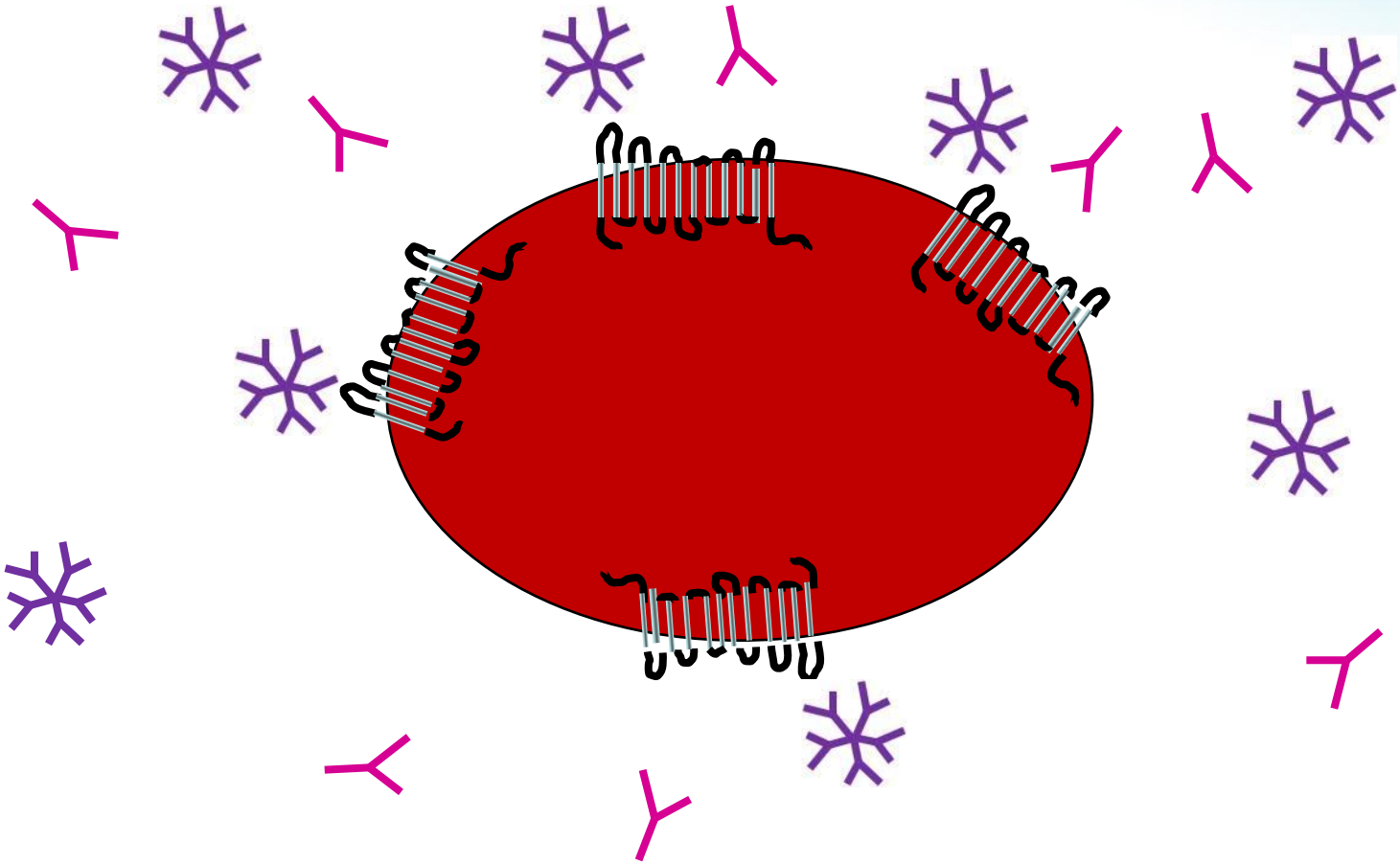
MONOCLONAL IgM/IgG ANTI-D



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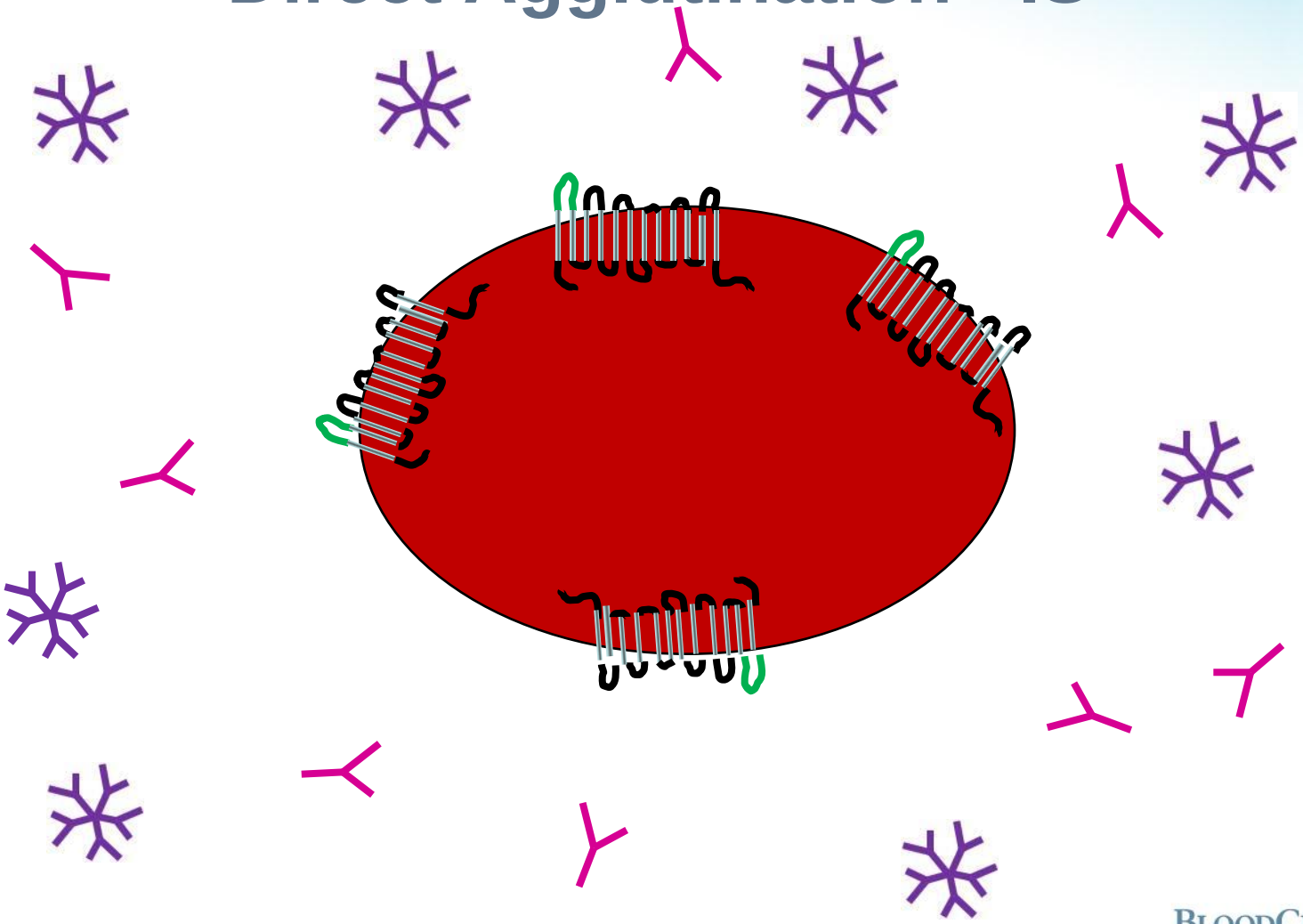
MONOCLONAL IgM/IgG ANTI-D #1
Direct Agglutination - IS



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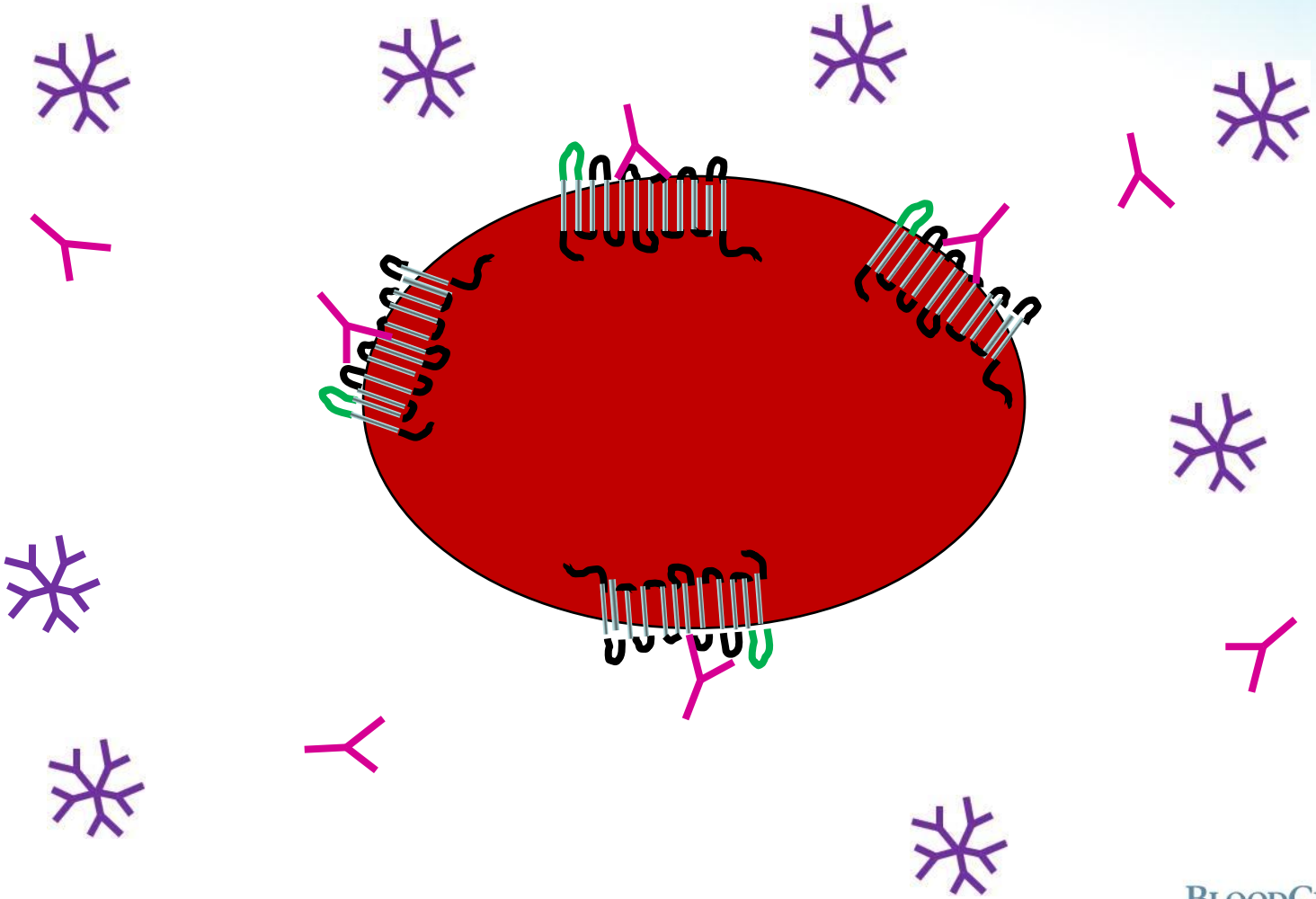
MONOCLONAL IgM/IgG ANTI-D #1
Weak D Test - IAT



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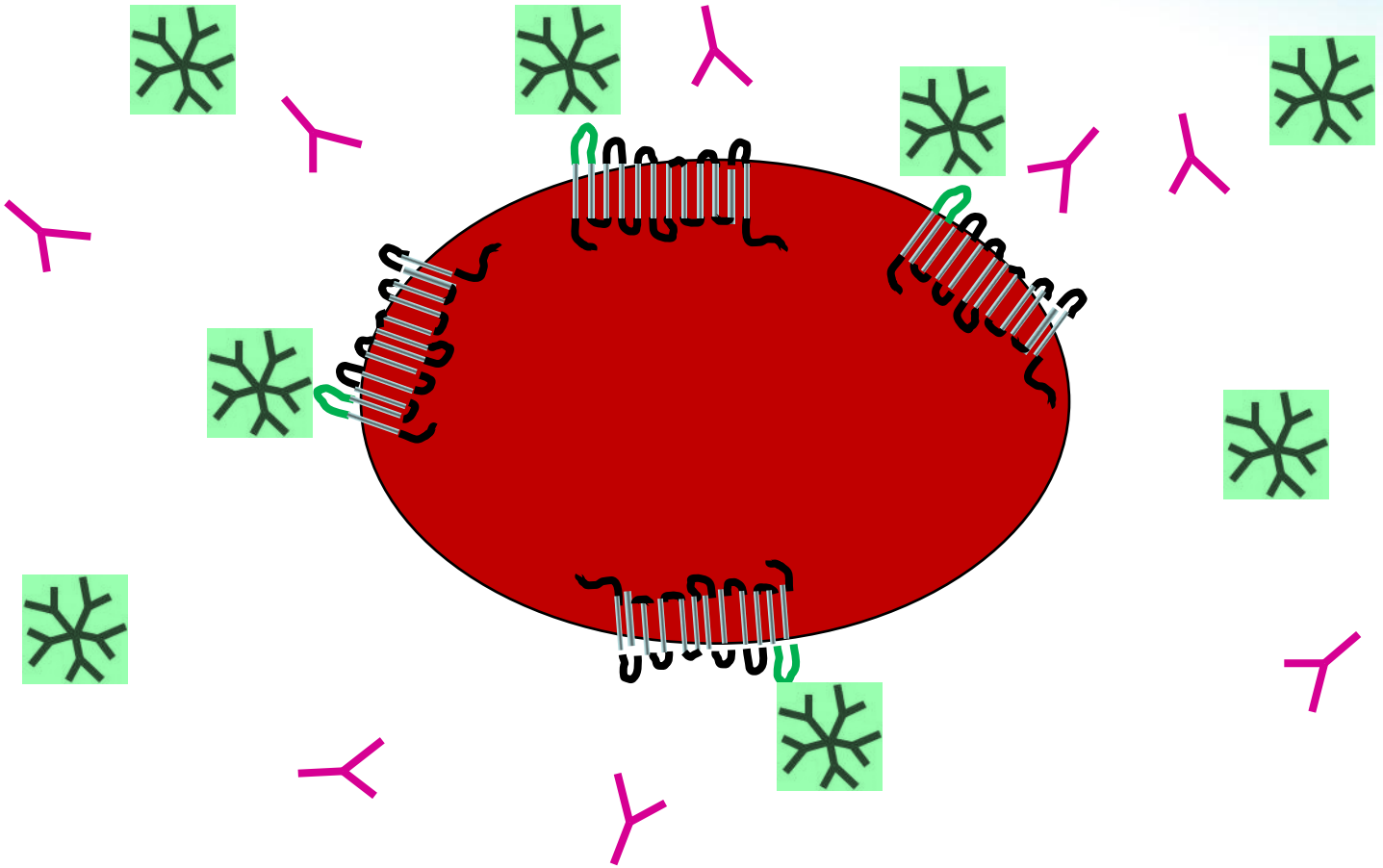
MONOCLONAL IgM/IgG ANTI-D #2



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Confusion Over Weak Expression of D

Donor	Rh+
Recipient	Rh-
Prenatal	**RhIG?**
Newborn	Postpartum RhIG?
Autologous Donor	@#!&*~?

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Reasons to Resolve Weak Expression

- Conserve Rh-negative blood for D-negative recipients (high risk of making anti-D).
- Avoid giving RhIG to women who do not need it (Rh status is confirmed for historical discrepancies)
- Resolve early in pregnancy to eliminate false-positive rosette tests.

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Rh Discrepancies - MSH, Toronto

Discrepancy between two anti-D direct tests

- 33,864 RhD phenotypings performed over an 18 month interval
- 55 of 5672 potential Rh-negative patients were tube test positive for one anti-D (0.98%)
  - 54 were tube test negative using one FDA-approved reagent but positive (2+ or less) using another government approved antisera

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Summary of the Toronto Study

20 functional *RHD* alleles detected; 1 wildtype (HDN)

- 34 Weak D Types (PCR-RFLP):
  - 16 weak D Type 1
  - 8 weak D Type 2
  - 1 weak D Type 3
  - 6 weak D Type 4
  - 1 weak D Type 5
  - 2 weak D Type 42
- 7 DAR (exon mapping plus sequencing)
- 6 D^{Va} or D^{Va}-like alleles:
  - 3 DVa(Kou.) 1 DVaHK(E233K) 1 DVa-like 1 DTO (Novel)
- DFR, DAU-4, DAU-5 (Novel), DAU-6 (Novel)
- DAR/DAU-2, DAU-0/*Cde*^{s} (compound heterozygotes)
- 1 not identified (possible DIIIa, DVa, DAR, DOL)

57% were Weak D types 1, 2, 3 and 4

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Impact if deemed Rh-negative

Inappropriate use of blood products

RHD Allele	OB	TR	NB	Impact
Weak D Types 1-4	12	8	5	12 OB patients received Rhig 4 transfusion recipients received 12 Rh-neg RBCs
Weak D Type 42	1	1	-	OB patient received Rhig Transfusion recipient received 11 Rh-neg RBCs
				Total: 21 RhIG 23 Rh-negative RBCs
DAR	3	1	3	3 OB patients received Rhig Potential transfusion recipient was not transfused.
DV^a and DV^a-like	1	1	5	1 OB patient an delivered an Rh-neg infant Potential transfusion recipient not transfused
DAU, DFR, DTO	3	2	2	2 OB patient delivered an Rh+ infant Neither potential transfusion recipient transfused
				Total: 7 Rhig 0 Rh-negative RBCs

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Summary of Alberta Study

Analysis '07 – '08 = 88,972

DNA Typing Results	# of Patients	Rh Status Assigned	RHIG Recommended	% of DNA Results Received
Weak D Type 1	60	Pos	No	29.0
Weak D Type 2	19	Pos	No	9.2 64%
Weak D Type 3	38	Pos	No	18.4
Weak D Type 4	15	Pos	No	7.2
DAR	2	Neg	Yes	1.0
Partial DVI Type I	3	Neg	Yes	1.3
Partial DVI Type II	1	Neg	Yes	0.5 36%
DVI Type II	2	Neg	Yes	1.0
DVa partial	1	Neg	Yes	0.5
Partial DVA-like	1	Neg	Yes	0.5
Unclassified	65	Neg	Yes	31.4
Pending	2	TBD	TBD	
TOTAL	**209**	(0.23% of total)		

Monoclonal Anti-D Panel

Anti-D cell line	Expected patterns of reactivity of different forms of partial D with the different monoclonal anti-D antibodies																Test results		
	Weak D type 1&2	DII & DNU	DIII	DIV	DV	DCS	DVI	DVII	DOL	DFR	DMH	DAR	DAR-E	DHK & DAU-4	DBT	Ro^{Hur}	Pos Cont	Neg Cont	Pt
LHM76/58	+	+	+	+	+/0	+	0	+	+	+	+	+	0	0	0	(+)/0	4	0	0
LHM76/59	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0	4	0	3
LHM174/102	(+)/0	+	+	0	0	+	0	+	0	0	+	0	0	0	0	0	4	0	0
LHM50/28	+	+	+	+	+	+	0	+	+	+	+	+	+	+	0	0	4	0	0
LHM169/81	+	+	+	0	0	+	0	+	+	+	+	0	0	0	0	0	4	0	0
ESD1	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0	4	0	4
LHM76/55	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0	4	0	3
LHM77/64	+	0	+	0	+	+	+	+	+	+	+	+	+	+/0	0	0	4	0	3
LHM70/45	(+)/0	+	+	0	0	0	0	+	0	0	0	0	0	0	0	0	4	0	0
LHM59/19	+	+	+	+	+	+	0	0	0	0	(+)	0	(+)	+	+	0	4	0	0
LHM169/80	+	+	+	+	+	+	0	+	+	+	+	+	+	0	0	0	4	0	0
LHM57/17	+	+	+	+	+	0	0	+	+	0	+	+	0	0	+	0	4	0	0

Interpretation: DVI

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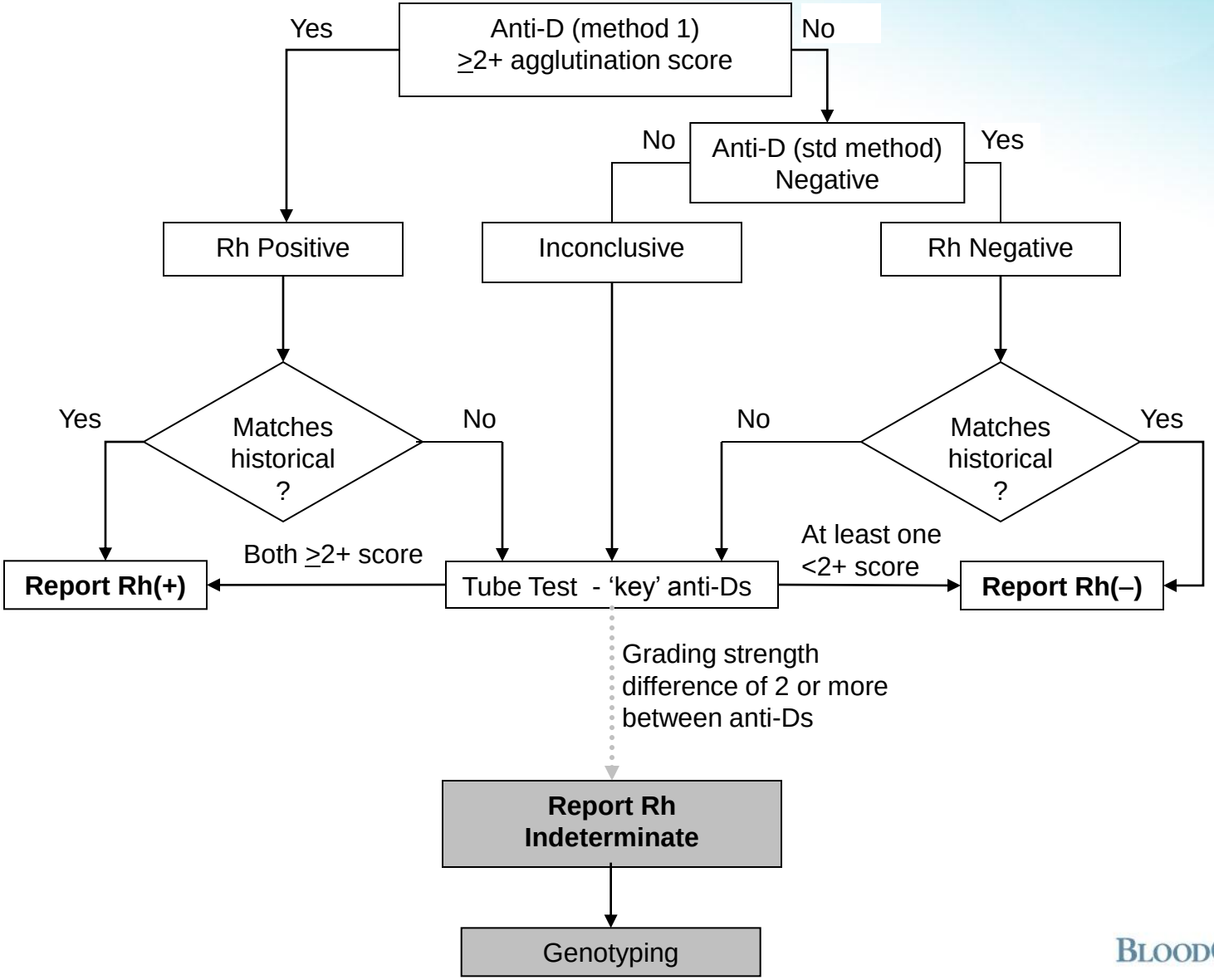
Rh Discrepancy Algorithm



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Bagene Weak D Worksheet

Worksheet und Auswertetabelle / *Worksheet and Evaluation diagram*

	Reaktions-Nr. / *Reaction No.*	1	2	3	4	5	6	7	8
	PCR-Produkt (Größe in bp) *PCR product (size in bp)*	150	126	165	101	130 83	112	198 83	153
	weak D Allele / *weak D alleles*								
	weak D type 1	+	–	–	–	–	–	–	–
	weak D type 2	–	+	–	–	–	–	–	–
⇨	weak D type 3	–	–	+	–	–	–	–	–
	weak D type 4.0, 4.1	–	–	–	+	–	–	–	–
	weak D type 4.2, DAR	–	–	–	+	130	–	–	–
	weak D type 5	–	–	–	–	–	+	–	–
	weak D type 11 (haplotype cDe)	–	–	–	–	–	–	198	–
	RHD(M295I) (haplotype $CD_{el}e$)	–	–	–	–	–	–	198	–
⇨	weak D type 15	–	–	–	–	–	–	–	+
	weak D type 17	–	–	–	–	83	–	83	–
	weak D type 4.2, 17	–	–	–	+	130 | 83	–	83	–
	Weak D type 11 / *RHD*(M295I), 17	–	–	–	–	83	–	198 | 83	–
	RHD pos. oder / or *RHD* neg.	–	–	–	–	–	–	–	–

Genotyp *Genotype*	1	2	3	4	5	6	7	8

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Investigation strategy for RhD typing discrepancies using a combination of PCR-SSP and serological techniques

Lay See Er, MSTM, (ASCP)SBB

- http://www.aabb.org/development/awardsscholarships/scholarships/Pages/pastwinners.aspx

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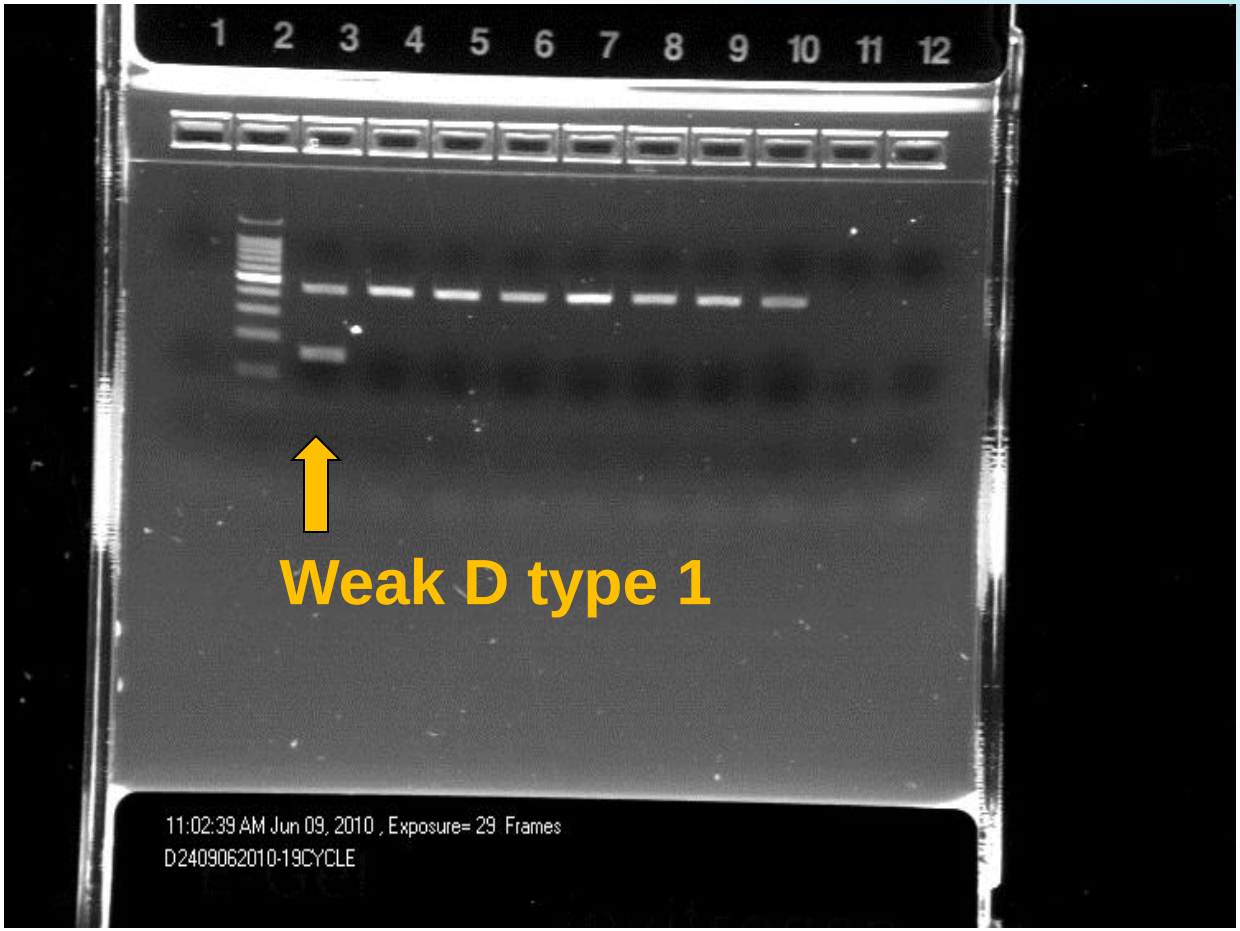
Bagene Weak D Kit Results



Lane 2: DNA ladder
Start reading from lane 3
Lane 1, 11,12: buffer load (no bands)

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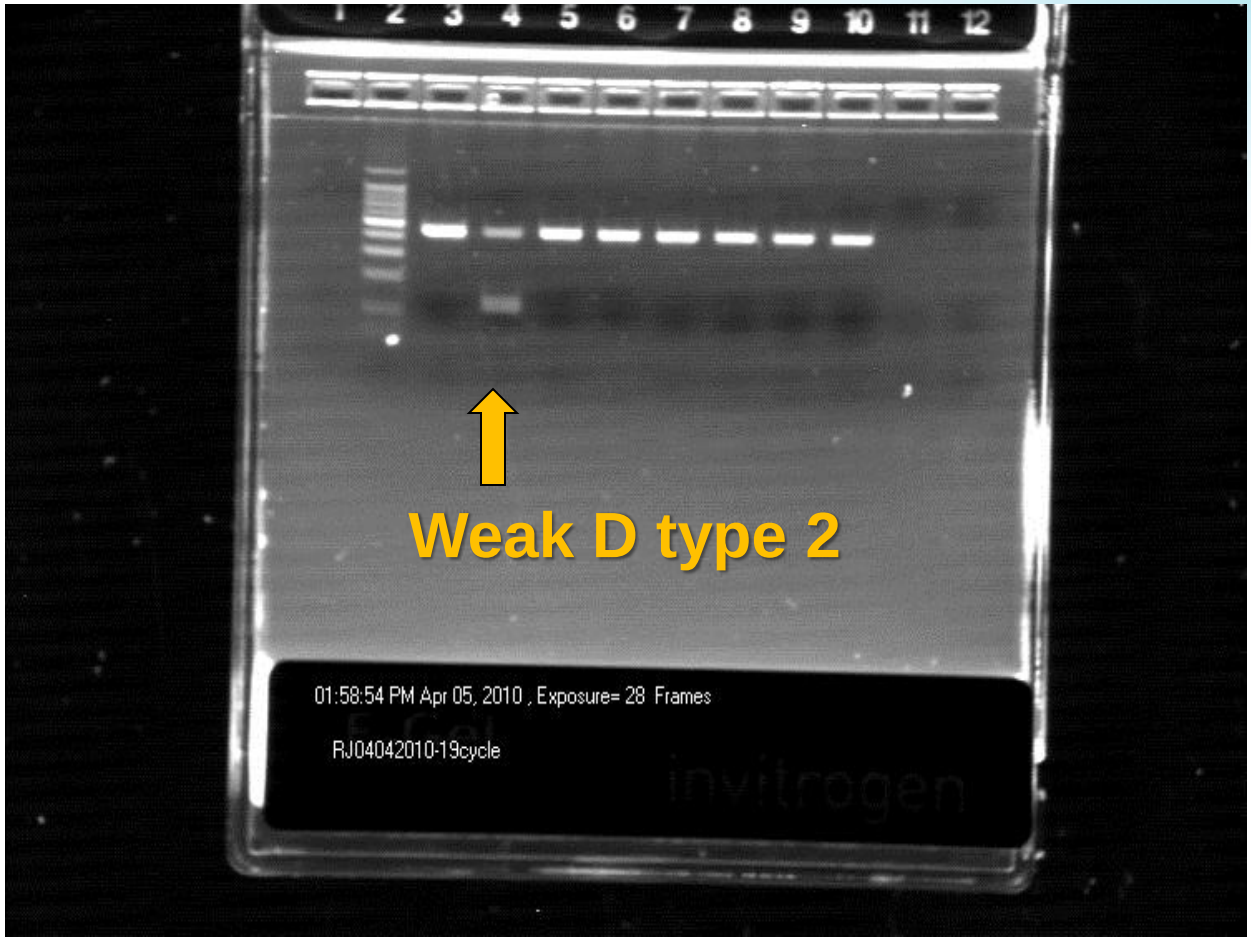
Bagene Weak D Kit Results



Lane 2: DNA ladder
Start reading from lane 3
Lane 1, 11,12: buffer load (no bands)

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Summary

- 3-5% RhIG doses go to women with Weak D Types
  - How often do you need to switch Rh status?
    - Molecular test is a permanent solution
    - Weak D Types 1 – 4 are Rh+ as a recipient and donor
  - Informed consent for administration of RhIG?
    - Avoid a blood product where it is not needed!
    - RhIG shortage, new infectious disease

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Summary, cont…

- Resolution $ Molecular Test < RhIG $
  - Rh allele pop'n frequencies
  - # of pregnancies

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Guideline for Interpreting Discordant Rh Typing Results

Rh typing results are evaluated at immediate spin (direct agglutination) and Rh typing is repeated with identical results

If individual types…	And individual is a….	And…	Then, consider molecular typing…
Rh-negative	Transfusion recipient	Donor record is Rh-positive	Interpret Rh-negative
Rh-negative	Obstetrical patient	Donor record is Rh-positive	Interpret Rh-neg or Rh-pos?
Rh-negative	Post delivery	Donor record is Rh-positive	Perform anti-D IAT*
Rh-negative	Transfusion recipient	Facility history is Rh-positive	Interpret Rh-negative
Rh-negative	Obstetrical patient	Facility history is Rh-positive	Interpret Rh-neg or Rh-pos?
Rh-negative	Post delivery	Facility history is Rh-positive	Perform anti-D IAT*

Modified from Transfusion Technology Report Vol. #013 Immucor, Inc.

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Guideline for Interpreting Discordant Rh Typing Results

Rh typing results are evaluated at immediate spin (direct agglutination) and Rh typing is repeated with identical results

If individual types…	And individual is a….	And…	Then, consider molecular typing…
Rh-positive	Transfusion recipient	Rh Negative at another facility	Type with different anti-D reagent
Rh-positive	Obstetrical patient	Rh Negative at another facility	Type with different anti-D reagent
Rh-positive	Post delivery	Rh Negative at another facility (regardless of history)	Type with different anti-D reagent

Modified from Transfusion Technology Report Vol. #013 Immucor, Inc.

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Conclusions

- Rh discrepancies are better resolved using a molecular approach.
  - MoAb approach is erroneous for some partial Ds
  - MoAb approach does not positively identify Weak D Types 1 and 2 and does not address Weak D Types 3, and Weak D Type 4 versus DAR.

- Laboratories who change methodologies or drop the IAT as a routine test on all patients have the appropriate support to resolve historical discrepancies through molecular testing.

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Objectives

- List the reasons for RhD typing discrepancies
- Discuss the biochemical and molecular characteristics of RhD
- Understand the differences among partial, weak, and Del variants
- Outline the advantage of a molecular resolution of Rh discrepancies

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References

- Wagner FF, Gassner C, Mu¨ ller TH, et al. Molecular basis of weak D phenotypes. Blood 1999; 93:385–393.
- Denomme GA, Wagner FF, Fernandes BJ, et al. Partial D, weak D types, and novel RHD alleles among 33 864 multiethnic patients: implications for anti-D alloimmunization and prevention. Transfusion 2005; 45:1554–1560.
- Flegel WA, Denomme GA, Yazer MH. On the complexity of D antigen typing: a handy decision tree in the age of molecular blood group diagnostics. J Obstet Gynaecol Can. 2007;29:746-52.
- Flegel WA. How I manage donors and patients with a weak D phenotype. Curr Opin Hematol 2006;13:476–483
- Flegel WA. Molecular genetics and clinical applications for RH. Transfusion and Apheresis Science 2011;44:81-91. 2.
- Sandler SG, Li W, Langeberg AL, Landy HJ. New Laboratory Procedures and Rh Blood Type Changes in a Pregnant Woman. Obstet Gynecol 2012;119:426–8.

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Thank You

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