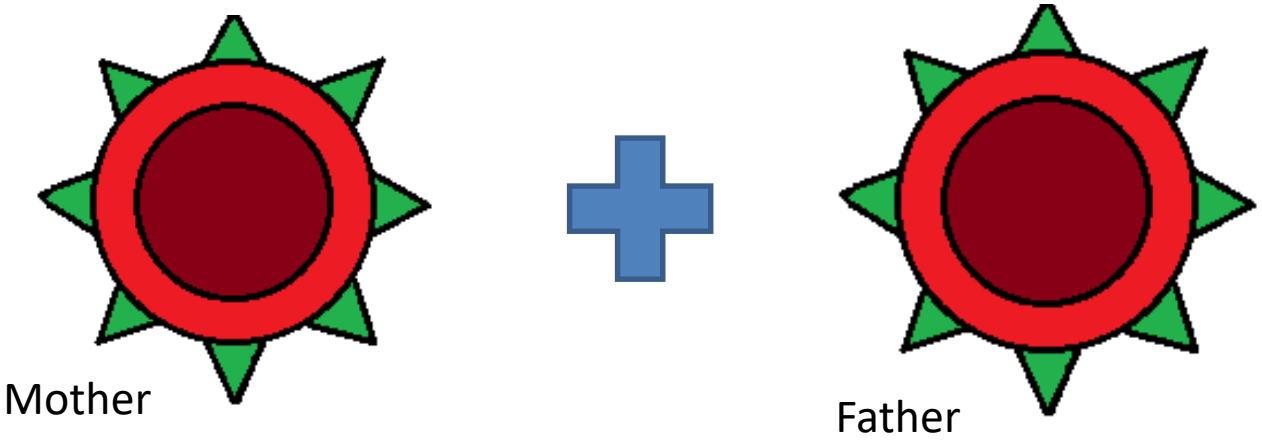
Cleveland Clinic

The Rh Blood Group System

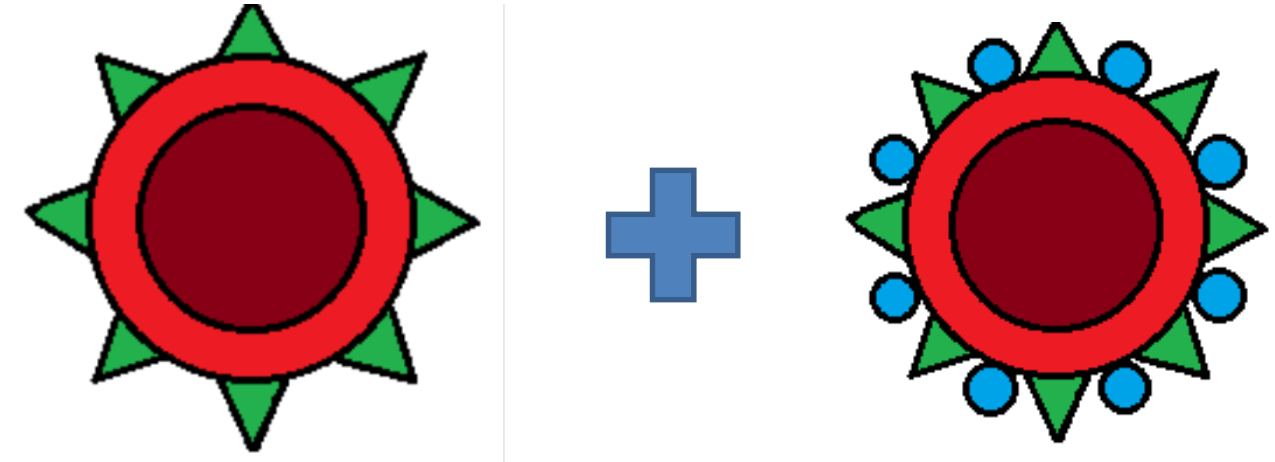
History

- 1939- Levine and Stetson discover anti-D (Rh)
- Women delivered stillborn infant
- Needed transfusion
- Given blood from husband who had same ABO
- After transfusion, had an acute hemolytic transfusion reaction



History

- Isolated antibody- react at RT and 37°C with husband's RBCs
- Fetus and father had common factor mother lacked
- Caused transfusion reaction



Mother

Father

One year later…

- Landsteiner and Wiener found antibody in guinea pigs and rabbits when transfused with blood from Rhesus monkeys
- Agglutinated 85% of human RBCs
- Named Rh after Rhesus monkey
- Years later realized the two were different
  - Human antibody remained Rh
  - Antibody in animals named anti-LW

Other Rh Antibodies

- Mid-1940s – 5 antigens in Rh system
- Today – Over 57 different antigens
- Only made after foreign exposure to antigen
  - Pregnancy
  - Transfusion
- Anti-D was a major cause of HDFN

Five Main Rh Antigens

- D, C, c, E, e
- d is considered a lack of D antigen
- Most frequent genotype is: DCe/dce

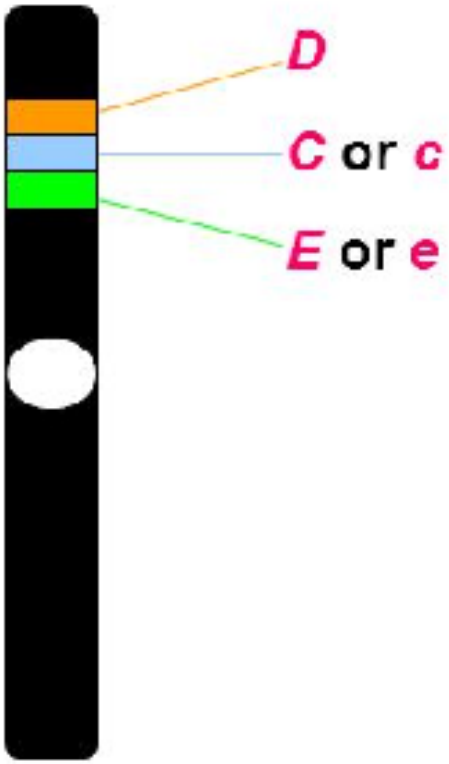
Antigen	Gene Frequency (%)
D	85%
d	15%
C	70%
c	80%
E	30%
e	98%

Four Nomenclatures

- Fisher-Race (DCE terminology)
- Wiener (Rh-Hr terminology)
- Rosenfield (alphanumeric terminology)
- International Society of Blood Transfusion or ISBT (Updated Numeric terminology)

Fisher-Race Terminology

- Antigens produced by 3 closely linked genes
- Major antigens are:
  - D
  - C and its allele c
  - E and its allele e



Fisher-Race Terminology

- Antigens and genes given same letter designation
  - Ags: D, C/c, E/e
  - Genes: *D, C/c, E/e* (genes are italicized)
- Inherit a set of 3 genes from each parent
- Rh genes codominant- both alleles will show
- Rh genotype: Rh genes inherited from parents
- Rh phenotype: Antigens expressed on RBCs

Fisher-Race Terminology

- D, C, c, E, e – represent actual antigens on RBCs
- “d” is the absence of “D” antigen
- Easy way to think about naming, but is no longer used

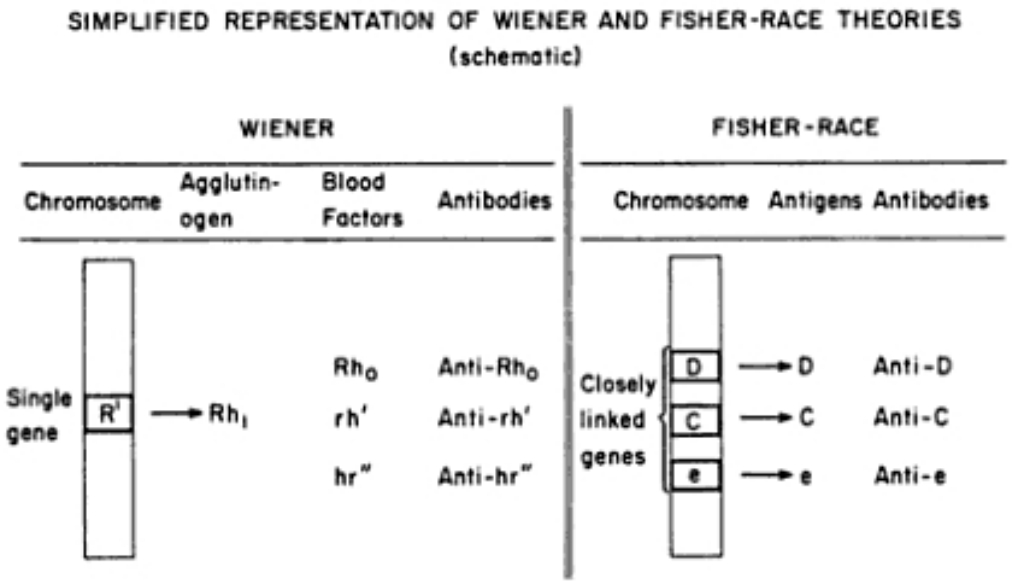
Haplotype	Caucasian Frequency
DCe	42%
dce	37%
DcE	14%
Dce	4%
dCe	2%
DcE	1%
DCE	<.01%
dCE	<.01%

Rare Genotypes

Deletion Genotypes	Phenotype
-De	D+ e+ E- C- c-
-DE	D+ E+ e- C- c-
CD-	D+ C+ c- E- e-
cD-	D+ c+ C- E- e-
D-	D+ C- c- E- e-
Rh null	No Rh antigens
Rh mod	Weakened expression of all Rh antigens

Wiener Terminology

- One gene for all Rh antigens
- This produces three factors within an agglutinogen
  - Agglutinogen: phenotypic expression of haplotype
  - Factor: antigen recognized by an antibody



Wiener Terminology

Symbol	Meaning
R	Presence of D antigen
r	Absence of D antigen
1 or ‘	Presence of C antigen
No 1 or ‘	Presence of c antigen
2 or “	Presence of E antigen
No 2 or “	Presence of e antigen
R_z	Presence of D, C, E antigens
r_y	Presence of C and E antigens

Wiener Terminology

Rh factors	Meaning
Single prime (‘)	C or c antigens
Double prime (“)	E or e antigens
r precedes h (rh’ or rh”)	C (rh’) or E (rh”) antigens
h precedes r (hr’ or hr”)	c (hr’) or e (hr”) antigens
Rh_0	D antigen
	No designation for absence of D

Gene	Agglutinogen	Blood Factor	Shorthand Designation	Fisher-Race Antigens
Rh^0	Rh_0	Rh_0 hr'hr''	R_0	Dce
Rh^1	Rh_1	Rh_0 rh'hr"	R_1	DCe
Rh^2	Rh_2	Rh_0 hr'rh"	R_2	DcE
Rh^z	Rh_z	Rh_0 rh' rh"	R_z	DCE
rh	rh	hr' hr''	r	dce
rh'	rh'	rh' hr"	r'	dCe
rh"	rh"	hr' rh"	r"	dcE
rh^y	rh_y	rh' rh"	r^y	dCE

*Genes described with italics and superscripts

Rosenfield Terminology

- 1960s- too many Rh antigens being discovered
  - Needed new system
- No genetic basis
- Assigns number to each Rh antigen
  - Generally in order of discovery
- System is used for other blood group systems as well

Rosenfield Terminology

Rosenfield Phenotype	Rh antigen
Rh: 1	D antigen
Rh: 2	C antigen
Rh: 3	E antigen
Rh: 4	c antigen
Rh: 5	e antigen
“-” sign in front of number for absence of antigen	

International Society of Blood Transfusion Terminology

- ISBT formed the Committee on Terminology for Red Cell Surface Antigens
- Goal: Establish nomenclature
  - Eye and machine readable
  - Genetic basis for blood groups
- Created 6 digit numbers
  - First 3= blood group system
  - Last 3= antigen specificty

ISBT Terminology

Numeric	Fisher-Race	Wiener	ISBT Number
Rh1	D	Rh_0	004001
Rh2	C	rh’	004002
Rh3	E	rh”	004003
Rh4	c	hr’	004004
Rh5	e	hr”	004005

004- assigned to Rh blood group system

ISBT Terminology

- When referring to individual antigens (similar to Rosenfield):
  - RH1- D
  - RH2- C
  - RH3- E
  - RH4- c
  - RH5- e
- Phenotype Designation:
  - D+ C- E+ c+ e+ is written RH:1, -2, 3, 4, 5
  - Minus sign is lack of the antigen

Common Genotypes and Nomenclature

Wiener	Fisher-Race	Rosenfield	Frequency (%)
R^1 r	DCe/dce	Rh:1,2,-3,4,5	34.9%
R^1 R^1	DCe/DCe	Rh:1,2,-3,-4,5	18.5%
rr	dce/dce	Rh:-1,-2,-3,4,5	15.1%
R^1 R^2	DCe/DcE	Rh:1,2,3,4,5	13.3%
R^2 r	DcE/dce	Rh:1,-2,3,4,5	11.8%
R^2 R^2	DcE/DcE	Rh:1,-2,3,4,-5	2.3%

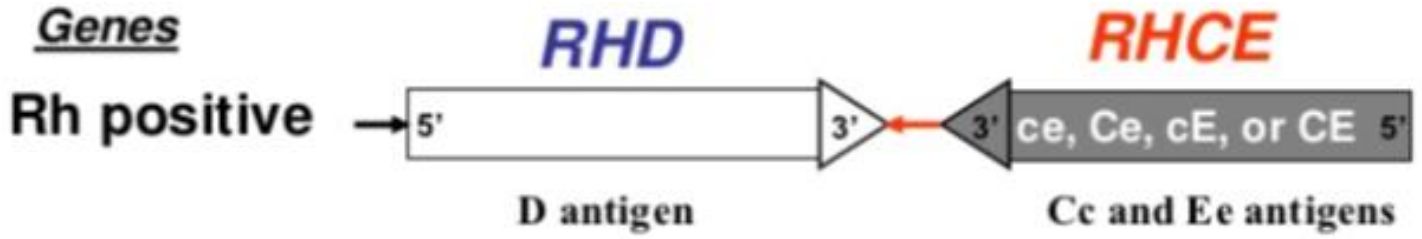
Full Chart on p. 153 Table 7-5

Use of Rh phenotypes/genotypes

- Parentage studies
- Population studies
- Predicting HDFN- Rh neg mother with anti-D
- Finding compatible blood for unusual phenotypes
  - Certain phenotypes are more common with different populations

Genetics of Rh System

- 2 closely linked genes on chromosome 1
  - *RHD* – presence or absence of D
  - *RHCE* – Codes for either Ce, cE, ce, or CE



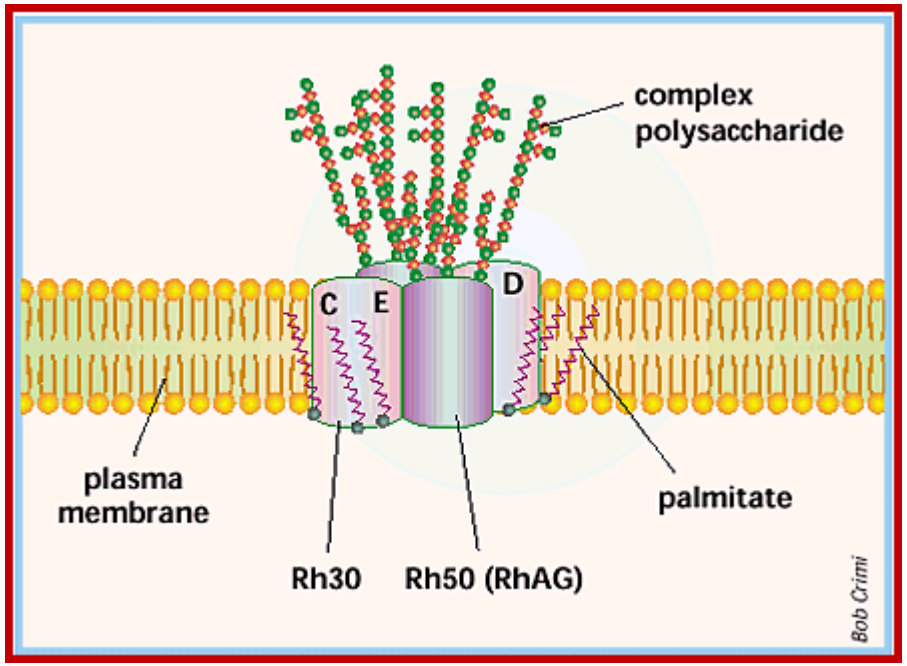
Rh Negative

- Results from 3 different mutations

European Ethnicity	African Ethnicity	Asian Ethnicity
• Most common • Deletion of *RHD* gene	• *RHD* pseudogene • Missense mutation • Can not produce protein	• D_{el} • Mutation • Low # of D antigen sites • Types as D negative • Adsorb and elute to detect

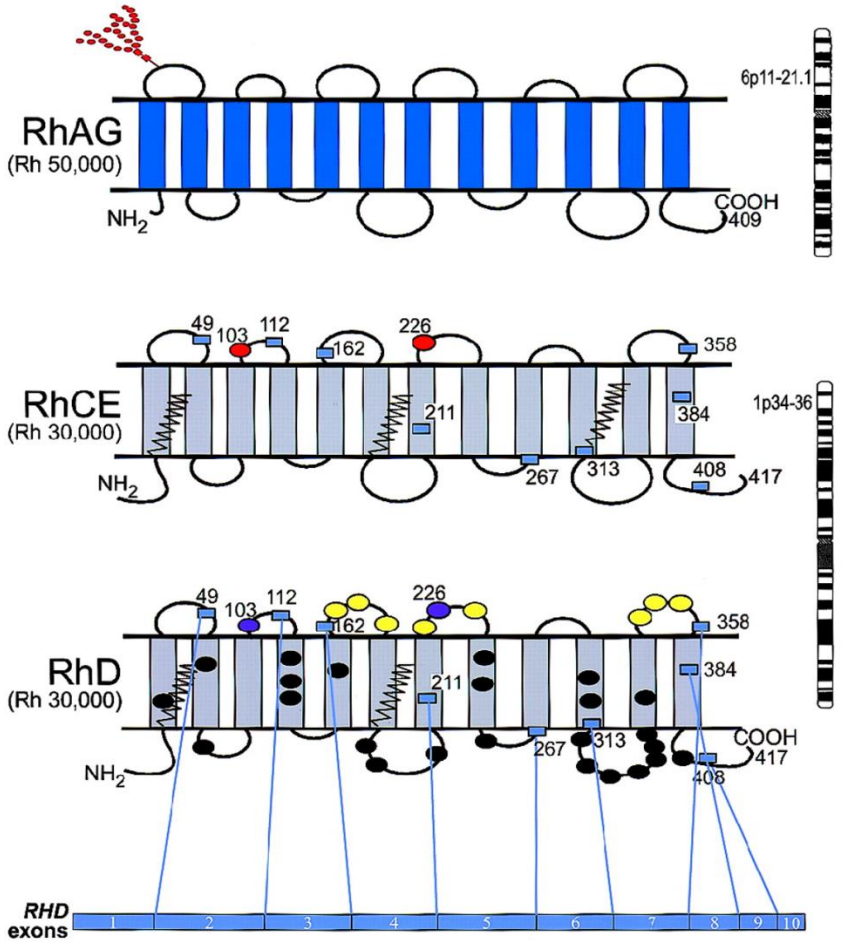
Rh-associated Glycoprotein (RhAG)

- Chromosome 6
- Forms complexes with Rh proteins
- Structure: similar to Rh but glycosolated (carbohydrates attached)
- Coexpressor- must be present for Rh antigens to be present



Rh Biochemistry

- Nonglycosylated proteins- no carbohydrate at the end
- Transmembrane proteins- spanning entirety of RBC membrane
- Function of Rh antigens- maintain RBC integrity

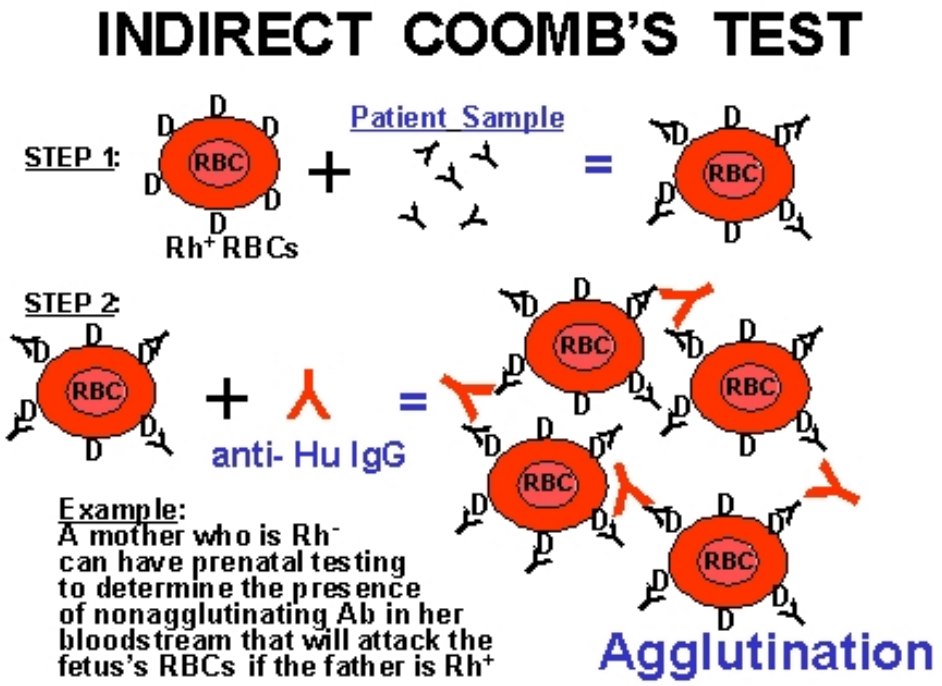


Rh Biochemistry

- RHD vs. RHCE:
  - 416 amino acids
  - Transverse membrane 12 times
  - Proteins differ by 32-35 amino acids
- C and c
  - Differ by 4 amino acids
- E and e
  - Differ by 1 amino acid

Testing for D antigen

- 1 drop 3-5% RBCs to 1 drop anti-D reagent
- Centrifuge 30 sec
- Weak D- Indirect antiglobulin test



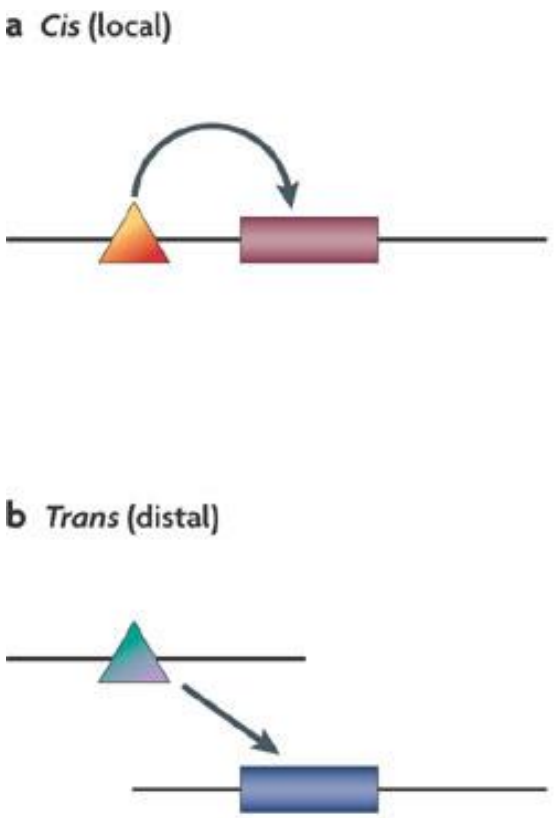
Weak D Antigen

- Weak expression of D antigen
- Test in antiglobulin phase to detect
- Previously called anti-D^u
- Different Mechanisms:

C in *Trans* *RHD*	Weak D
Partial D	D_{el}

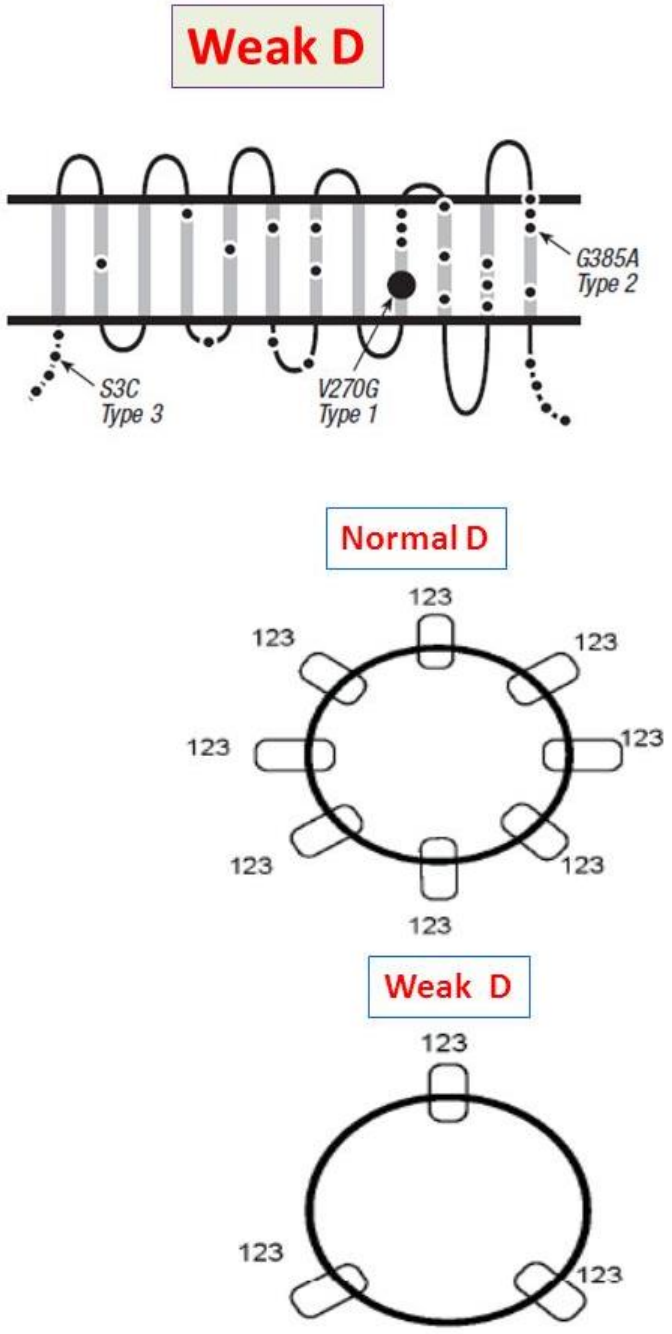
C in *Trans* to *RHD*

- Allele carrying *RHD* is *trans* (in opposite haplotype) to allele carrying C
- D antigen structurally complete
- Interferes with expression of D antigen
- Can receive D positive RBCs
  - *Trans: Dce/dCe*
  - *Cis: DCe/dce*



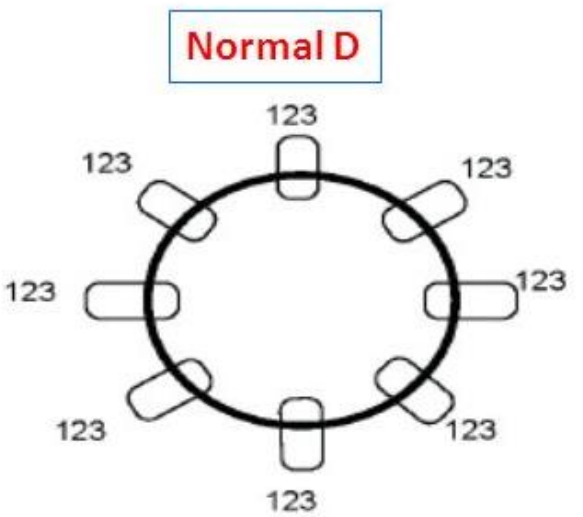
Weak D

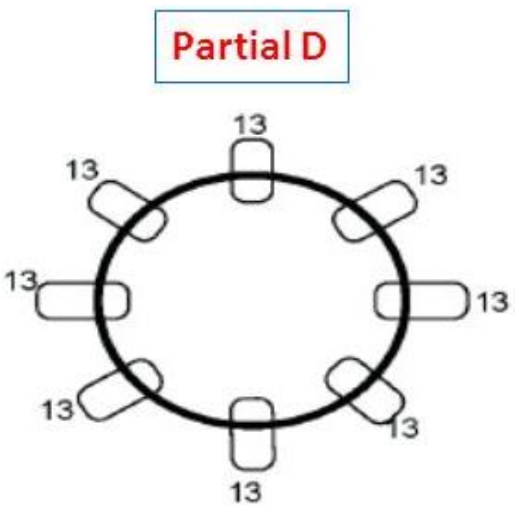
- *RHD* genes code for weakened expression
- Mutations causing changes in intracellular region of protein
- Complete antigens, few in number

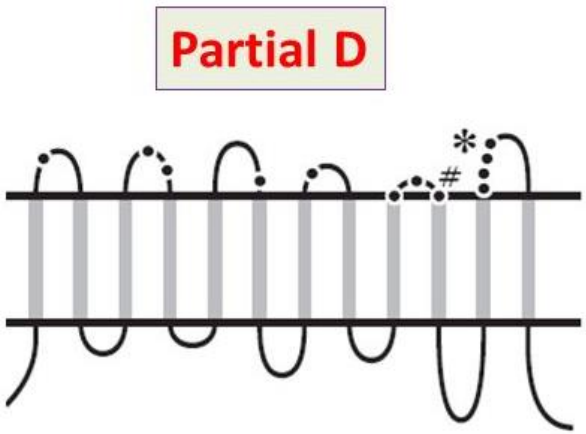


Partial D (D Mosaic)

- One or more D epitopes within D protein are missing or altered
- React weaker or not at all with anti-D reagents
- Can form alloanti-D causing HDFN or transfusion reactions
- Transfuse Rh Neg blood







Comparing Weak D antigens

	All epitopes present	Make anti-D	Patient considered	Location of Changes
C in *Trans*	Yes	No	D+	None
Weak D	Yes	No	D+	Internal
Partial D	No	Yes	D-	External

D_{el}

- Extremely weak Rh D expression
- Mutation/deletions
- Adsorption and elution of anti- D from RBCs needed to detect D antigen

When to Perform Weak D Testing

- IS D neg donors to determine D+ or D-
- IS D neg OB patients – Do they need Rhogam?
- Newborns of D neg mothers – Do they need Rhogam?
- Many times more testing needed

Rh Antibody Characteristics

- Produced after exposure (transfusion, pregnancy)- only need 0.1mLs
- Show dosage – react more strongly to double dose (E+e- vs. E+e+)
- Highly immunogenic – D antigen most potent
- Immunogenicity: D>c>E>C>e

	Rh
Enzymes	Increased
IgM vs. IgG	IgG
Cold or Warm	37°C
Natural vs. Immune	Immune
HTR	Yes
HDN	Yes

Rh Antibody Characteristics

- IgM formed initially- transition to IgG
- In circulation for years
- Titer can fall below detectable levels
  - Can experience secondary antibody response
- Clinically significant – if make antibody, must give antigen negative blood

Rh Antibody Characteristics

- Do not bind complement
- To fix complement:
  - 2 IgG immunoglobulins need to attach in close proximity
  - Rh antigens are not close on cell surface
- RBC destruction from Rh antibodies is extravascular
  - Abnormal RBCs removed by liver/spleen

Transfusion Reactions with anti-D

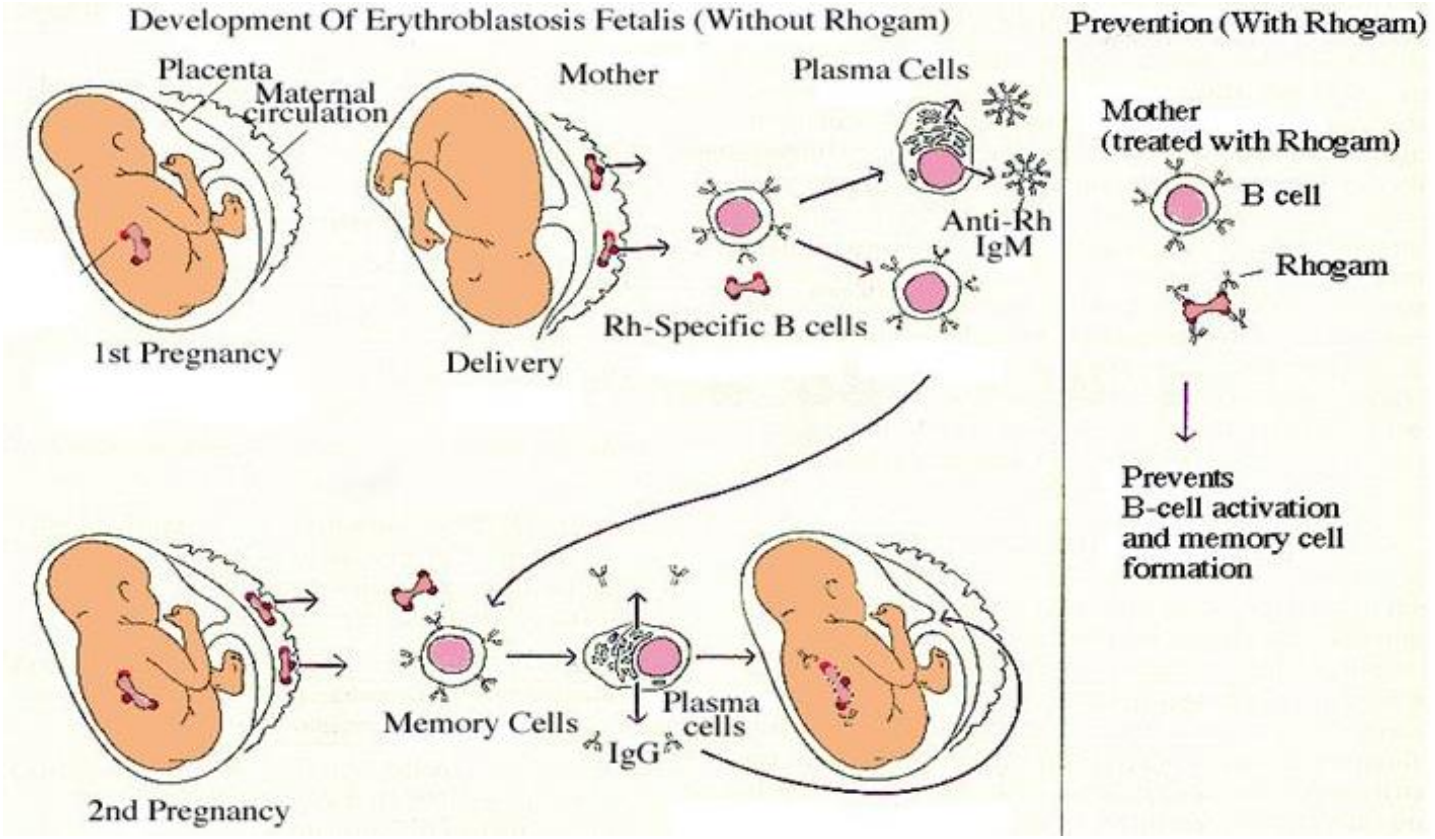
- Most immunogenic outside ABO system
- Antibody appears within 120 days (primary exposure)
- Once an antibody is made, transfuse antigen neg.
- Extravascular destruction

Fever	Mild bilirubin elevation
Decreased hemoglobin and haptoglobin	Positve DAT

Once you make one Rh antibody, usually make more

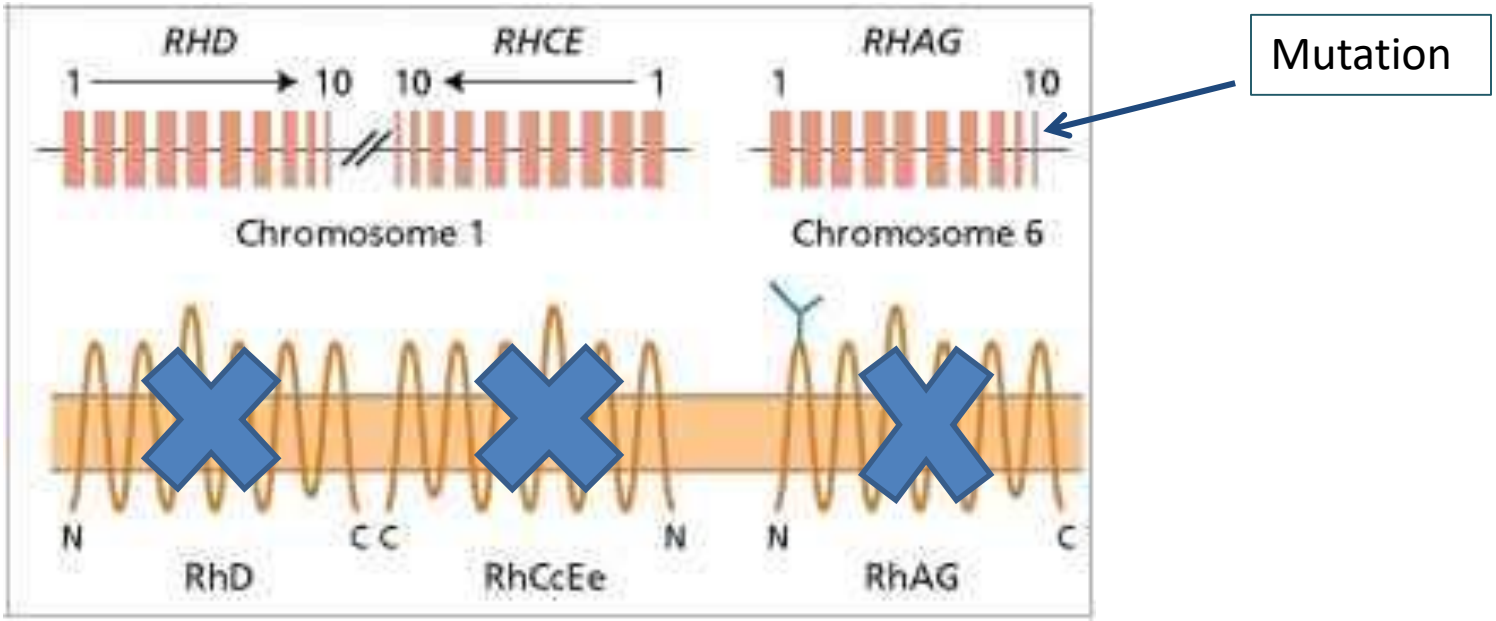
Hemolytic Disease of the Newborn

- Rh antigens well developed on fetal cells
- Primarily IgG- can cross placenta
- Rh-immune globulin (IgG anti-D) prevents mothers from forming anti-D



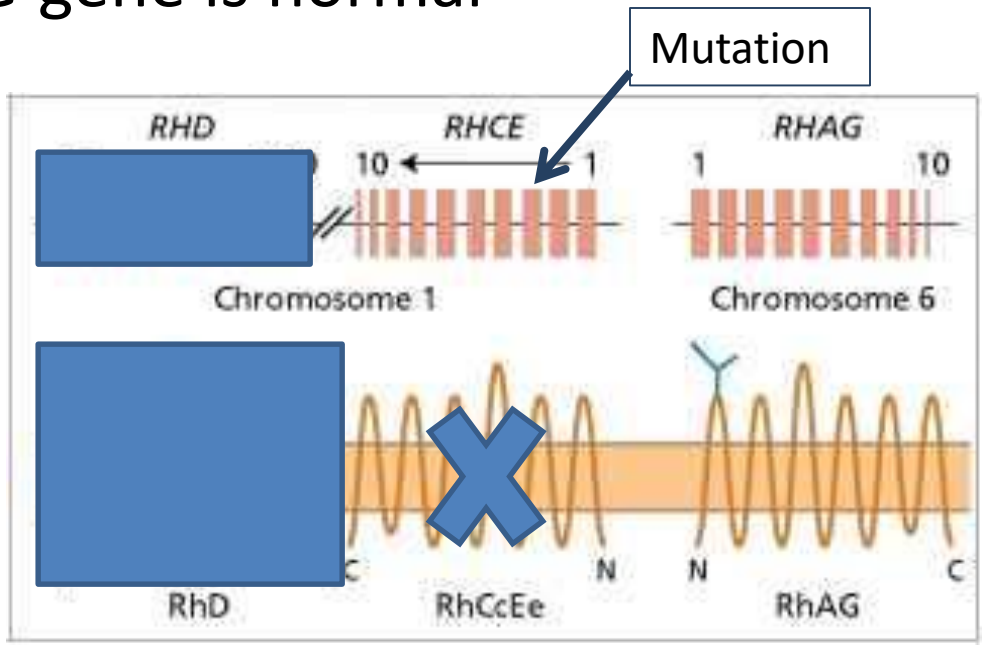
Rh null

- No expression of Rh antigens
- Must receive Rh_{null} RBCs for transfusion
- Regular type Rh_{null} - *RHAG* gene mutation
  - No RHAG protein expression
  - Therefore no RHD or RhCE expression



Rh null

- Amorphic type Rh_{null}
  - Mutation in both *RHCE* genes inherited
  - Common deletion of *RHD* gene
  - *RHAG* gene is normal

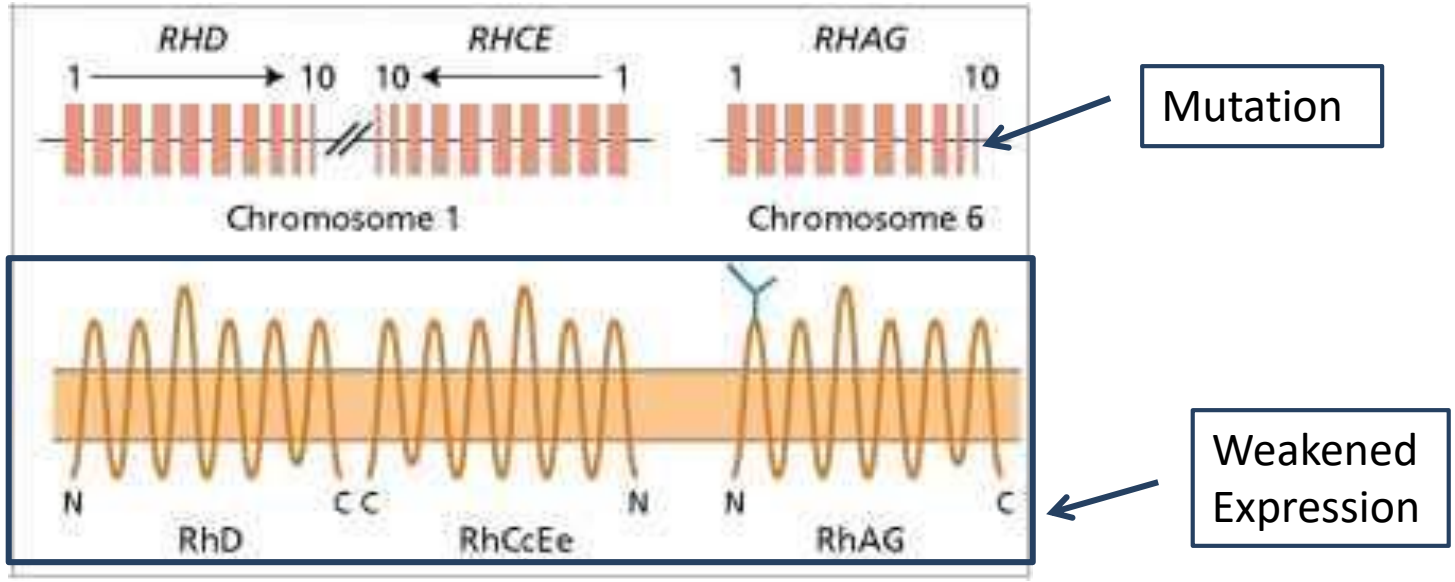


Symptoms of Rh null

- Mild compensated hemolytic anemia
- Reticulocytosis
- Stomatocytosis
- Increase Hgb F
- Decrease serum haptoglobin
- Sometimes elevated bilirubin

Rh_{mod}

- Partial suppression of *RH* gene expression
- Cause: mutations in *RHAG* gene
- Less severe clinical symptoms than Rh_{null}



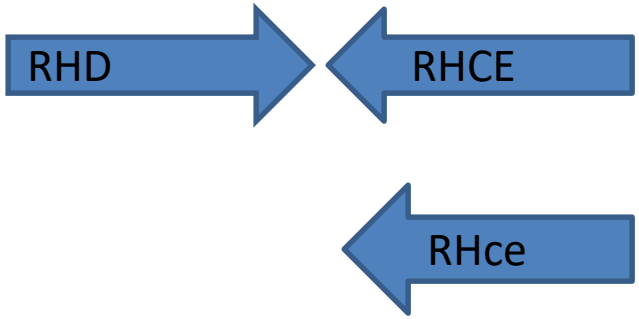
C^w Antigen

- Low prevalence antigen- occur in <1% of individuals
- Antithetical to high-prevalence MAR antigen
- Single amino acid change in *RhCe*
- 2% of whites positive
- Very rare in African descent

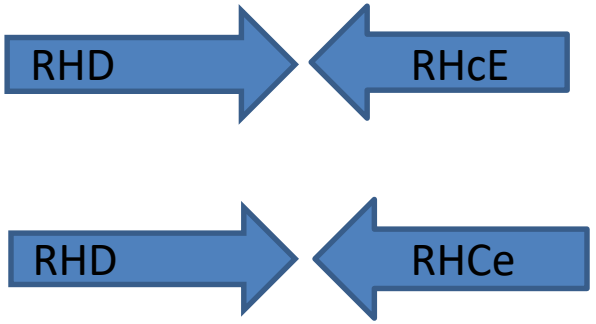
f(ce) Antigen

- Expressed when c and e are present on same haplotype (cis position)
- Expressed on Rhce protein
- Give c or e negative RBCs for transfusion

Cis- can make f antigen



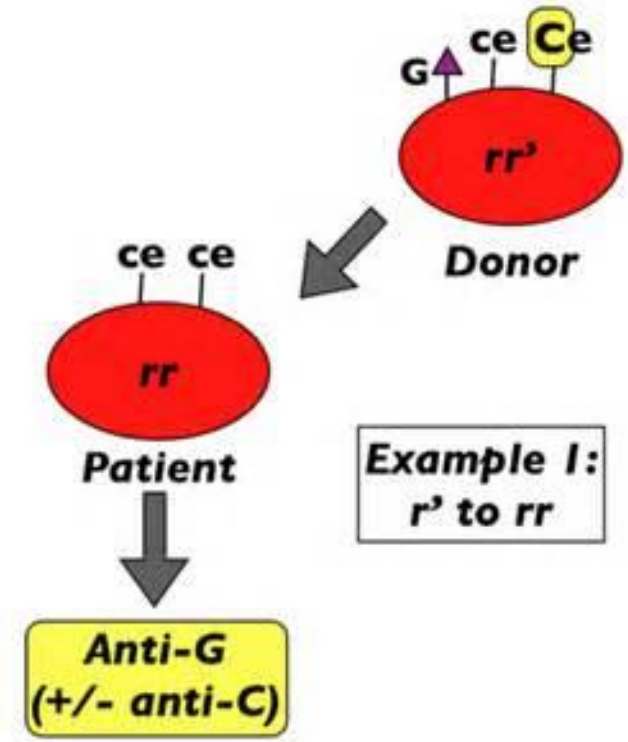
Trans- cannot make f antigen



Same phenotype: D+C+E+c+e+

G Antigen

- Present on most D+ and all C+ RBCs
- 1 amino acid change on RHD, RHCe, or RHCE proteins
- Reacts as combination of anti-C and anti-D
- Transfuse D and C neg. RBCs
- Important to distinguish D, C, and G in OB patients
  - Determine if Rhogam is necessary



e Variants

- Similar qualities to those with partial-D
- Can phenotype e positive, still make anti-e
- 2 altered RHCE genes
- Mutated e antigen

V and VS

- Low prevalence antigens in Caucasians
- 30-32% of African Americans are positive
- Mutation in Rhce

Deletion Phenotype

- Very uncommon phenotypes
- No Cc and/or Ee reactivity
- Unusually strong D expression
- Phenotype written as D-
- D-/D- people make anti-Rh17 or anti-Hr_0
- If antibodies present, must give D- RBCs

LW System

- Phenotypically similar with Rh system
- Frequently presents as autoantibody

Reactions with various RBCs:

	D+ RBCs	D Neg RBCs	Rh null RBCs	Cord cells
Anti-LW	Strongly	Weakly or not at all	None	Equal reactivity
Anti-D	Strongly	None	None	Yes with + no with -

Cleveland Clinic

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