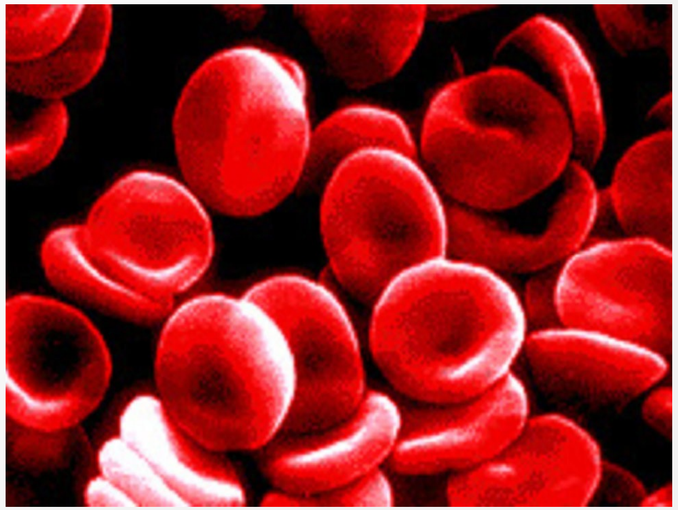
Transfusion Medicine

Immunohematology I

D. Joe Chaffin, MD

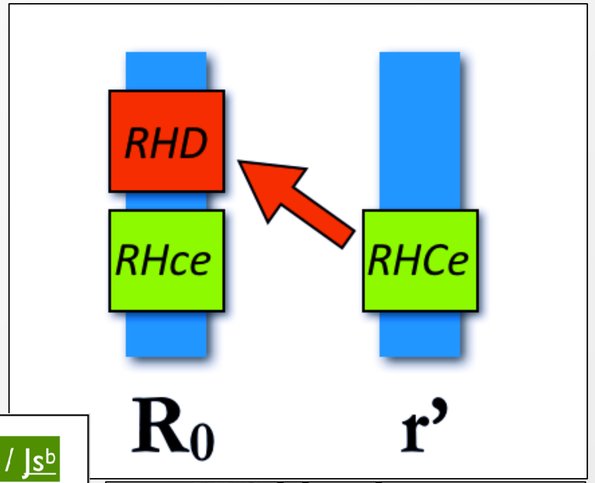


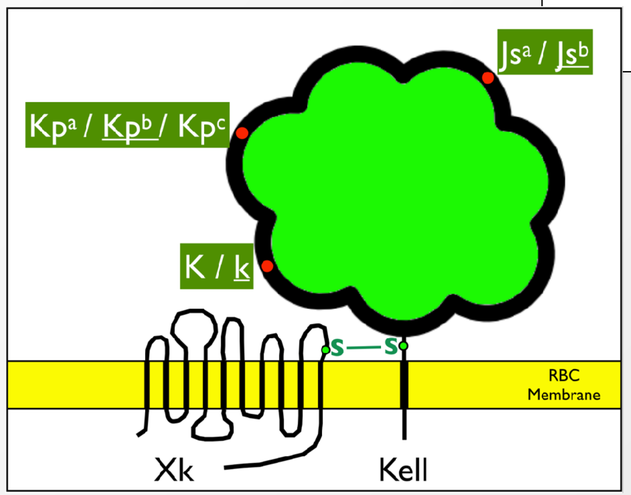
What to Expect

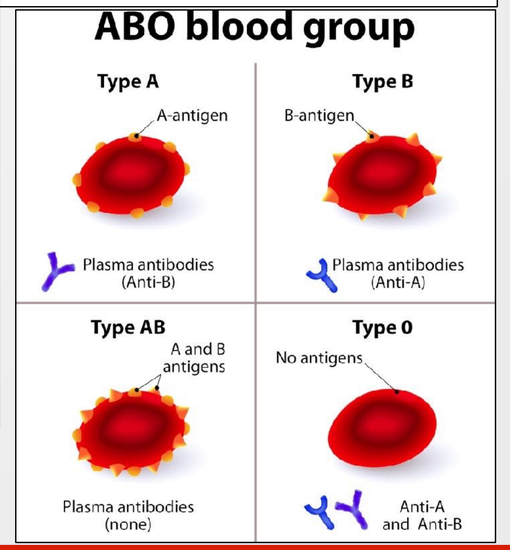
- Today: Immunohematology I
- 1/22: Immunohematology II (PT testing, Ab ID)
  - Interactive session!
- 2/5: Blood Products and Their Uses
- TBD: Transfusion Reactions

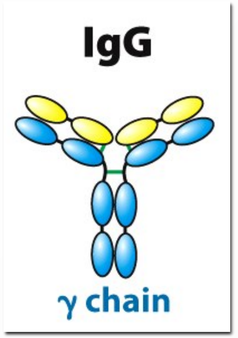
Immunohematology I

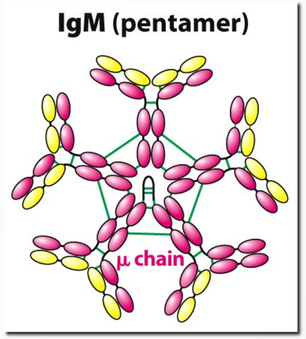
- Basic antigen-antibody testing
  - Basic tests
  - Principles to know
- Blood Groups

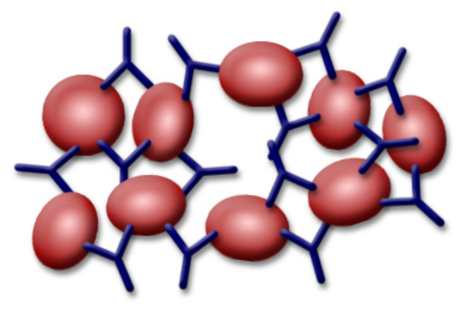












Best at body temp (“warm”)	Best below body temp (“cold”)
Coats RBCs	Agglutinates/lyses RBCs
Pregnancy, transfusion	“Naturally occurring”
Crosses placenta (HDFN)	Does not cross placenta (no HDFN)
Protein-rich antigens	Carbohydrate-rich antigens

Basic Reactions

4+ → 0

Tube

Solid phase

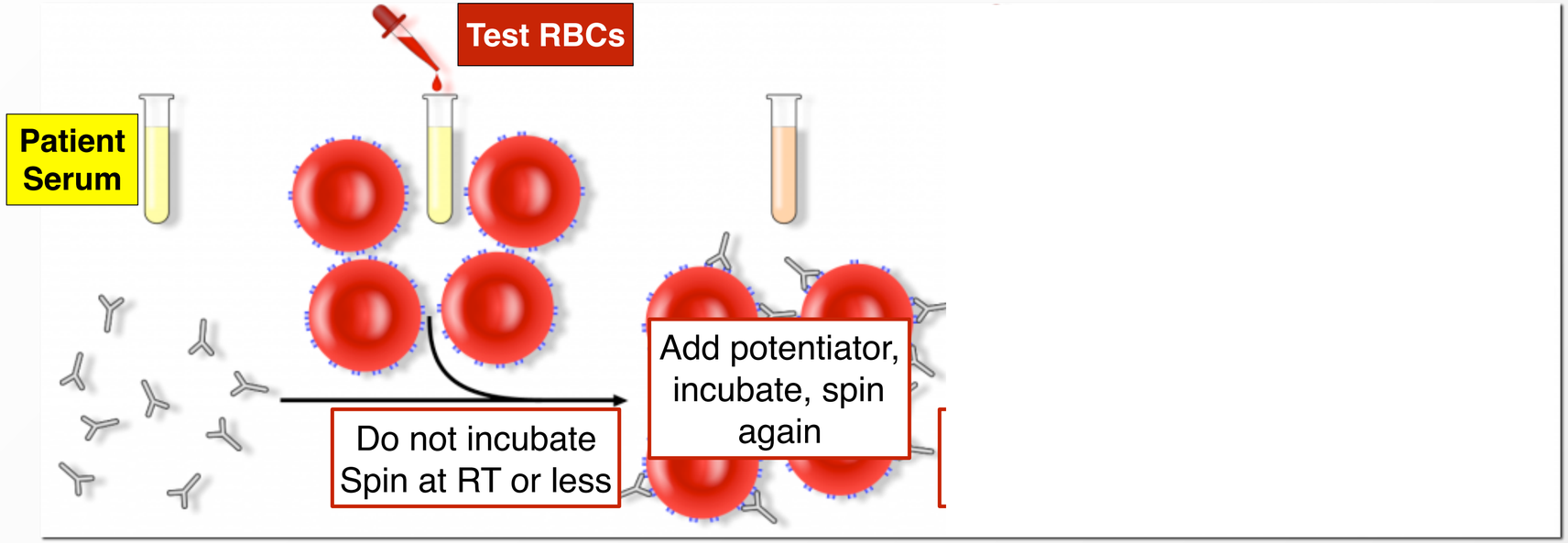
Gel
(column agglutination)

Three "Phases":

- Immediate Spin
- 37C Phase
- AHG Phase

Tube Testing

Immediate Spin Phase

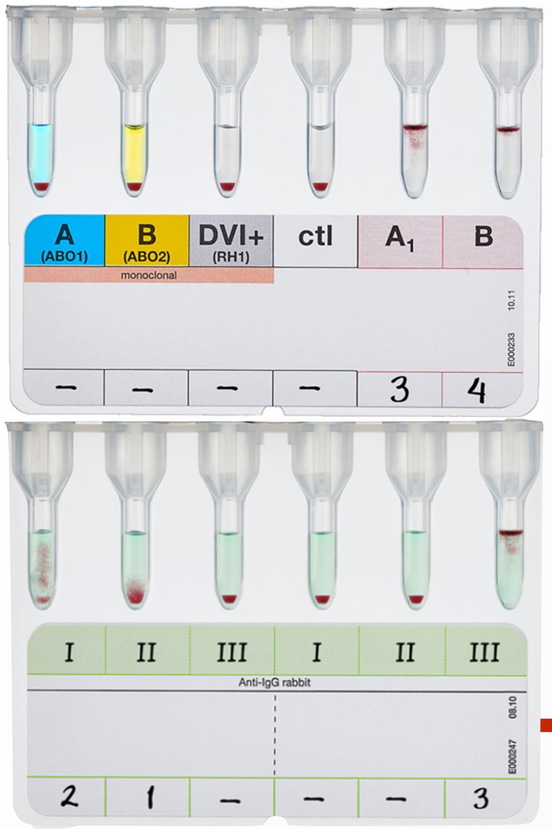


37C Phase

AHG Phase

Lewis			Results		
Le^a	Le^b	Cell	IS	37	AHG
0	+	1	0	1+	3+
0	+	2	0	1+	3+
0	0	3	0	1+	3+
0	0	4	0	1+	3+
+	0	5	0	0	0 ✓
+	0	6	0	0	0 ✓
0	+	7	0	0	0 ✓
+	0	8	0	0	0 ✓
0	+	9	0	0	0 ✓
+	0	10	0	0	0 ✓
0	+	11	0	1+	3+
		AC			

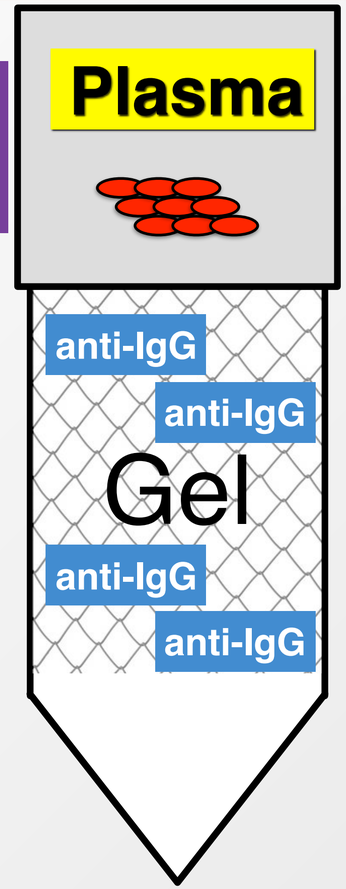
Gel Testing



3+ 2+ 1+

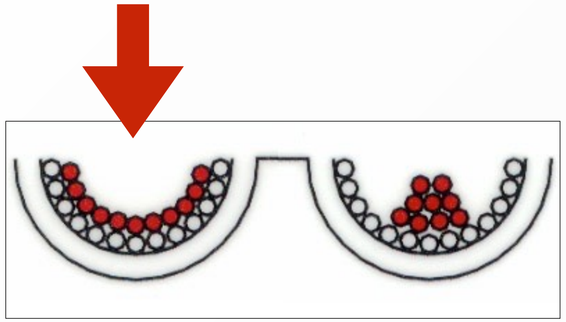
Negative

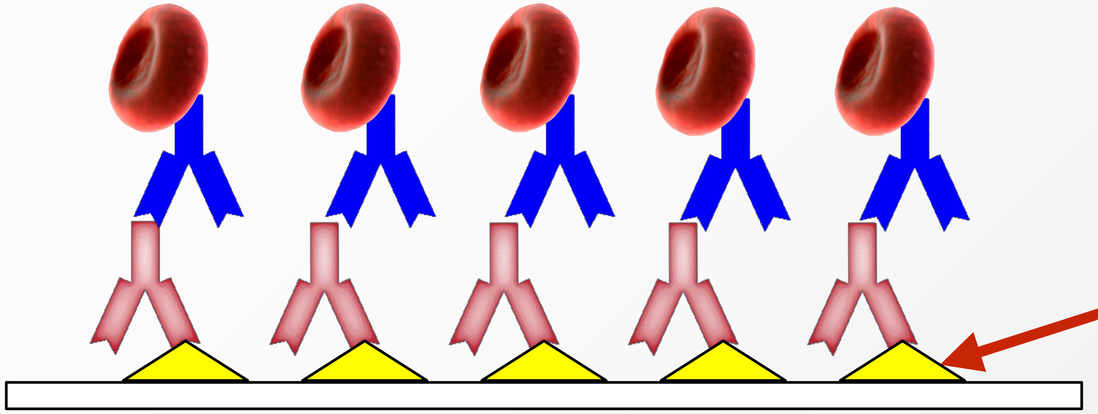
Incubate & Spin



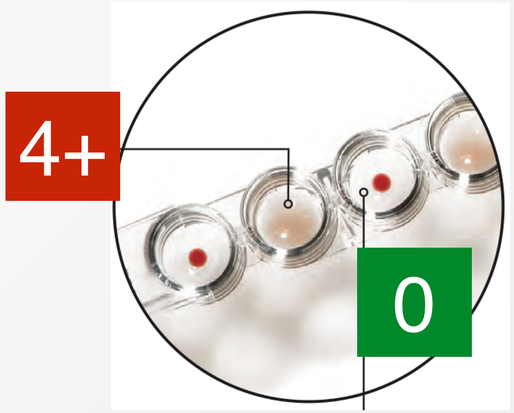
Images Courtesy of Bio-Rad

Solid Phase Testing

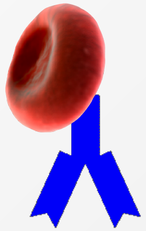




Microwell



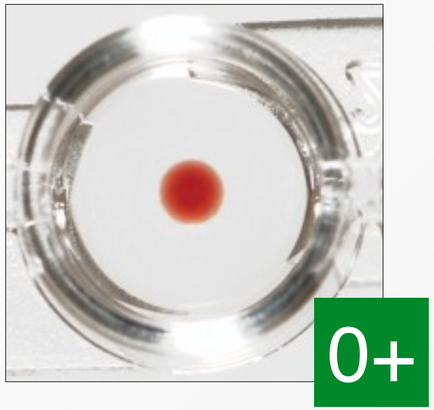
Patient antibody



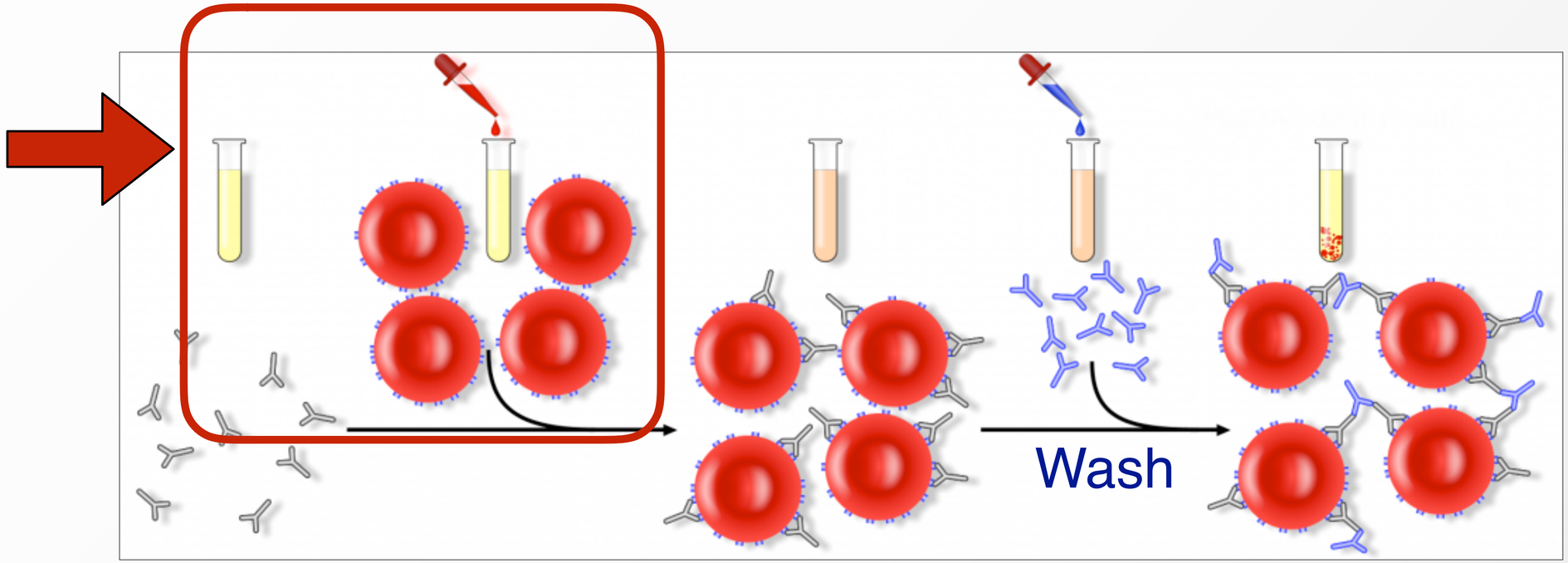
Indicator RBC
with anti-IgG

Image: Courtesy of Immucor

Solid Phase Testing



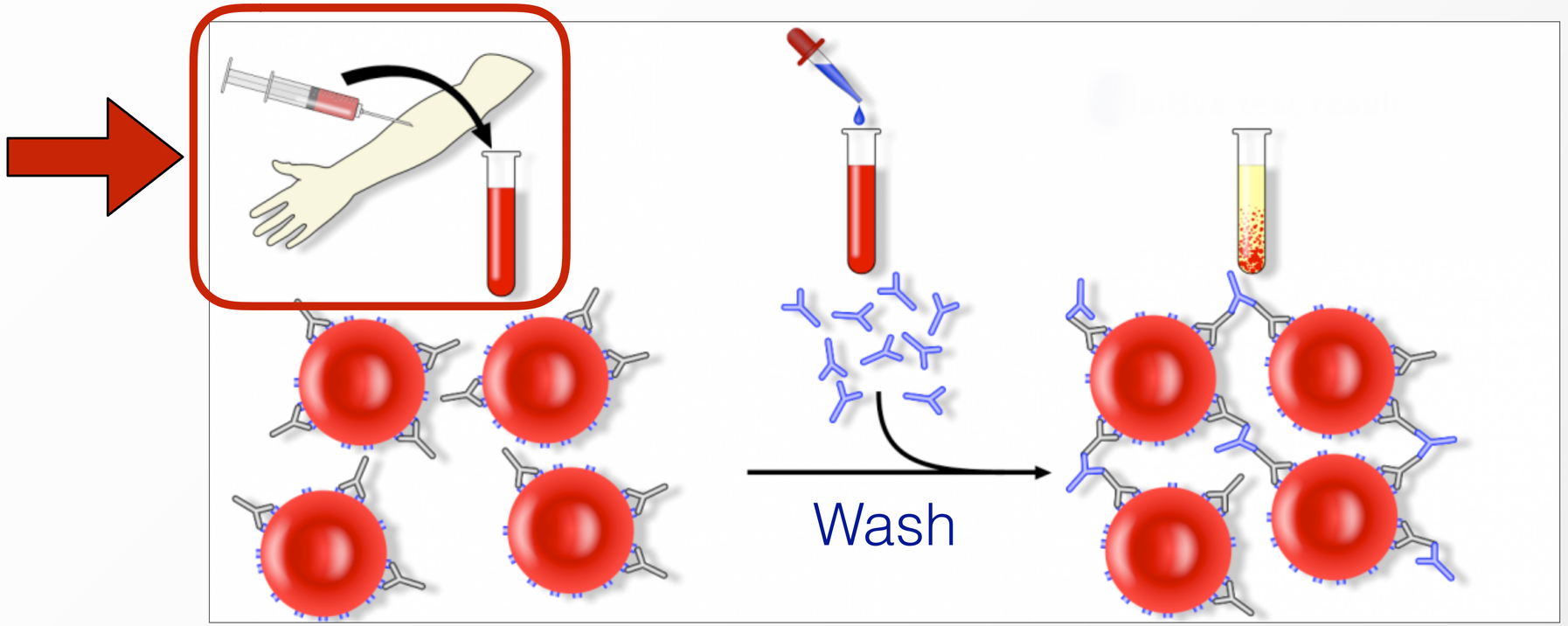
Indirect Antiglobulin Test



IAT: Antibody coating happens in test system

Image credit: Wikipedia Commons, A. Rad, 2006

Direct Antiglobulin Test



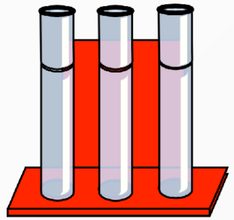
IAT: Antibody coating happens in test system

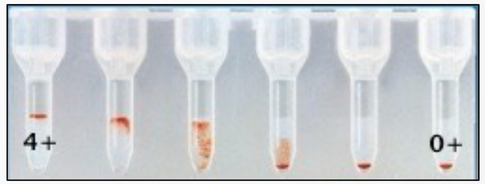
DAT: Antibody coating happens in the body

Image credit: Wikipedia Commons, A. Rad, 2006

Types of Antiglobulin

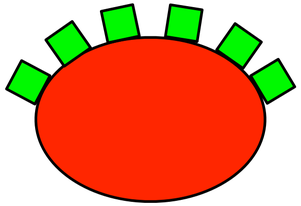
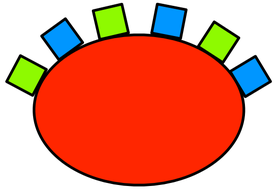
- **Anti-IgG, -C3d**
  - "Polyspecific"
- **Anti-IgG**
- **Anti-C3d**
  - IgM hemolysis, CAD





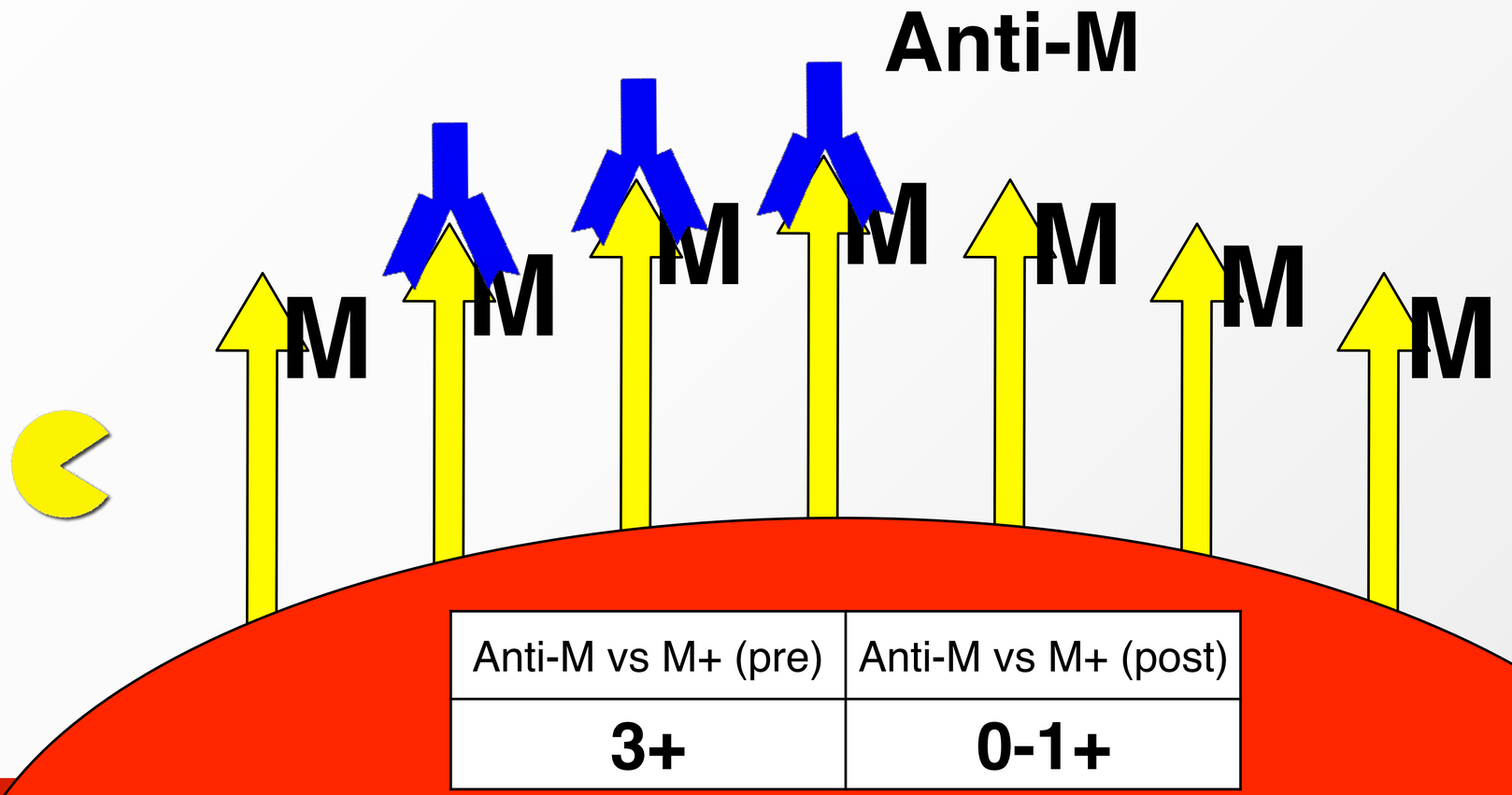
Dosage

Kidd
Duffy
Rh

Antibody	RBCs	Reaction
Anti-Fy^a	Fy(a+b–) 	3+
Anti-Fy^a	Fy(a+b+) 	0-1+

Proteolytic Enzymes

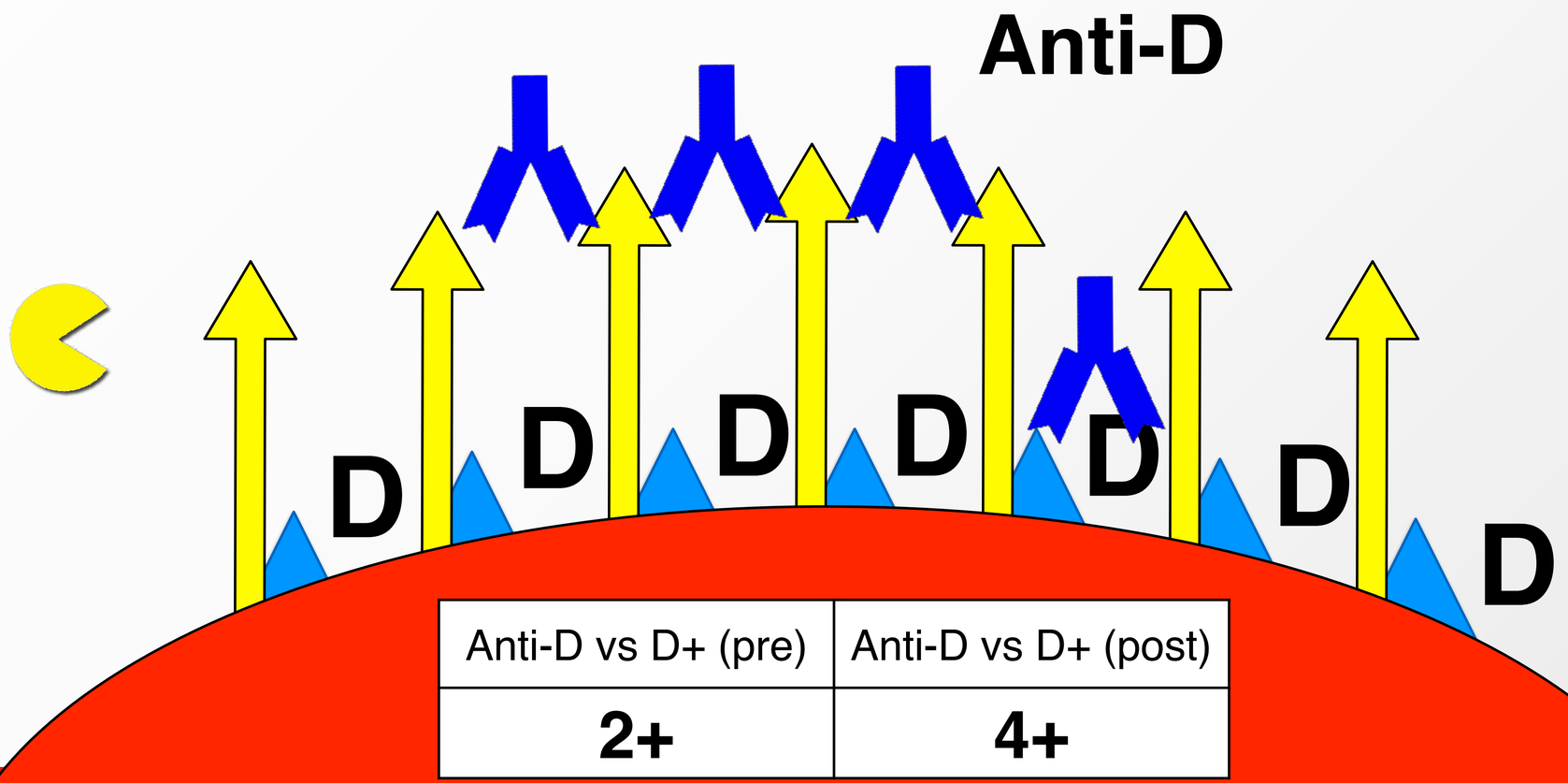
Papain/ficin/bromelain cleave proteins



Anti-M vs M+ (pre)	Anti-M vs M+ (post)
3+	**0-1+**

Proteolytic Enzymes

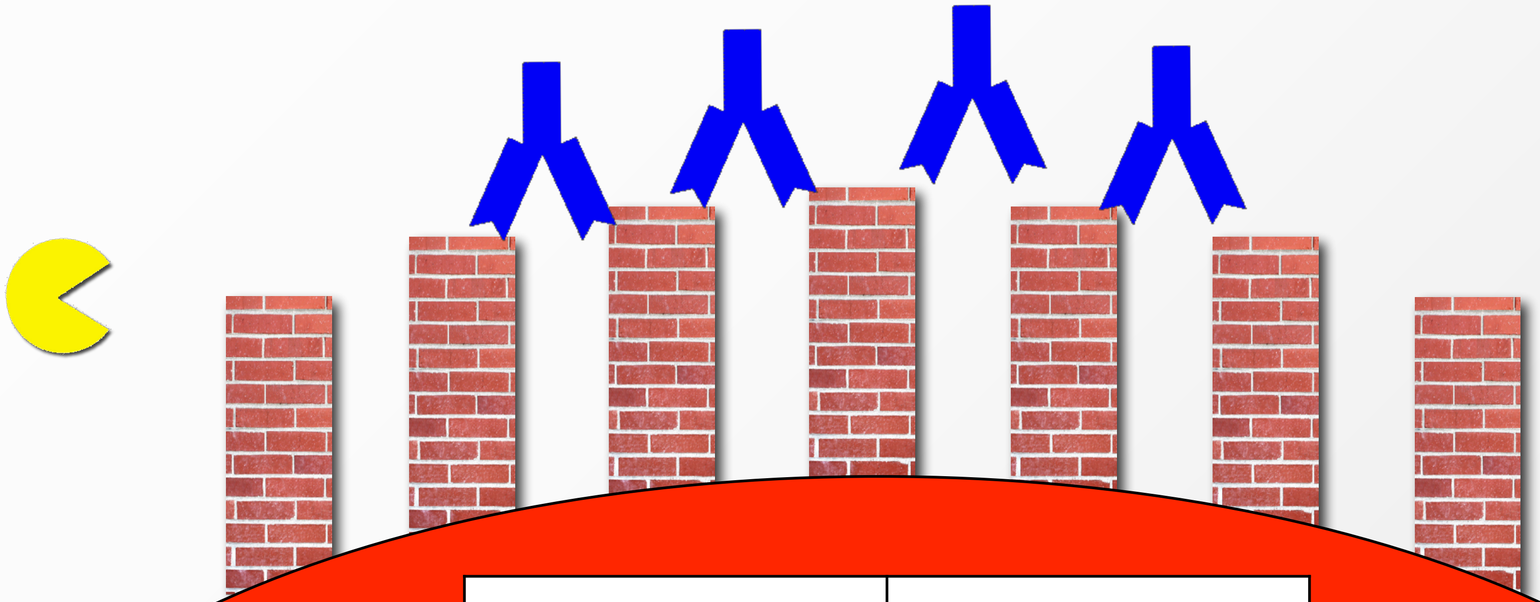
Papain/ficin/bromelain cleave proteins



Anti-D vs D+ (pre)	Anti-D vs D+ (post)
2+	**4+**

Proteolytic Enzymes

Papain/ficin/bromelain cleave proteins



Anti-K vs K+ (pre)	Anti-K vs K+ (post)
3+	**3+**

Table of blood group antigens v.8.1_181111

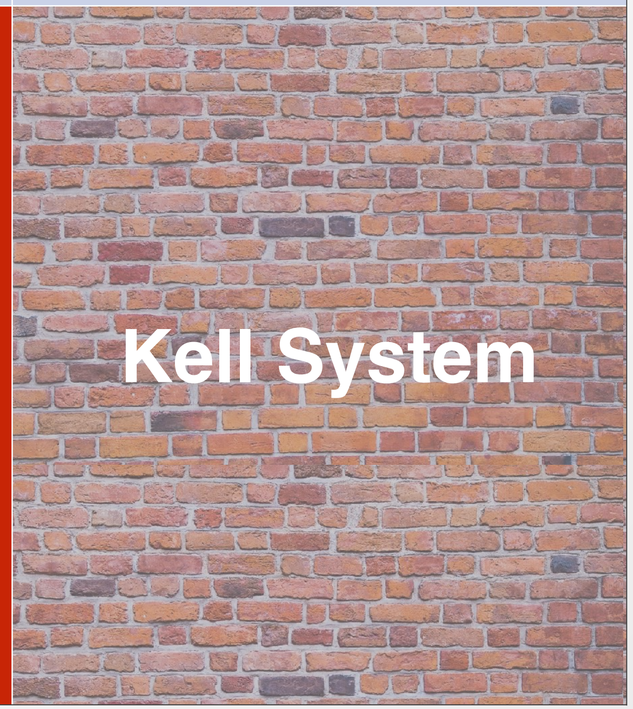
System		Antigen number		
		001	002	003
001	ABO	A	B	A,B
002	MNS	M	N	S
003	P1PK	P1	...	P^k
004	RH	D	C	E
005	LU	Lu^a	Lu^b	Lu3
006	KEL	K	k	Kp^a
007	LE	Le^a	Le^b	Le^{ab}
008	FY	Fy^a	Fy^b	Fy3
009	JK	Jk^a	Jk^b	Jk3
010	DI	Di^a	Di^b	Wr^a
011	YT	Yt^a	Yt^b	YTEG
012	XG	Xg^a	CD99	
013	SC	Sc1	Sc2	Sc3
014	DO	Do^a	Do^b	Gy^a
015	CO	Co^a	Co^b	Co3
016	LW	...	...	...
017	CH/RG	Ch1	Ch2	Ch3
018	H	H		
019	XK	Kx		

Table of blood group antigens v.8.1_181111 2(4)

System		Antigen number												Total in system
		001	002	003	004	005	006	007	008	009	010	011	012	
020	GE							An^a	Dh^a	GEIS	GEPL	GEAT	GETI	11
021	CROM							IFC	WES^a	WES^b	UMC	GUTI	SERF	20
022	KN							Sl2	Sl3	KCAM				9
023	IN													6
024	OK													3
025	RAPH													1
026	JMH						Q							6
027	I													1
028	GLOB													2
029	GIL													1
030	RHAG													3
031	FORS													1
032	JR													1
033	LAN													1
034	VEL													1
035	CD59													1
036	AUG													4

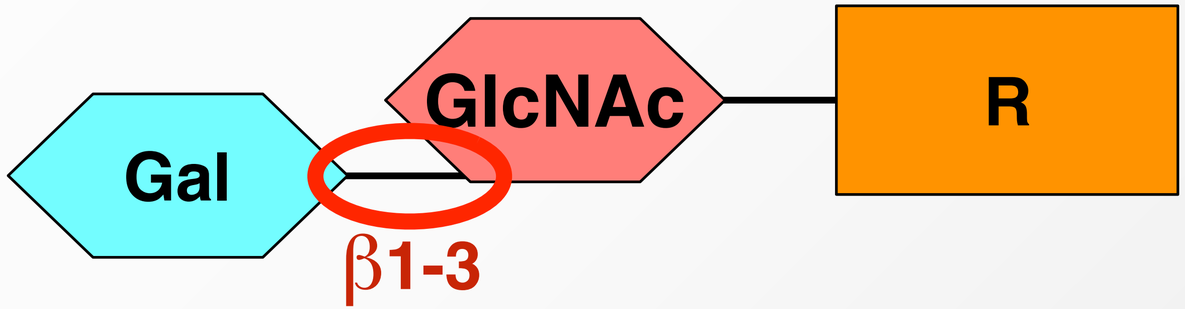
Source: International Society for Blood Transfusion (ISBT); Accessed Jan 2019

Enzyme Classification

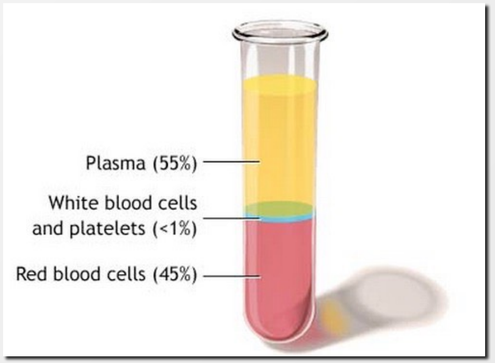
Enhanced	Decreased	Unaffected
ABO-related -ABO/H -Lewis -I -P1PK/GLOB Rh System Kidd System	MNS System Duffy System	

ABO-related Systems

- Type 1 chains

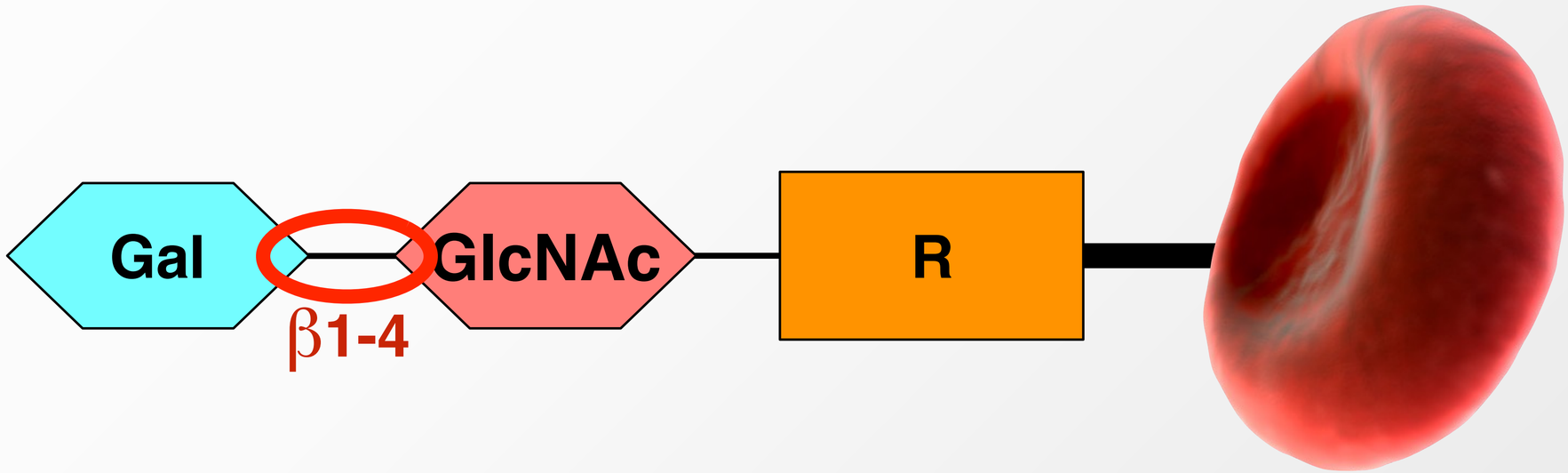


Secretions, primarily glycoprotein
Plasma, primarily glycolipid



ABO-related Systems

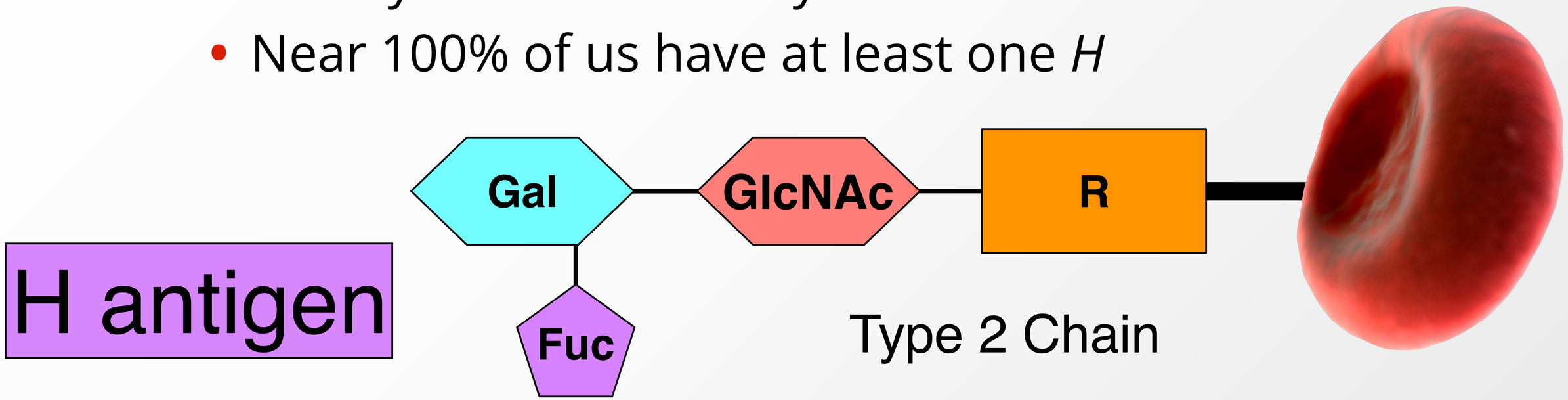
- Type 2 chains



RBC Membranes, <u>glycoprotein/glycolipid</u>

H (FUT1)

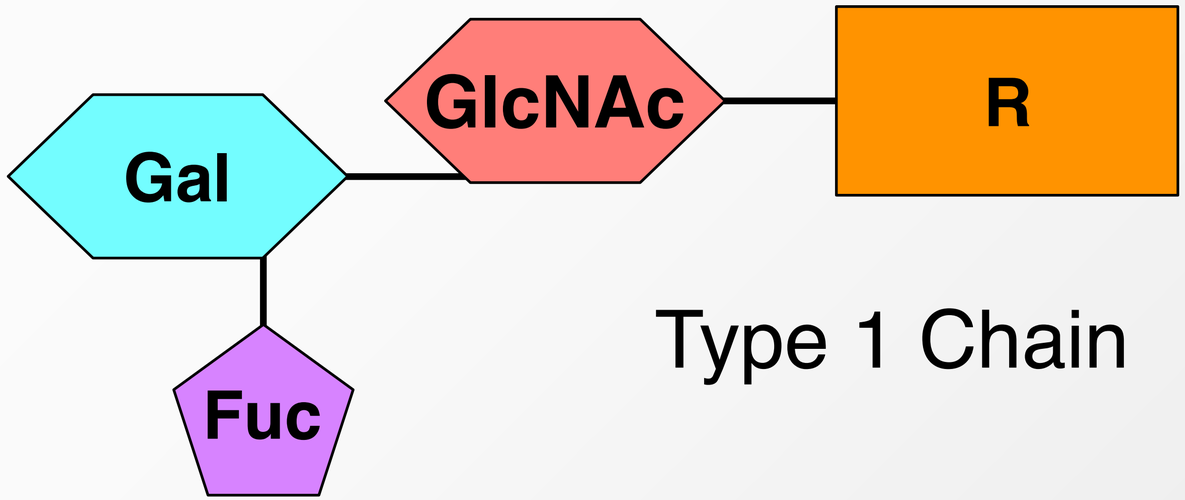
- *H* and *h* alleles
- Fucosyltransferase enzyme
- Near 100% of us have at least one *H*



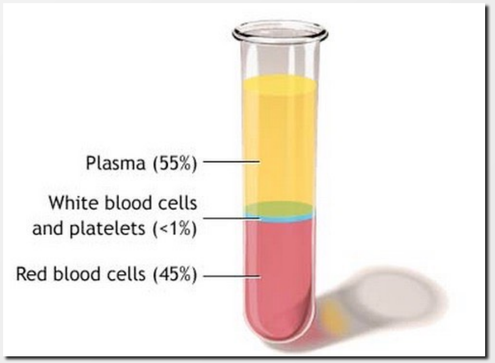
Se (FUT2) - "Secretor"

- *Se* and *se* alleles
- Fucosyltransferase enzyme
- 80% of us can make H in secretions

H antigen

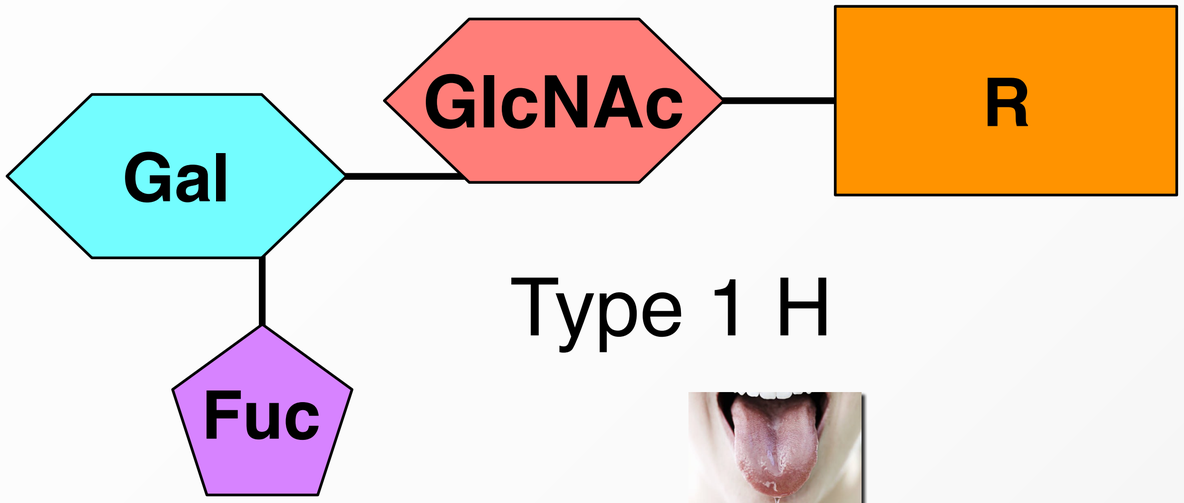


Type 1 Chain

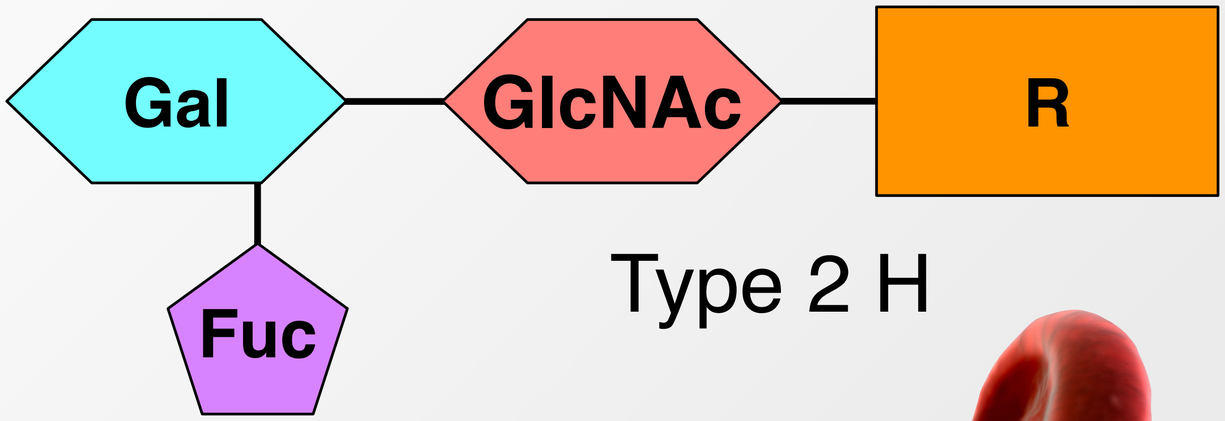


H Antigen

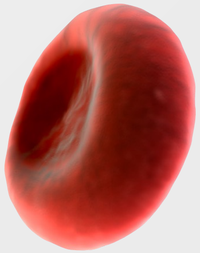
- Precursor to A or B - Just add sugar!



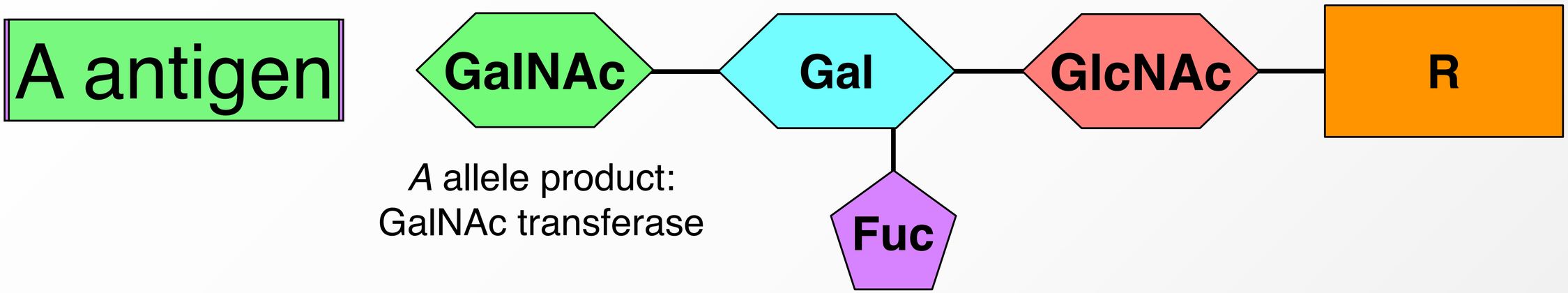
Type 1 H



Type 2 H

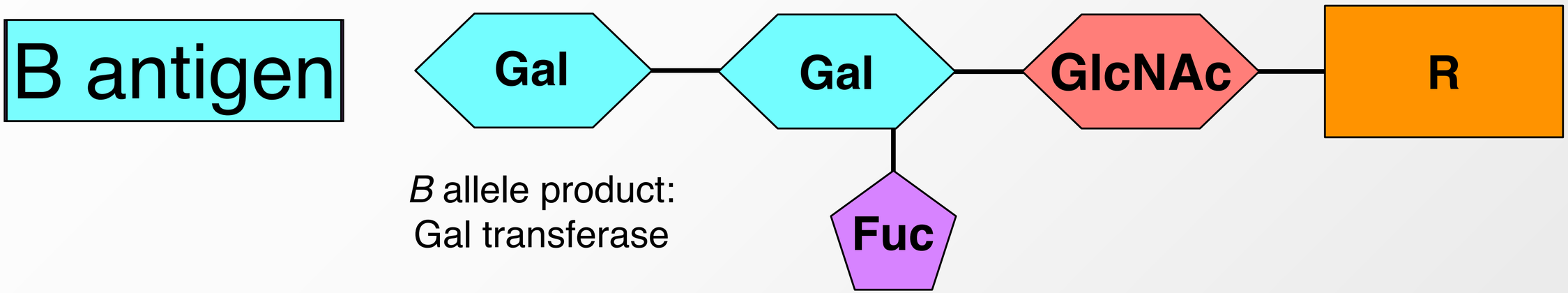


Blood Group A



A allele product:
GalNAc transferase

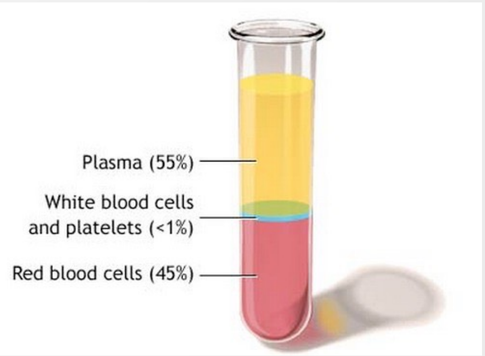
Blood Group B



B allele product:
Gal transferase

ABO Antigens

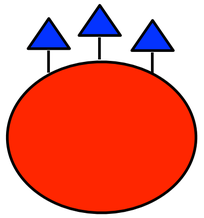
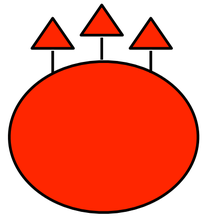
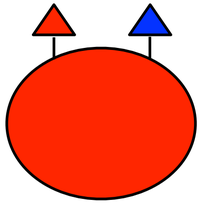
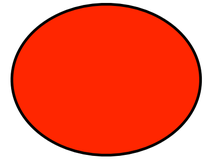
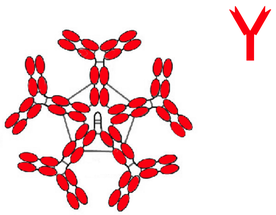
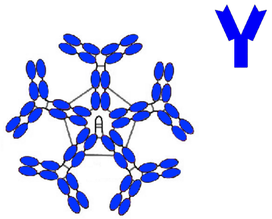
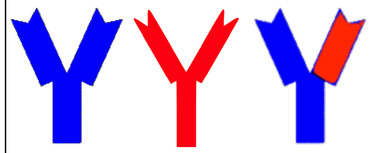
- Antigens start early (6 weeks EGA)
  - Still poorly formed at birth
  - Adult levels by age 4
- Antigens are not limited to RBCs
  - Platelets
  - Endothelial and epithelial cells
    - Lung, GI tract, heart, kidney, etc.
  - Plasma and secretions

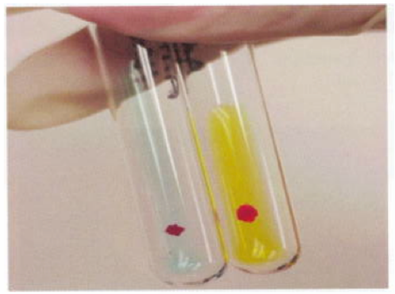


ABO Antibodies

- Babies have only Mom's IgG
  - Best from Group O
- Production by 6 months of age
- Adult levels by age 10

“Landsteiner’s Law”

	A	B	AB	O
Antigens	 A antigen	 B antigen	 A, B antigens	 None
Antibodies	 Anti-B	 Anti-A	None	 Anti-A Anti-B Anti-A,B



ABO Testing

Cell Group		Serum Group		ABO Interp.
Anti-A	Anti-B	A1 RBC	B RBC	
4+	0	0	4+	A
0	4+	4+	0	B
4+	4+	0	0	AB
0	0	4+	4+	O

Images: Harmening D "Modern Blood Banking & Transfusion Practices"

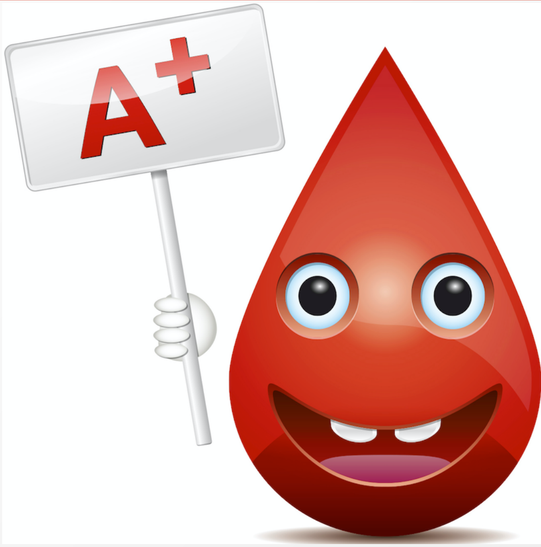
ABO Types by Race

Type	Whites	Blacks	Asians	Native Americans
O	45%	49%	40%	79%
A	40%	27%	28%	16%
B	11%	20%	27%	4%
AB	4%	4%	5%	<1%

Group O

- Most common across racial lines
- Antigen = H
  - *Ulex europaeus* lectin
- 3 mostly IgG antibodies = anti-A, anti-B, anti-A,B
  - Most common form of HDFN 
  - Antibodies tend to be stronger than grp A or B

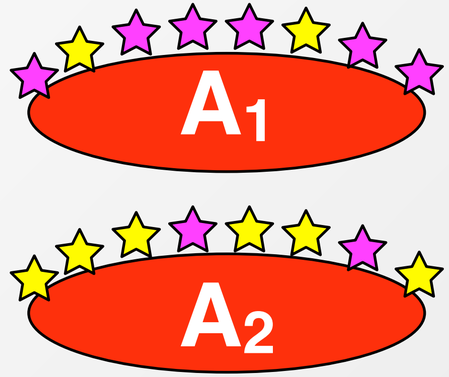
Group A



- Antigens = A, H
  - Varying amounts depending on subgroup
- Antibody = anti-B
  - Naturally occurring IgM

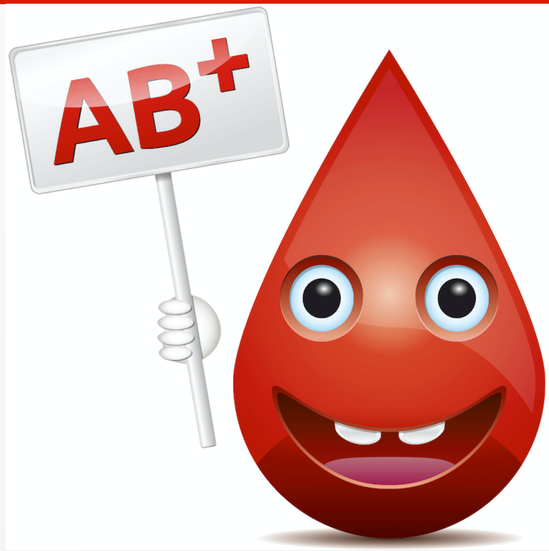
Group A Subgroups

- **A_1** (80%) and **A_2** (~20%)
  - A_1 has 5x more A antigen than A_2
  - 1-8% A_2, 25% A_2B form **anti-A_1**
    - ✓ Insignificant unless at 37 C
  - *Dolichos biflorus* lectin + with A_1 not A_2



Cell Group		Serum Group	
Anti-A	Anti-B	A1 RBC	B RBC
4+	0	1+	4+

Group AB



- Least frequent across all racial lines
  - 4%
- Antigens = A, B (little to no H)
  - Subgroups of A (A_1B, A_2B, etc.)
- Antibodies: NONE
  - “Universal recipient”

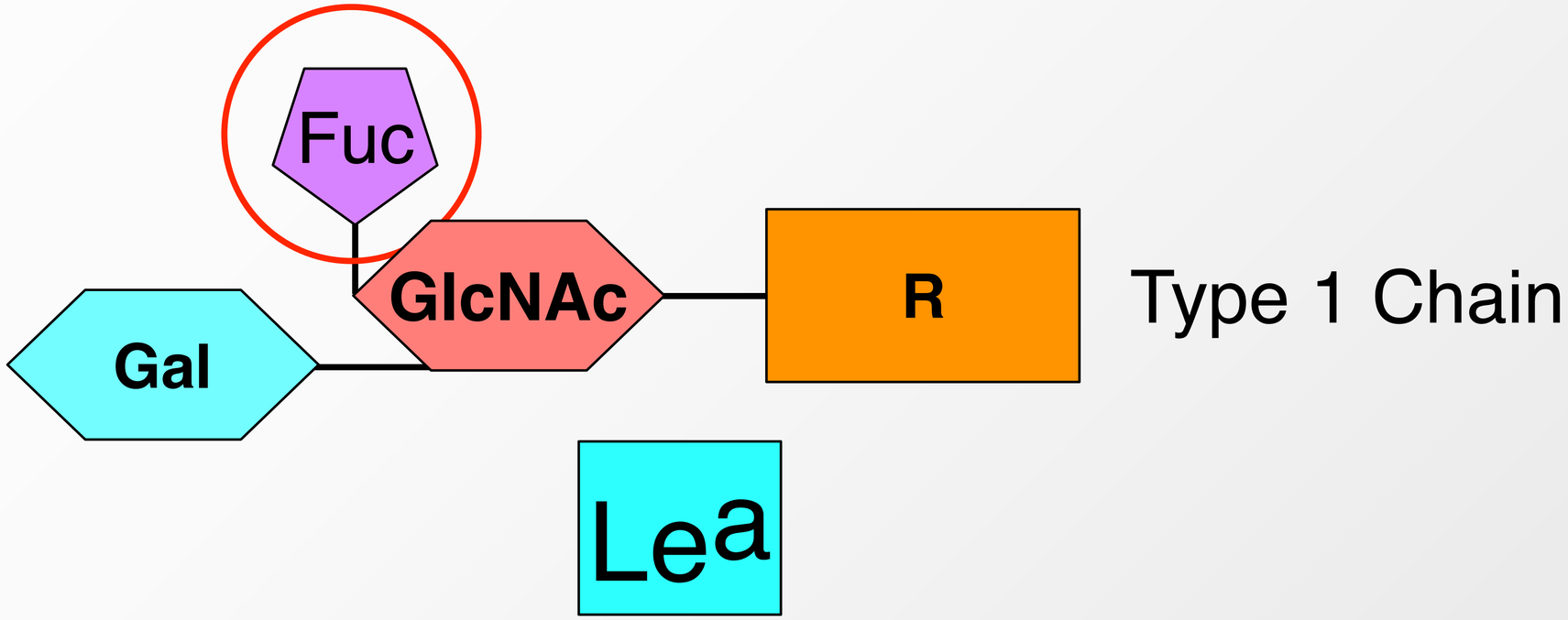
ABO Discrepancies

- Red cell grouping ≠ serum grouping
- Something's wrong with antigen testing
- Something's wrong with antibody testing
- Somebody messed up the testing

- Bottom line: **Until you KNOW, give Group O!**

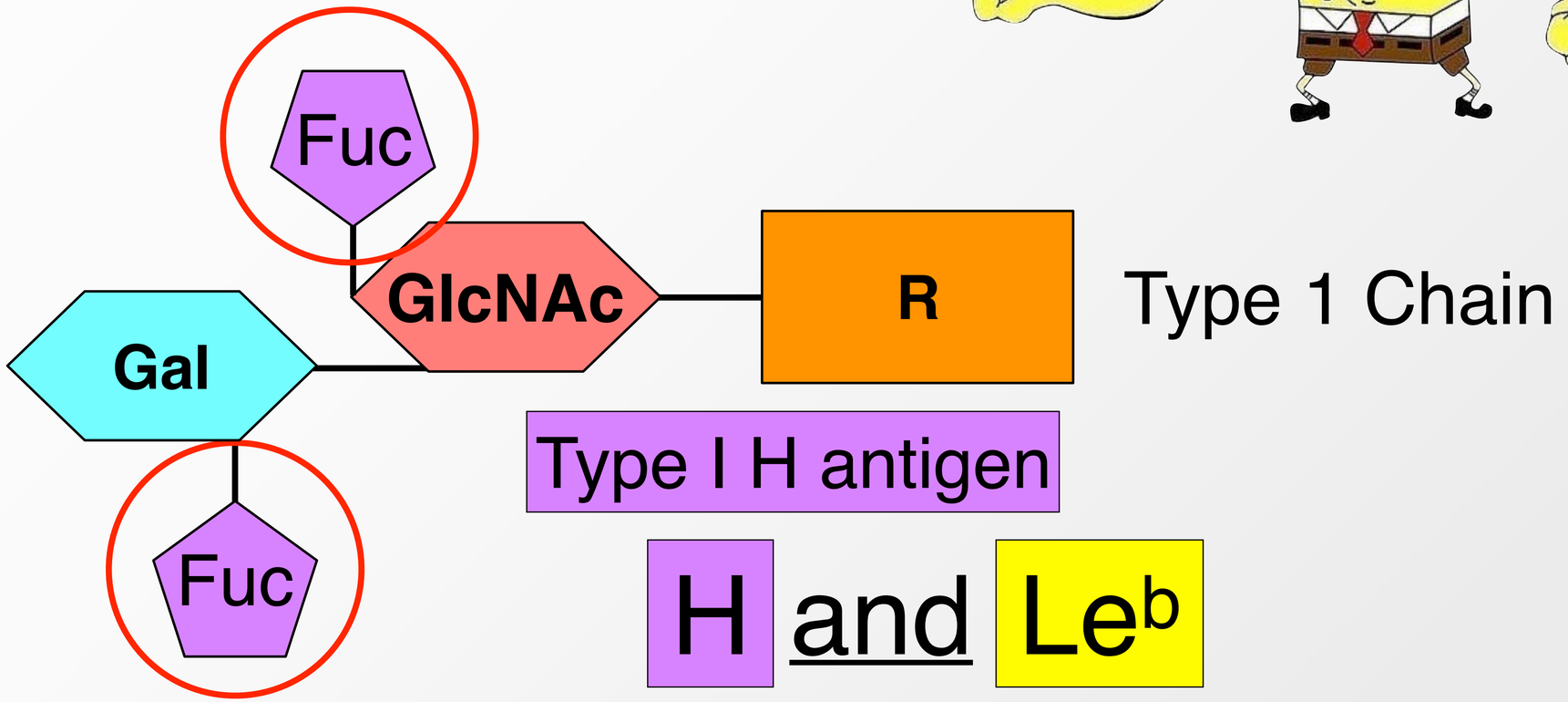
Lewis System

- *Le* allele

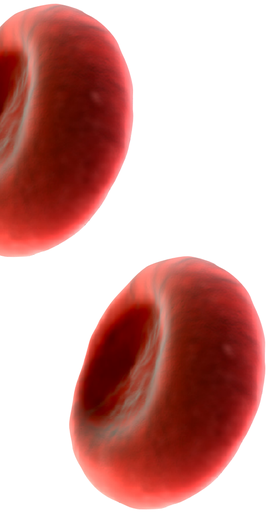


Lewis System

- Wait, *Se* works here, too!



Lewis Antigens



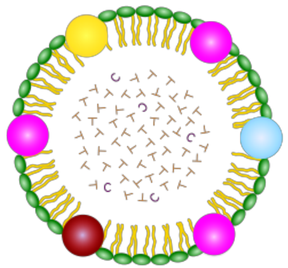
Le(a-b+)

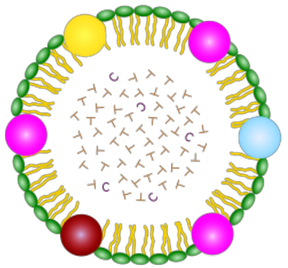
RBCs

Le^b Le^b
Le^b Le^b
Le^b Le^b
Le^b Le^b
Le^b Le^b

Le^a

Plasma Glycolipids



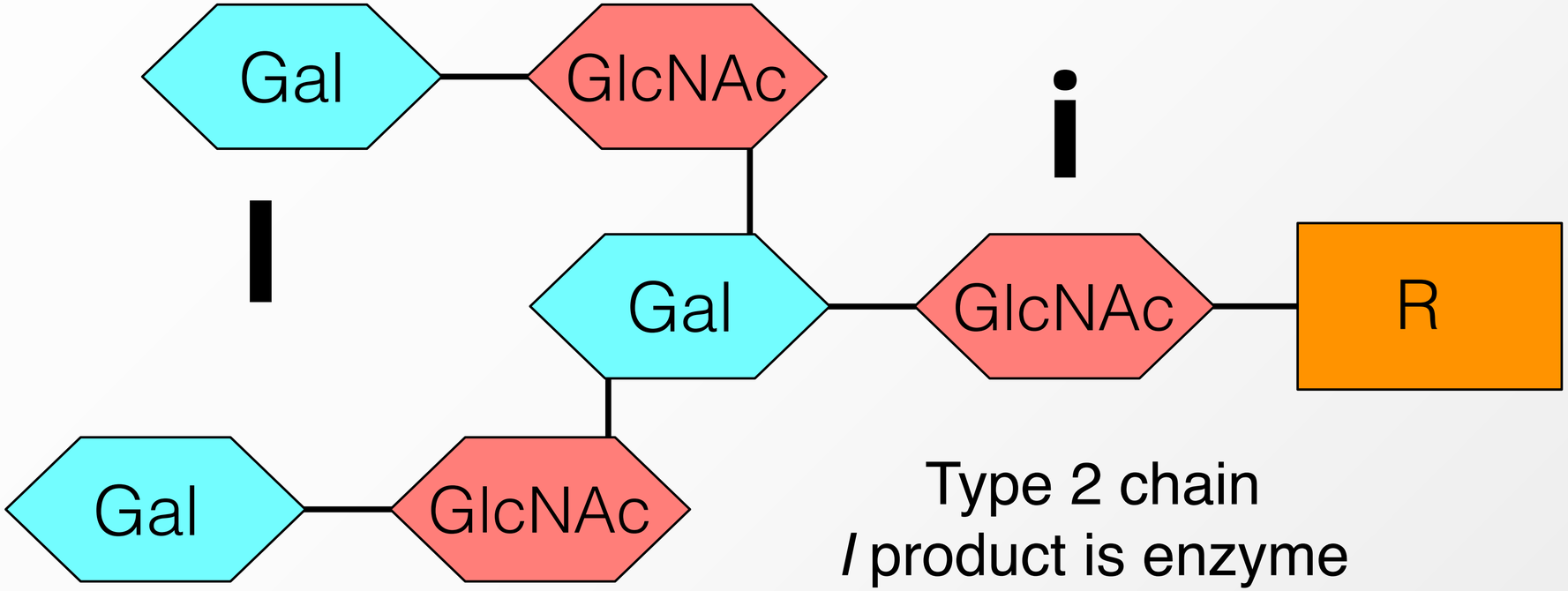


Lipoproteins

Mismatch

- Antibodies are insignificant (cold IgM)
- HTR's are rare (anti-Le^a)
- HDFN not seen
  - IgM antibodies
  - Fetal RBCs lack Le antigens
    - Cord blood is Le[a-b-]

I System



Type 2 chain
I product is enzyme

ABO antigens get stronger in parallel with I

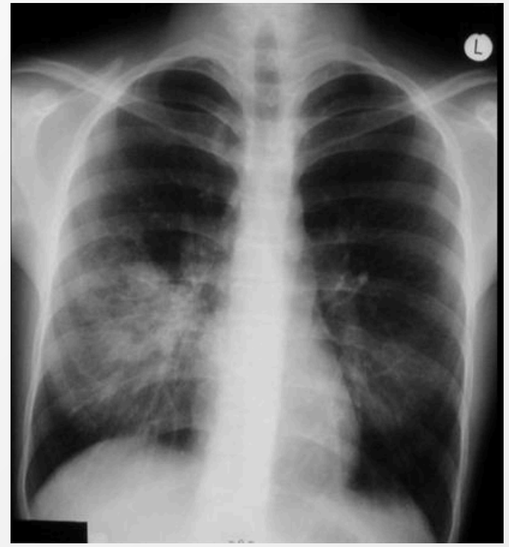
I System

I  i 

“Big I in big people, little i in little people”

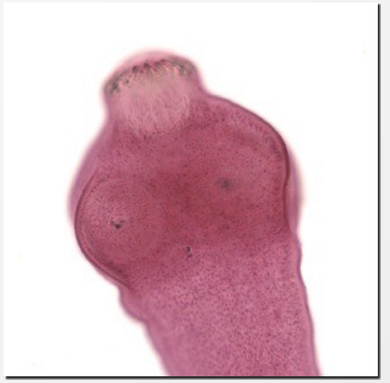
Classic Associations

- Auto-anti-I
  - *Mycoplasma* pneumonia 
  - Cold Agglutinin Disease
    - ✓ CLL, NHL, Waldenstroms
- Auto-anti-i
  - Infectious mononucleosis 
  - Cold Agglutinin Disease



P1PK/GLOB Systems

- The weirdest blood group around
- Three historic antigens:
  - P1 and P^k in P1PK system
  - P now a globoside (“GLOB”) antigen
  - Missing all 3: “p phenotype”
- P1 famous: Hydatid cysts and pigeon eggs!
- P: Parvovirus point of entry



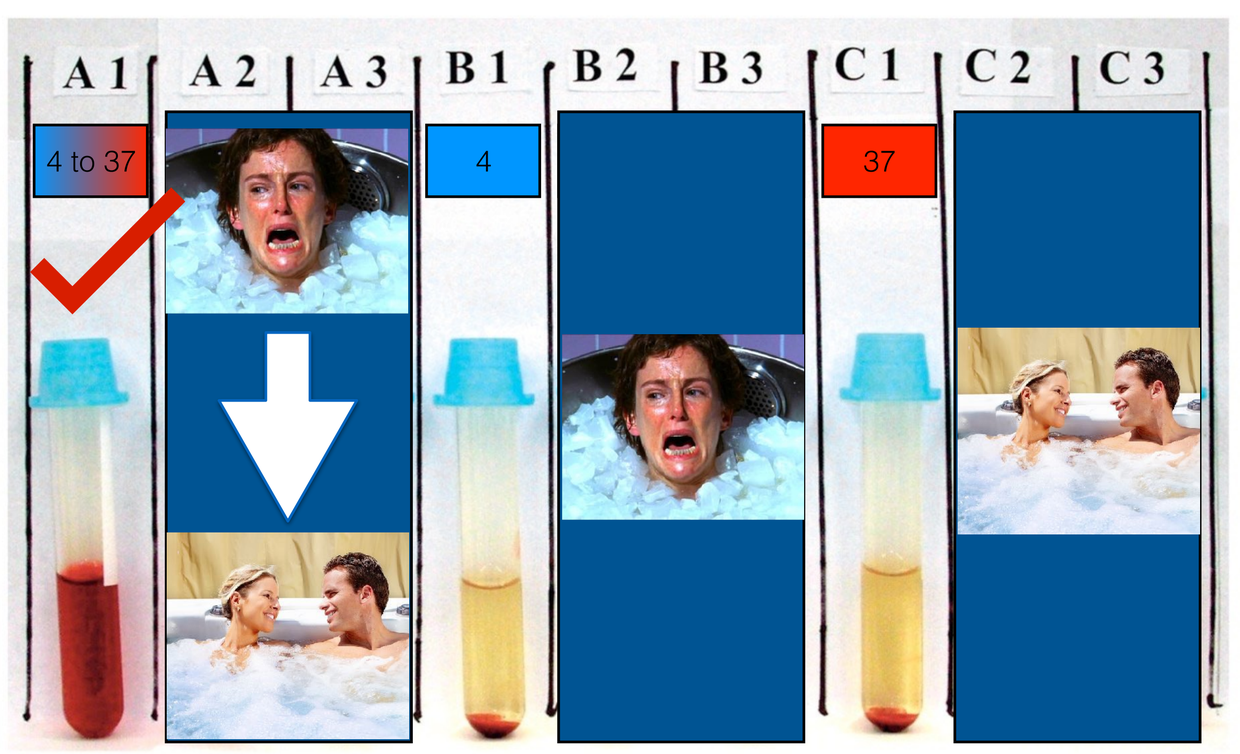
P1PK Antibodies

- Antibodies usually cold, insignificant IgMs
- Auto-anti-P: “Biphasic IgG hemolysin” (next)
- p phenotype antibodies:
  - Rare anti-PP1P^{k}
    - ✓ Acute HTRs
    - ✓ Spontaneous abortions (placenta)

P1PK Antibodies

- Paroxysmal Cold Hemoglobinuria
  - Biphasic IgG autoanti-P (Donath-Landsteiner)
    - ✓ *Clings when it's cold, hurts when it's hot*
  - Seen after viral infection in children

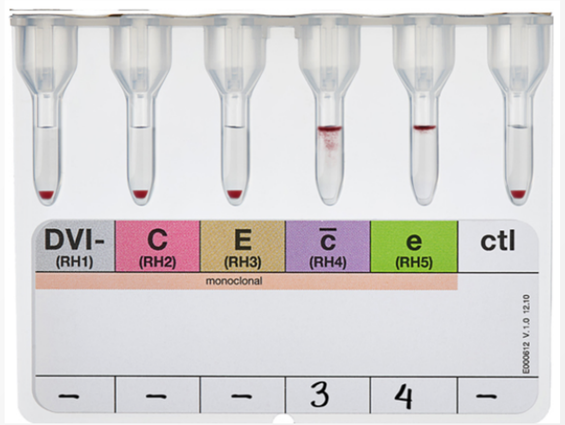
Donath-Landsteiner Test



Sanford KW and Roseff SD_Detection and significance of Donath-Landsteiner antibodies in a 5 year old female presenting with hemolytic anemia_Lab Medicine_0410

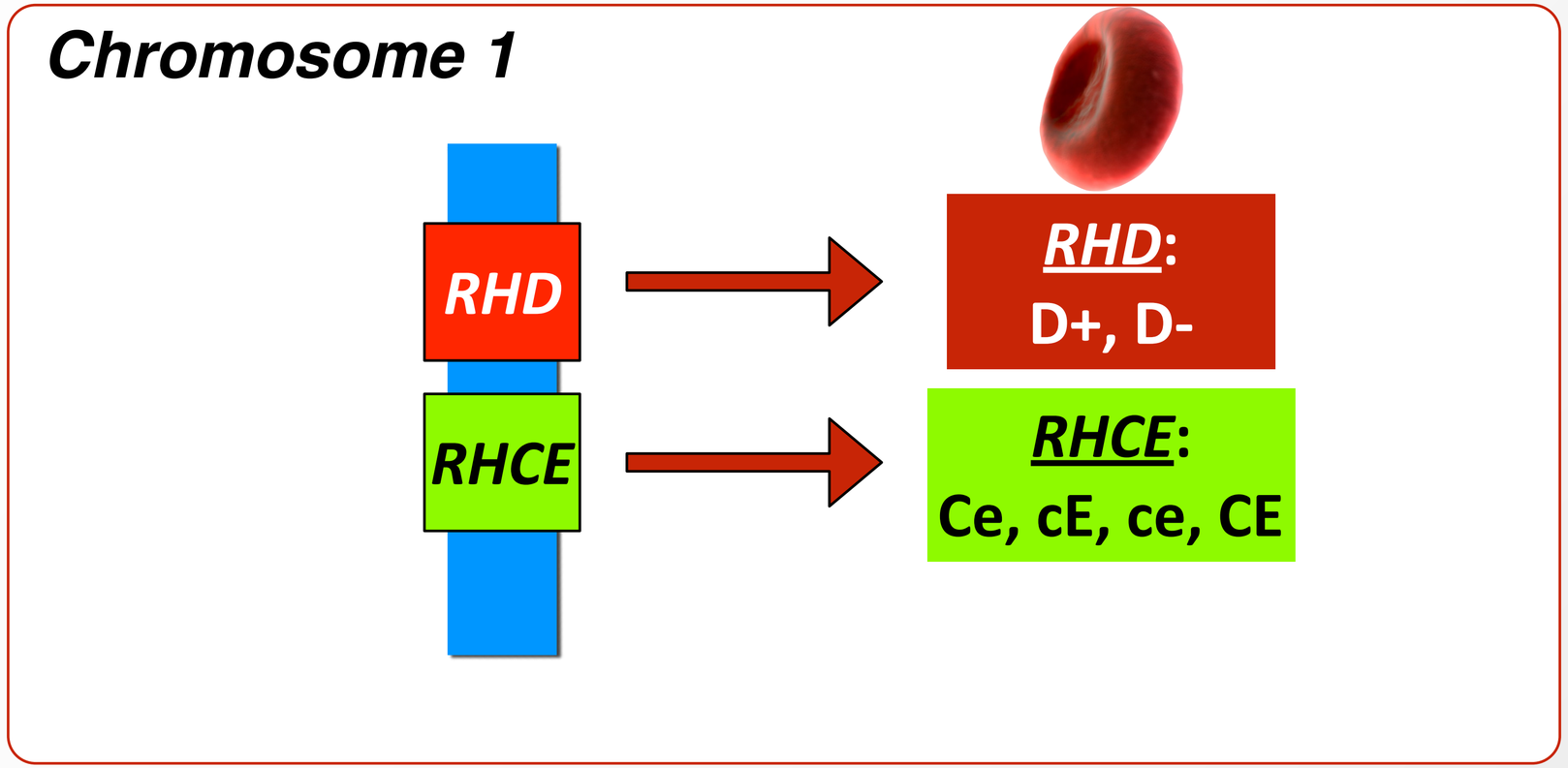
A: 4C to 37C
B: 4C only
C: 37C only

Rh System

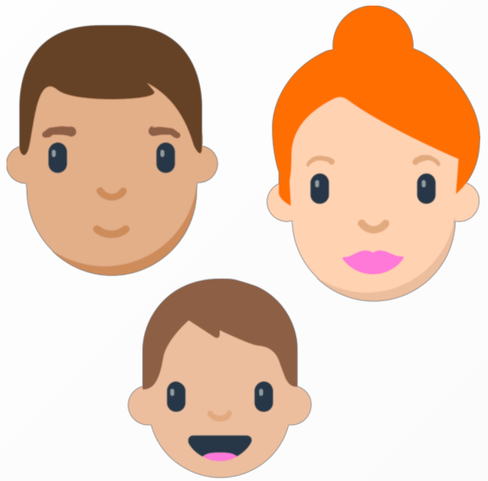


- Five main antigens (50+ overall)
  - D, C, E, c, e
  - “Rh+” means “D+”
  - Rh– described as “d”

Rh System Genetics



Rh System



"Haplotype"

Mom

D — *RHD*

C e — *RHCE*

→ **DCe = R_1**

Dad

d — *RHD* (Mutated/deleted)

c e — *RHCE*

→ **dce = r**

Rh System Haplotypes

R_1: D	r’:d
R_2: D	r”:d
R_0: D	r:d
R_z: D	r^y:d

Ce cE ce CE

The “Big Four”

- R_1, R_2, R_0, and r (97%)

Whites	R_1	r	R_2	R_0
Blacks	R_0	r	R_1	R_2

1. R_0 1st in blacks, last in whites
2. r always second
3. $R_1 > R_2$

The “Big Four”

- R_1, R_2, R_0, and r (97%)

Whites	$R_1 > r > R_2 > R_0$
Blacks	$R_0 > r > R_1 > R_2$
Asians	$R_1 > R_2 > R_0 > r$

RhD-negative

- Mutations and deletions, not *d*
- Caucasians most likely D-negative
- Asians RARELY D-negative

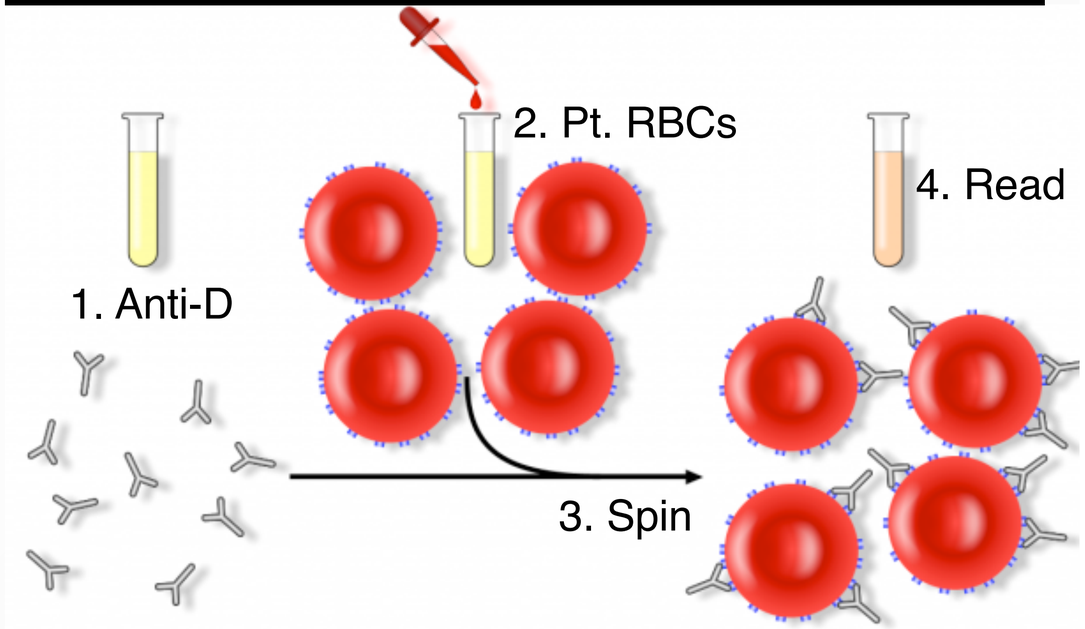
Caucasians (15%)	**African-Americans (3-5%)**	**Asians (<0.1%)**
Deleted *RHD*	Deleted *RHD* “Psi” pseudogene	Mutated *RHD* DEL

Rh Antibodies

- Exposure-requiring warm IgG
- Rh antigens stimulate antibodies well
  - Old: 80% of D– make anti-D
  - New: 22% of D– hospital pts make anti-D
- Consequences:
  - HTRs, primarily extravascular
  - Prototypical HDFN with anti-D
    - ✓ Anti-c: Severe HDFN, others mild

What's "Weak D?"

Routine D Testing (Steps 1-4)

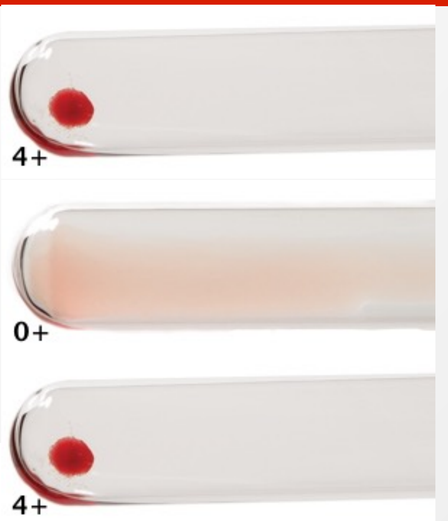


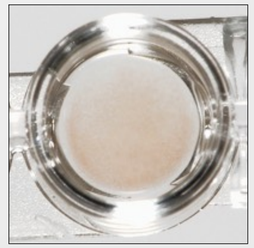
Mix of IgM/IgG

5. Incubate 15-30 min, spin

6. Wash, add AHG, spin

Weak D Test (Steps 5-6)

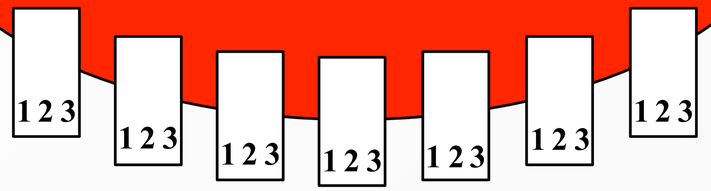


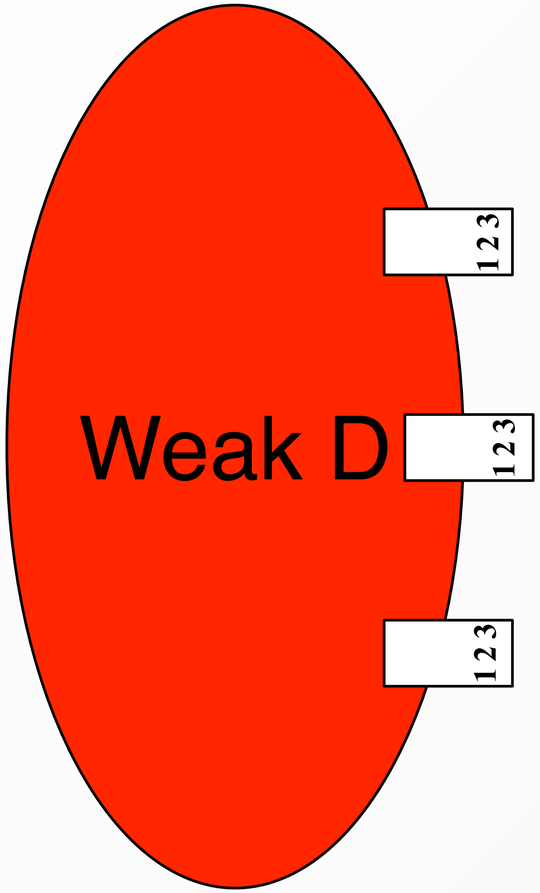


= RhD

123 = Epitopes

D-positive





Typical D+

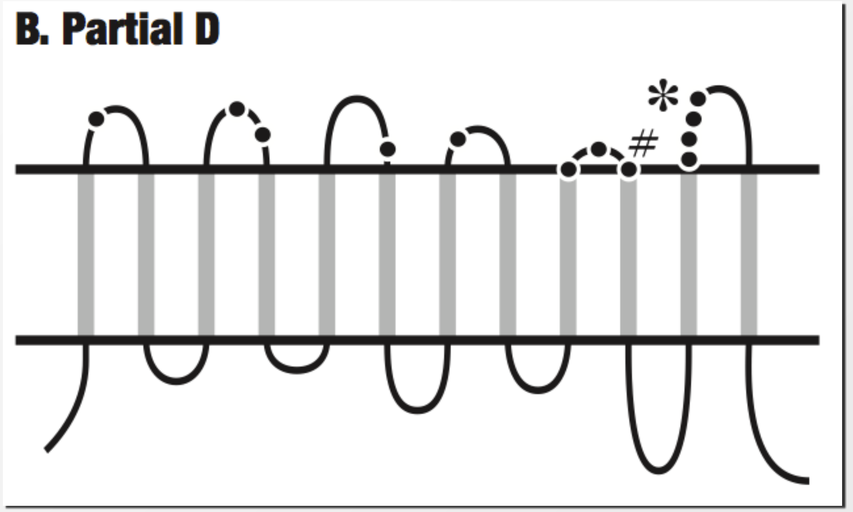
True Weak D

"Serologic Weak D"

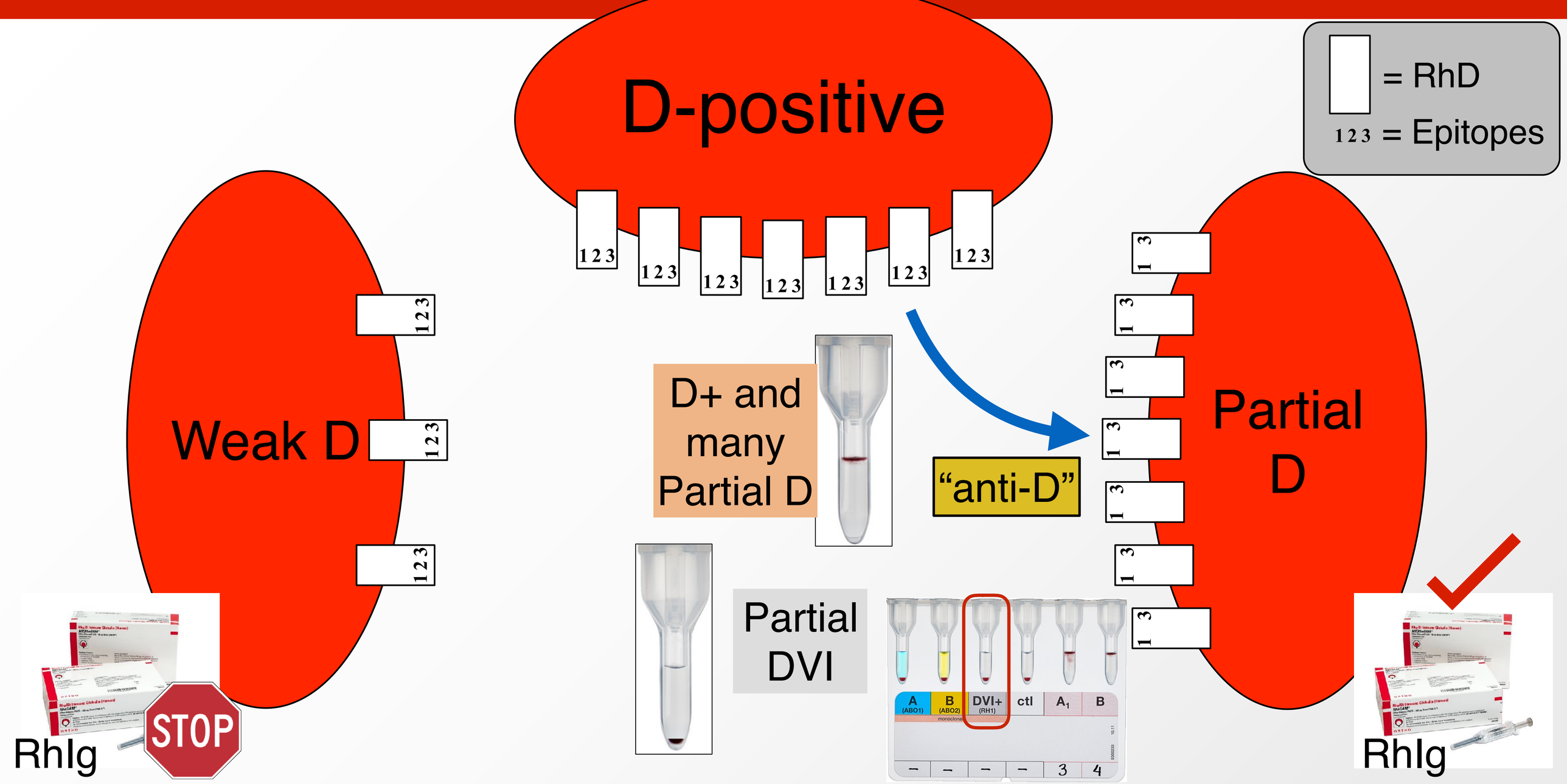
<u>Current AABB Recommendation</u>:
Consider Rh genotyping with serologic weak D in Tx service
-Saves Rh neg supply
-Saves RhIg injections for moms

Partial D

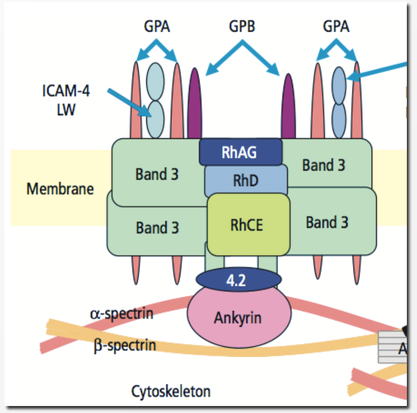
- Qualitative D defect
  - Parts of D on outside of RBC via *RHD* mutation
- Abs vs. missing parts ~ anti-D
  - Classic: Anti-D in a D+ person



Westhoff CM, Review: the Rh blood group D antigen...dominant, diverse and difficult. Immunohematology 2005;21(4):155-6.


D-positive
= RhD
123 = Epitopes
Weak D
123
123
123
123 123 123 123 123 123 123
D+ and many Partial D
“anti-D”
Partial D
123 123 123 123 123 123 123
Partial DVI
A (ABO1)
B (ABO2)
monoclona
DVI+ (RH1)
ctl
A1
B
– – – – 3 4
STOP
RhIg
RhIg

Weird Stuff



- Rh_{null}
  - Mutation in *RHAG* gene (3rd Rh gene)
    - ✓ Structural problem
  - No Rh antigens
  - Stomatocytic hemolytic anemia

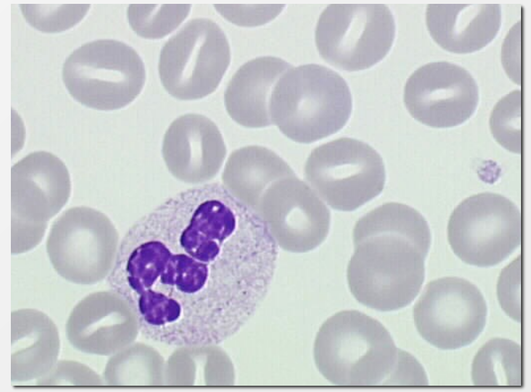
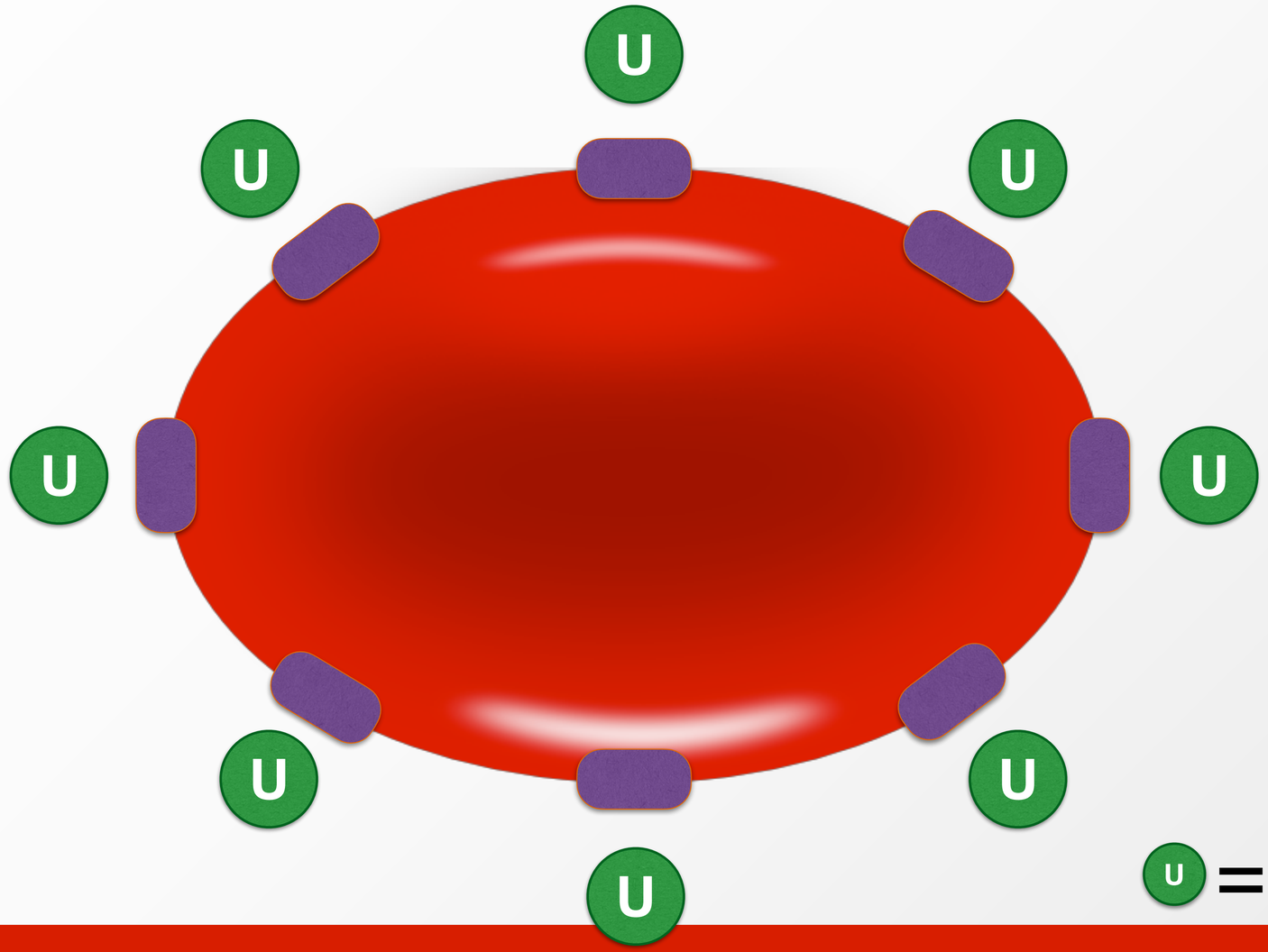


Image: http://i212.photobucket.com

Kidd System

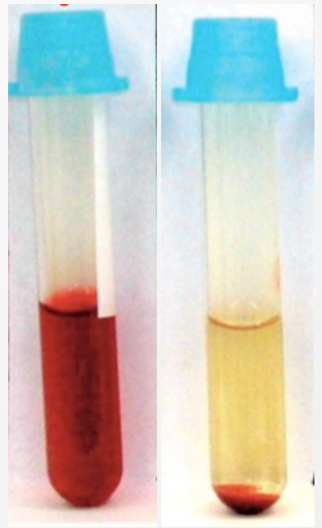
- Antigens:
  - Jk^a and Jk^b
    - ✓ Jk3: Absent in Jk(a–b–)
  - Urea transport antigen
    - ✓ Jk(a–b–) resistant to 2M urea

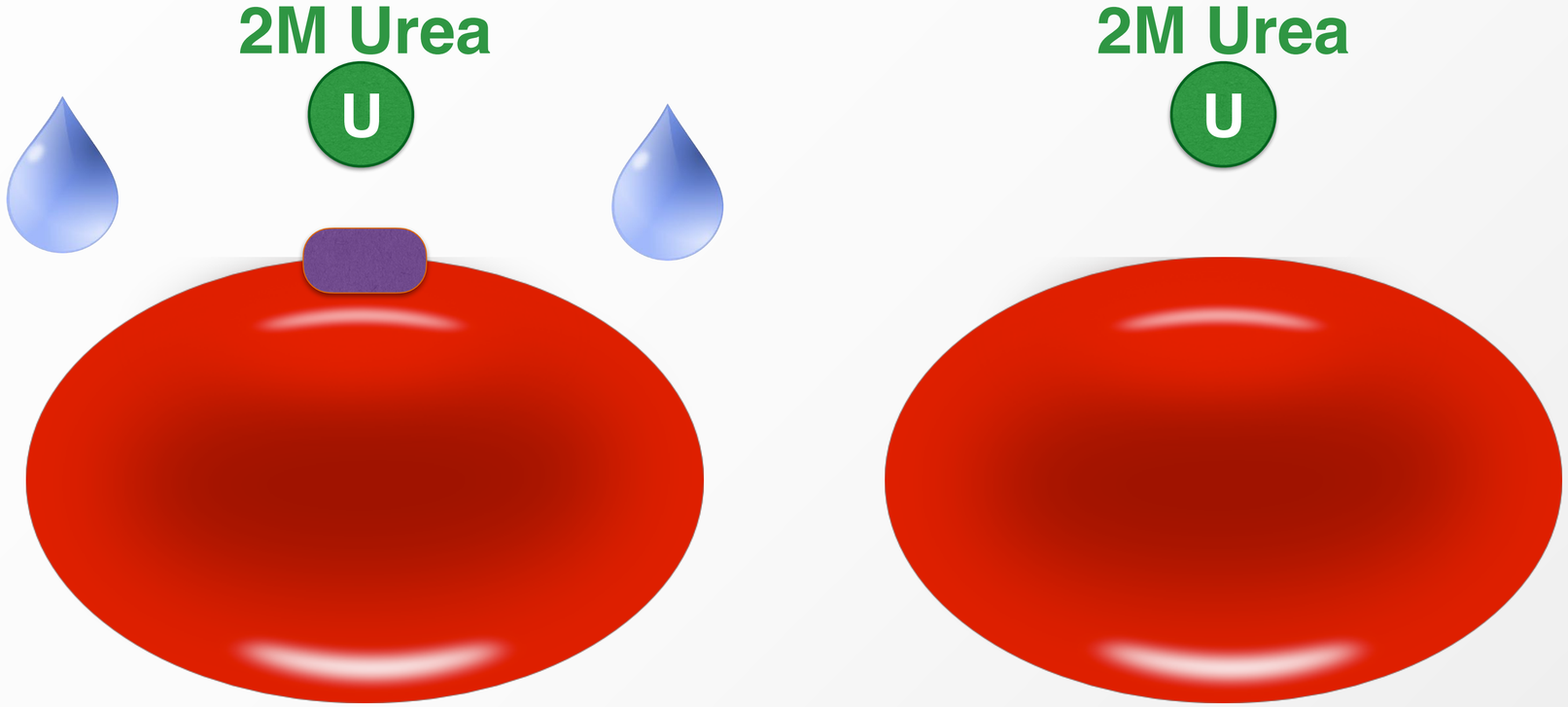
Urea Transport



U = Urea

Weird Stuff





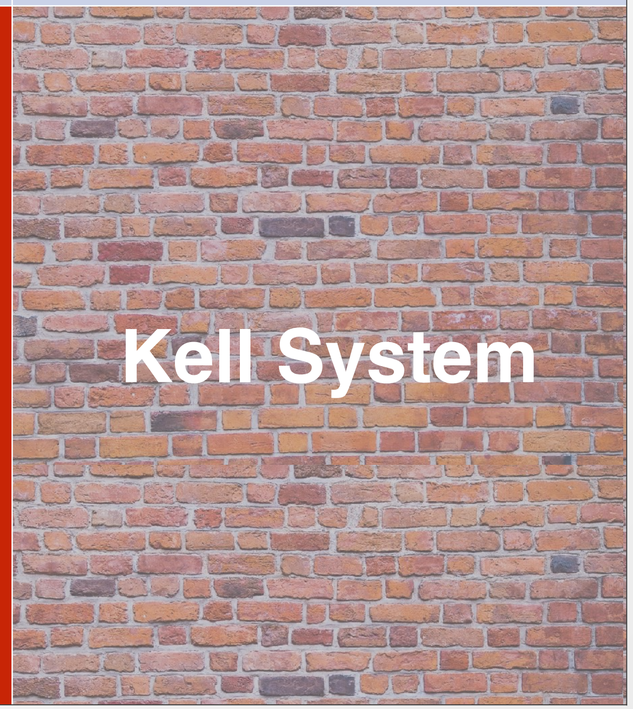
Jk(a+) or Jk(b+)

Jk(a-b-)/Jk3–

Kidd Antibodies

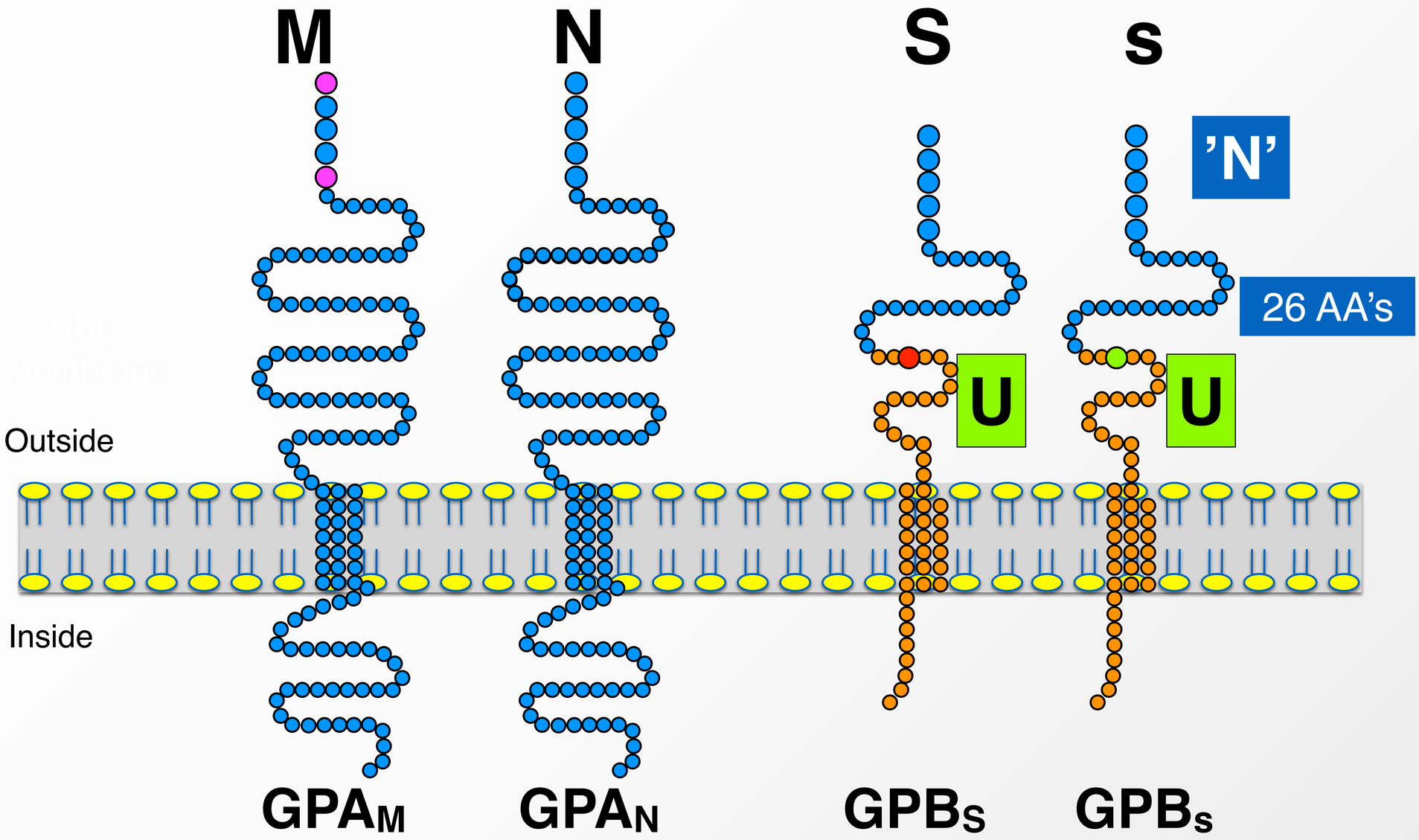
- Warm IgG (+/- with IgM component)
  - May fix complement
- Rarely found alone
- Marked dosage
- Variable expression with time
  - “Hide and seek”
- Immediate or Delayed hemolytic reactions
  - Disappearing antibody that comes roaring back

Enzyme Classification

Enhanced	Decreased	Unaffected
ABO-related -ABO/H -Lewis -I -P1PK/GLOB Rh System Kidd System	MNS System Duffy System	

MNS System

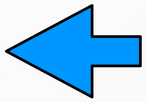
- 49 recognized antigens
- Glycophorin A and B
  - Glycophorin A carries M and N
  - Glycophorin B carries S, s, and U
  - Both are receptors for malaria parasite
    - ✓ P. falciparum attaches here


M
N
S
s
'N'
26 AA's
U
U
Outside
Inside
GPA$_M$
GPA$_N$
GPB$_S$
GPB$_s$
Ser ↔ 1 ↔ Leu
Gly ↔ 5 ↔ Glu
Met ↔ 29 ↔ Thr

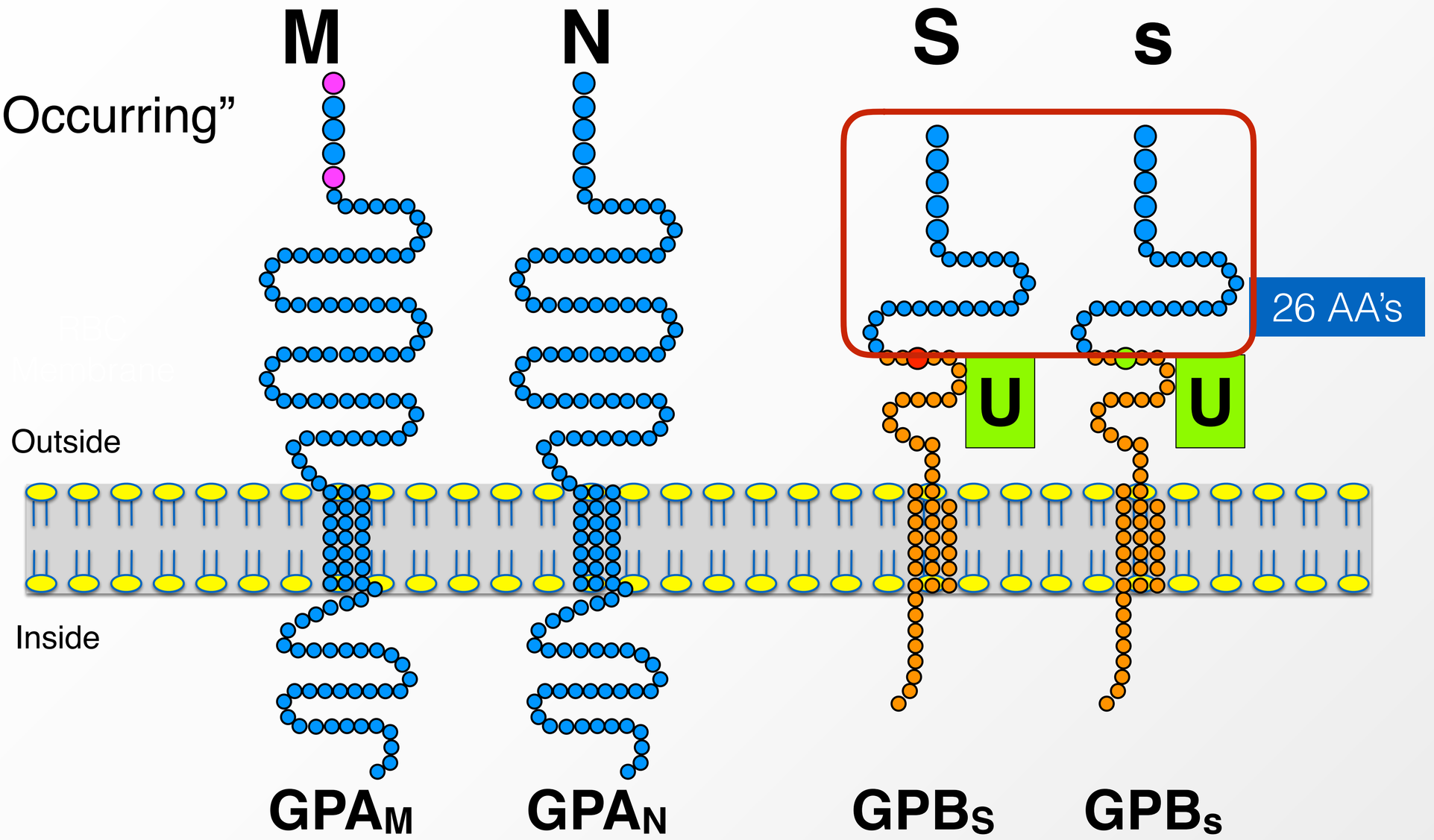
MNS Antibodies

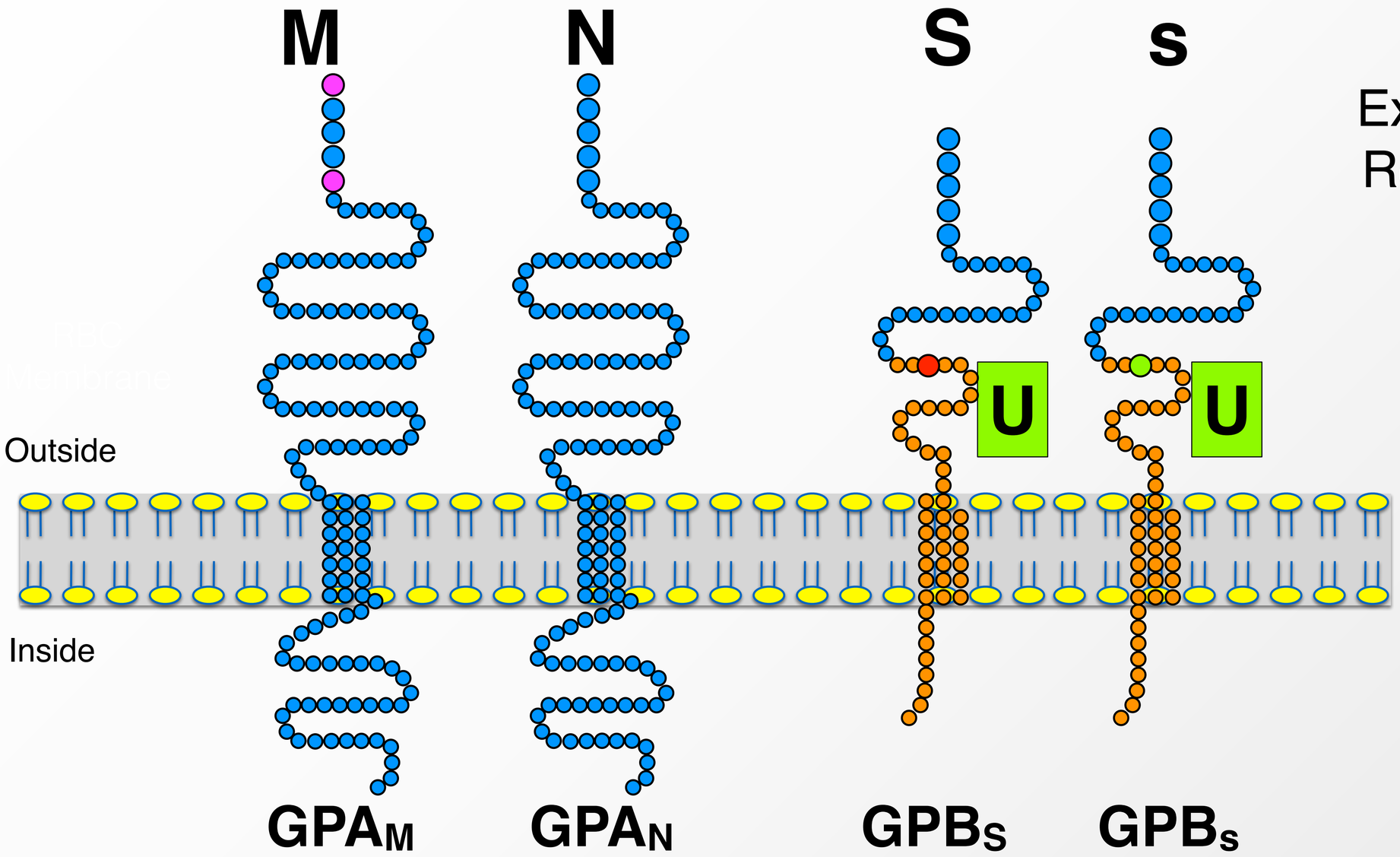
Anti-M and -N	Anti-S, -s, & -U
“Natural”	Exposure
IgM/IgG	IgG
Cold-reactive	37 and AHG
Us. Insignificant	Significant

Occasional anti-M at 37C



“Naturally Occurring”





Exposure Required

MNS Antigens

- S–s–U–
  - 1% of African-Americans, never in Caucasians
  - Anti-U is significant risk
    - ✓ Can cause HTR and HDFN

Duffy System

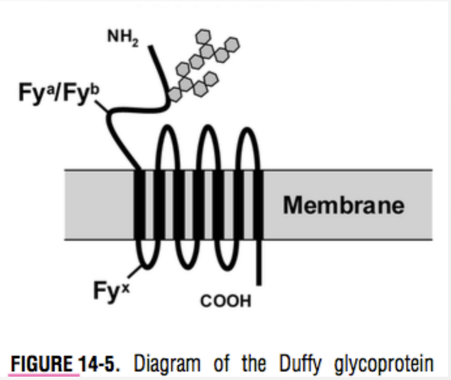
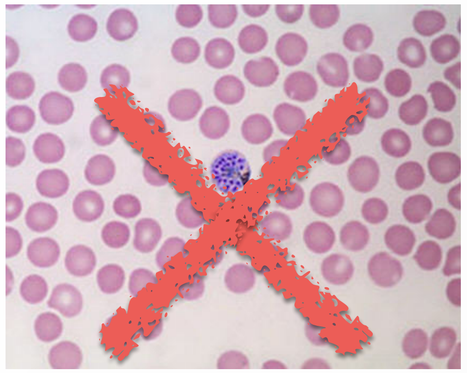


FIGURE 14-5. Diagram of the Duffy glycoprotein

- Fy^{a}, Fy^{b} main antigens (*FY*A*, *FY*B* alleles)
  - Carried on RBC membrane protein (ACKR1)
  - Also on endothelial cells of:
    - Heart, lung, kidney, spleen, GI tract 
- *FY*B* is commonly mutated in African-Americans
  - This mutation impacts Fy^{b} expression ON RBCs 
  - <u>BUT NOT everywhere else!</u>

Image: AABB Technical Manual, 17th ed, p 422



Fy(a-b-)

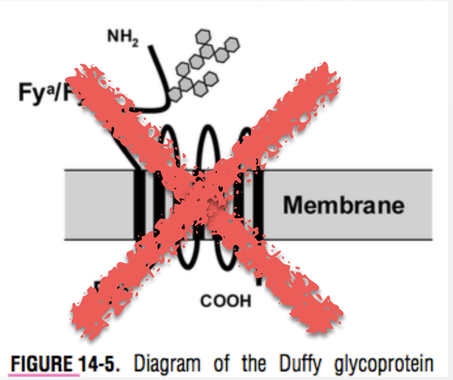


FIGURE 14-5. Diagram of the Duffy glycoprotein

	C	AA
Fy(a+b-)	17	9
Fy(a+b+)	49	1
Fy(a-b+)	34	22
Fy(a-b-)	rare	68

Fy(a–b–) individuals of African descent rarely make anti-Fy^{b}

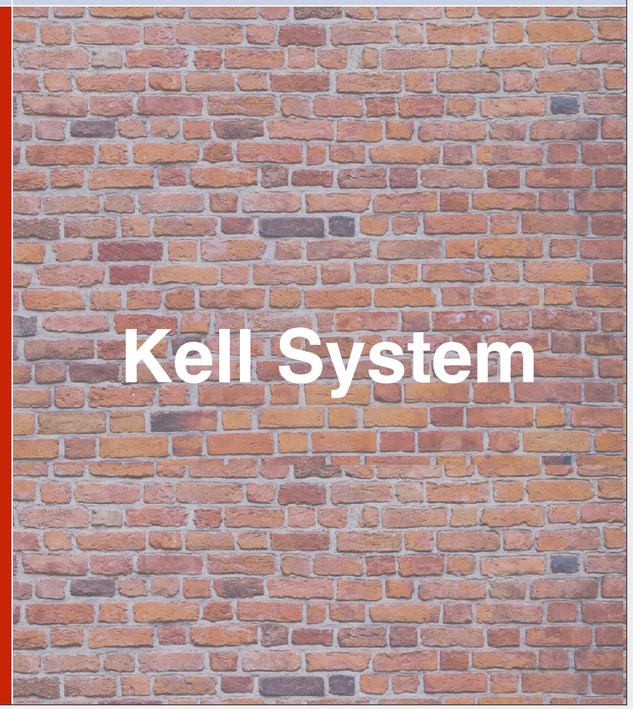
Fy(a–b–) individuals of African descent are resistant to *P. vivax*

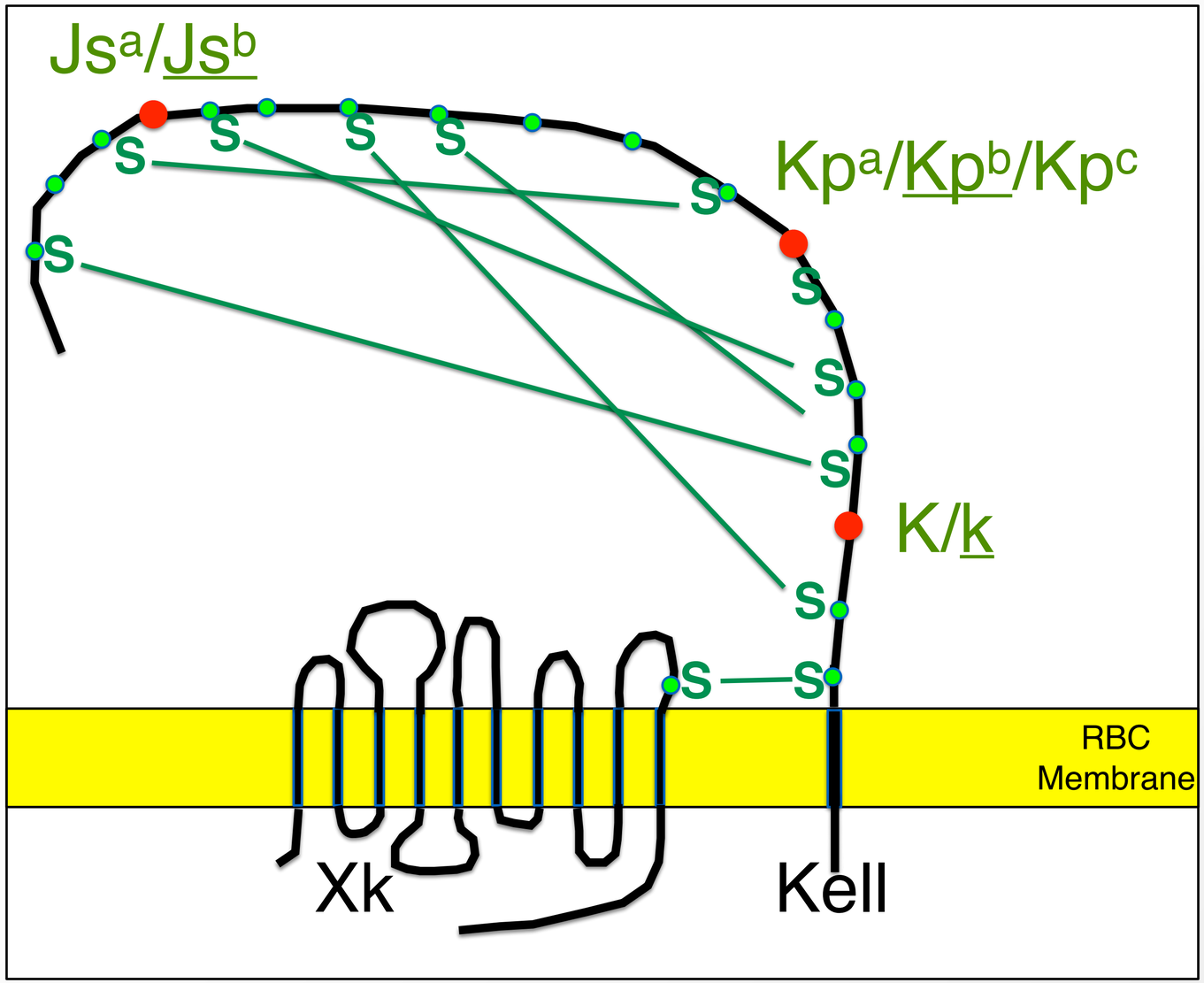
Image: AABB Technical Manual, 17th ed, p 422

Duffy Antibodies

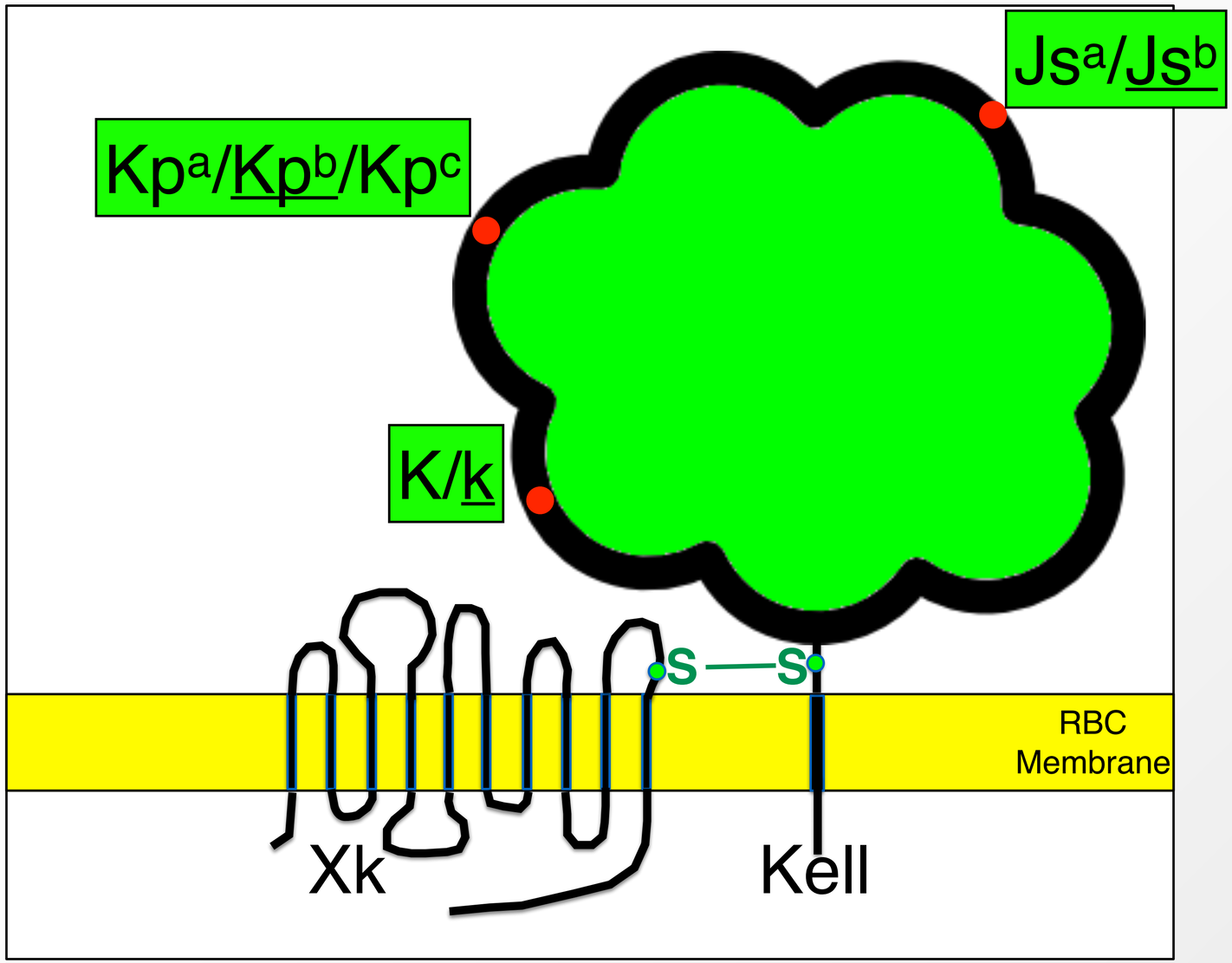
- Exposure-requiring warm IgG
  - Marked dosage (like Kidd) 
  - Variable expression (like Kidd) 
  - Delayed HTRs (like Kidd) 
  - Anti-Fya >> anti-Fyb

Enzyme Classification

Enhanced	Decreased	Unaffected
ABO-related -ABO/H -Lewis -I -P1PK/GLOB Rh System Kidd System	MNS System Duffy System	


Js^a/Js^b
Kp^a/Kp^b/Kp^c
K/k
RBC
Membrane
Xk
Kell

Dithiothreitol (DTT)



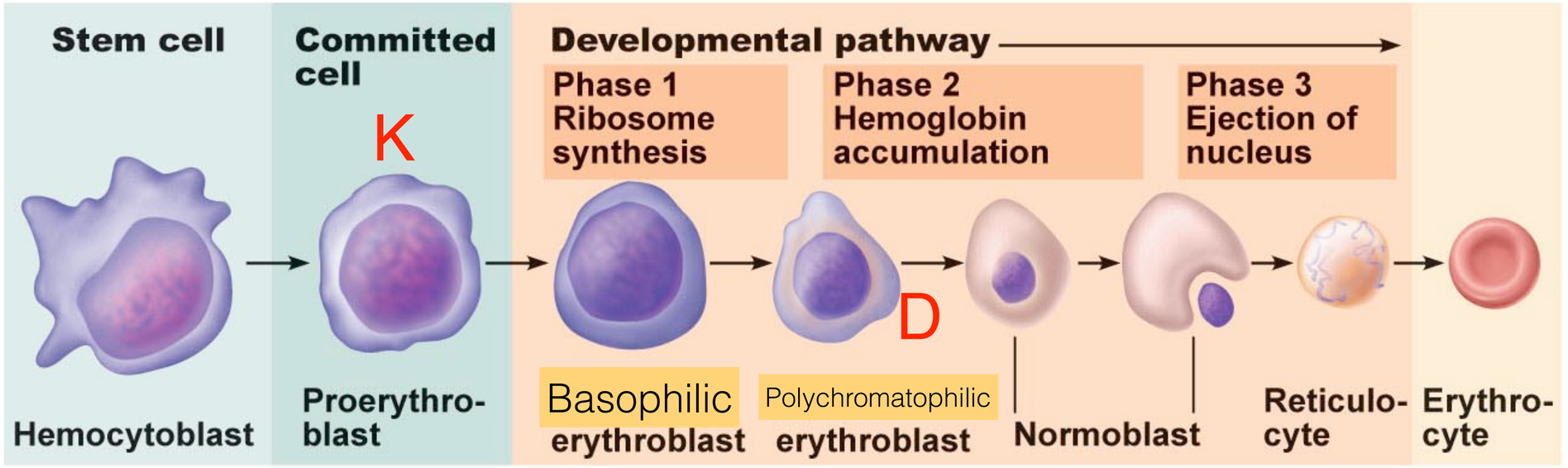
Kell Antigens

- K1 (aka K, NOT “Kell”)
  - 9% Caucasians, 2% African-Americans

- High frequency:
  - k (aka KEL2); 99.8%
  - Js^b, Kp^b

Kell Antibodies

- Anti-K
  - After D, most immunogenic non-ABO antigen
  - Exposure-requiring warm IgG
  - Severe HTRs
  - Severe HDFN* 
- Anti-k
  - Like anti-K, just very uncommon

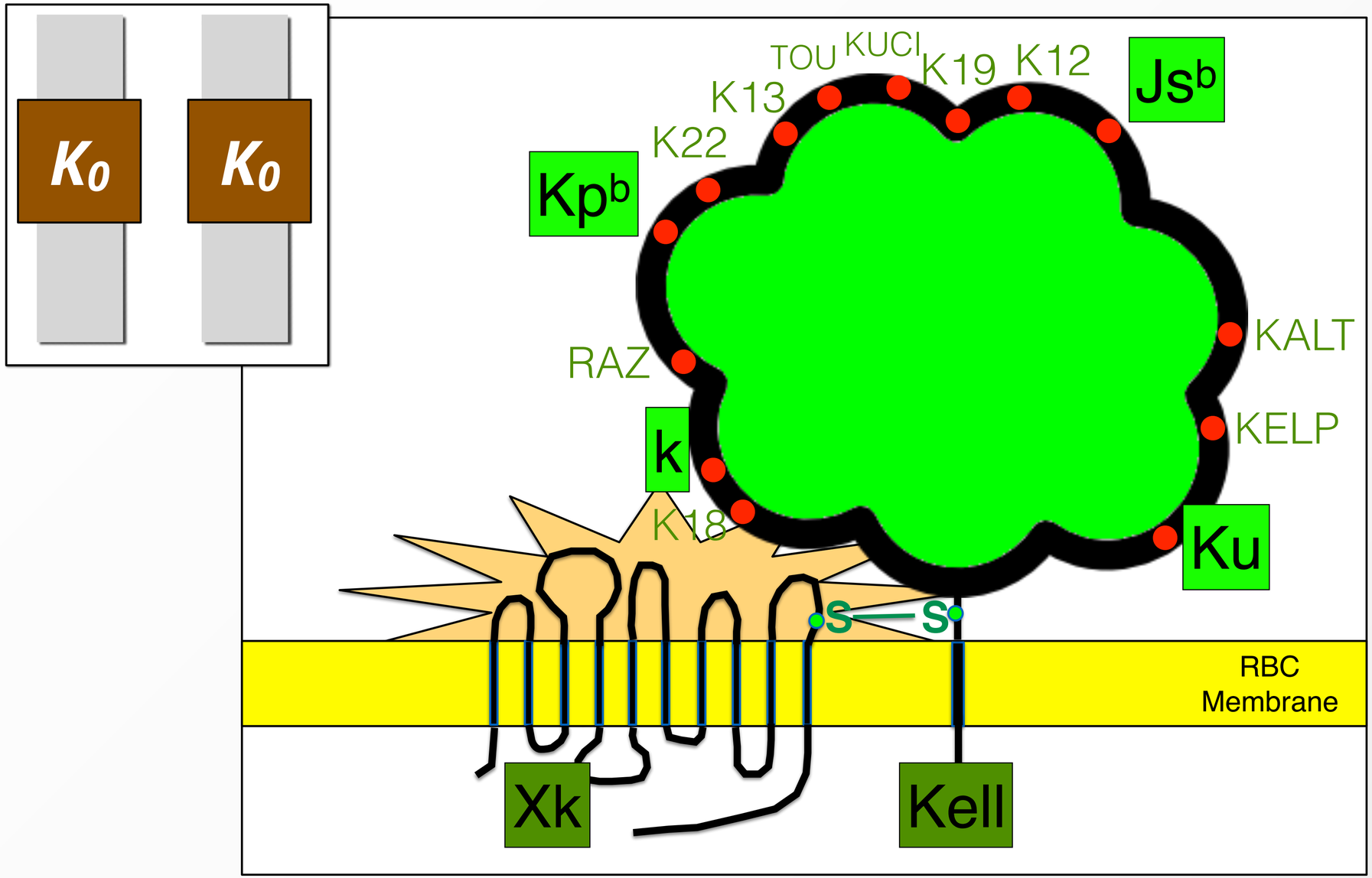


Anti-K HDFN

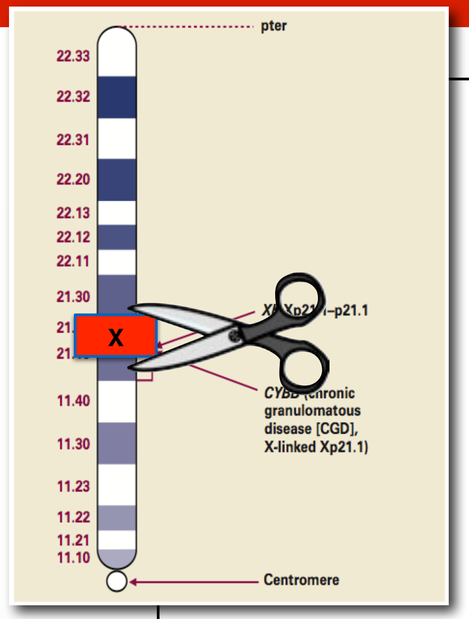
-Suppression
-Reticulocytopenia
-ANEMIA

RhD HDFN

-Hemolysis
-Reticulocytosis
-Hyperbilirubinemia
-Anemia



Ku = Kell “universal”


pter
22.33
22.32
22.31
22.20
22.13
22.12
22.11
21.30
21
21
11.40
11.30
11.23
11.22
11.21
11.10
x
p21.1
CYBB (chronic granulomatous disease [CGD], X-linked Xp21.1)
Centromere

Js^b
Kp^b
k
Ku
S—S
RBC Membrane
Xk
Kell

McLeod Neuroacanthosis

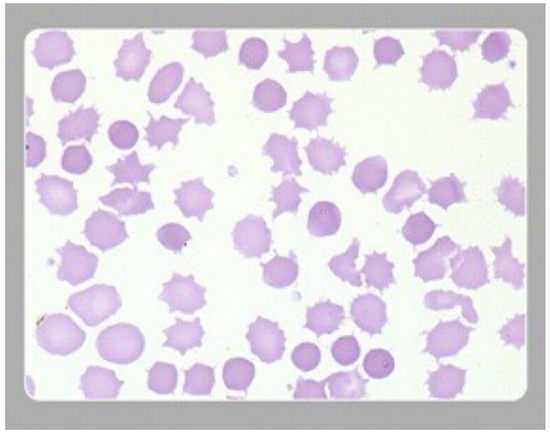
McLeod Phenotype	**McLeod Syndrome**
RBCs lack Kx RBCs have decreased Kell Ags	RBCs lack Kx RBCs have decreased Kell Ags
	Acanthocytic hemolytic anemia X-linked CGD (minority) Chorea, seizures Psychiatric disorders Muscle wasting Cardiac arrhythmia

Image: Blood 108:5;1433 (Maslak P)