BLOOD GROUPS

ABO AND Rh Serology

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Karl Landsteiner

Discovered blood groups in 1901

Nobel Prize in 1930 for Blood Groups

Austria: 1868 - 1943

ISBT nomenclature		ISGN nomenclature		Gene product name
Symbol	Number	Gene	Chromosome	
ABO	001	*ABO*	9q34.1	α1,3-*N*-acetylgalactosaminyltransferase (A antigen) α1,3-galactosyltransferase (B antigen)
MNS	002	*GYPA* *GYPB* *GYPE*	4q28.2	Glycophorin A Glycophorin B Glycophorin E
P_1	003	*α4GalT1*	22q13	α1,4-galactosyltransferase (P^k, P_1 antigens)
RHD RHCE	004	*RHD* *RHCE*	1p36.1	RhD protein RhCE protein
LU	005	*LU*		19q13.2 Lutheran glycoprotein, B-CAM
KEL	006	*KEL*	7q33	Kell glycoprotein
LE	007	*FUT3*	19p13.3	α-3/4-fucosyltransferase
FY	008	*DARC*	1q22	Duffy associated receptor cytokine glycoproteir
JK	009	*SLC14A1*	18q11	Urea transporter (HUT11)
DI	010	*SLC4A1*	17q12	Anion exchanger 1 (AE1, Band 3)
YT	011	*ACHE*	7q22	Acetylcholinesterase
XG	012	*XG*	Xp22.3	Xg glycoprotein
SC	013	*HERMAP*	1p34	Human erythroid membrane associated protein
DO	014	*ART4*	12p13.2	ADP-ribosyltransferase
CO	015	*AQPI*	7p14	Aquaporin 1 (CHIP)
LW	016	*LW*	19p13.3	LW glycoprotein
CH/RG	017	*C4A, C4B*	6p21.3	C4A, C4B complement glycoproteins
H	018	*FUT1*	19q13.3	α1,2-fucosyltransferase
XK	019	*XK*	Xp21.1	Kx glycoprotein
GE	020	*GYPC*	2q14	Glycophorin C and glycophorin D
CROM	021	*DAF*	1q32	CD55 (decay-accelerating factor)
KN	022	*CR1*	1q32	CD35 (complement receptor 1)
IN	023	*CD44*	11p13	CD44
OK	024	*CD147*	19p13.3	CD147, extracellular matrix metalloproteinase i
MER2	025	*MER2*	11p15.5	Not defined
JMH	026	*SEMA-L*	15q22.3	Semaphorin CD108
I	027	*IGnT*	6p24	β1,6-*N*-acetylglucosaminyltransferase
GLOB	028	*β3GalT3*	3q25	β1,3-*N*-acetylgalactosaminyltransferase
GIL	029	*AQP3*	9p13	Aquaglyceroporin

Terminology for Blood Group System

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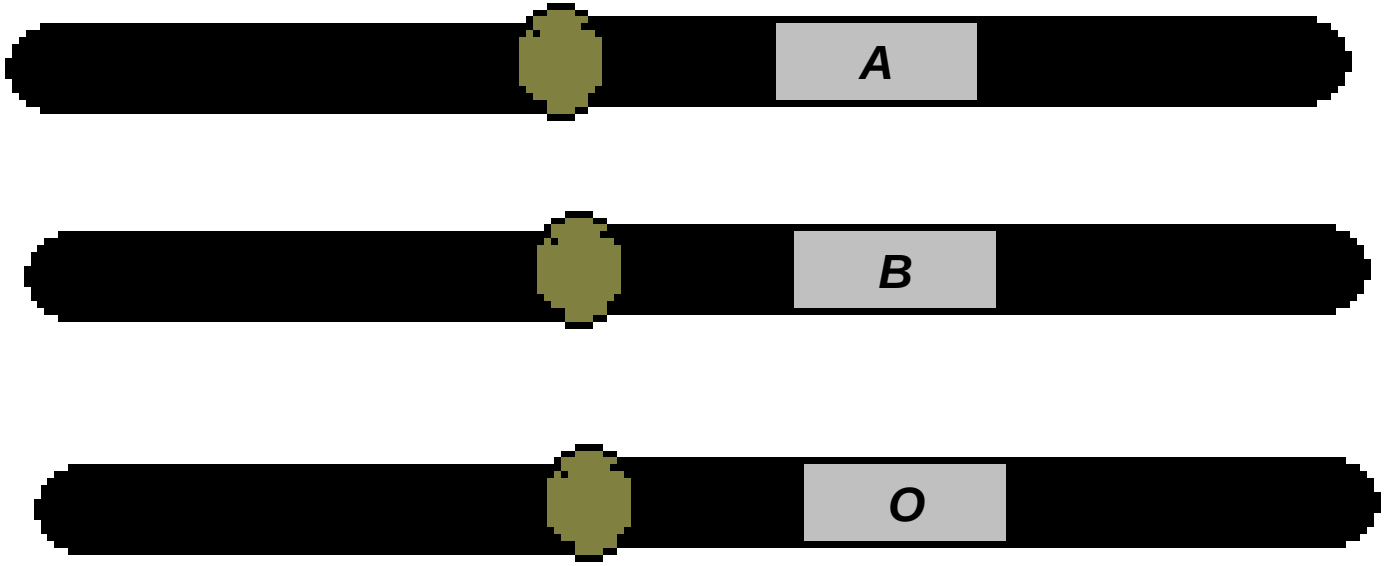
ABO Alleles

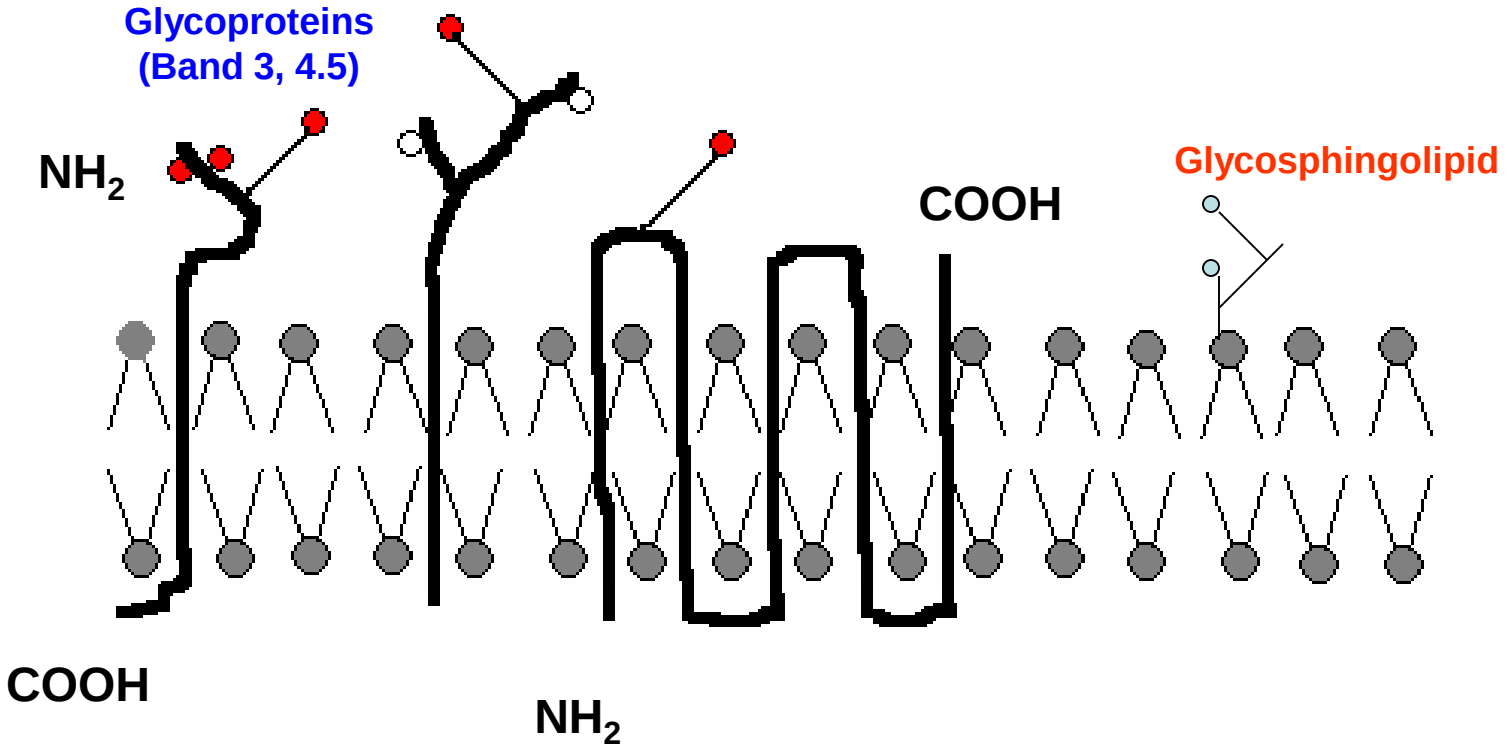
Chromosome 9, Locus ABO



Hh/Sese Alleles

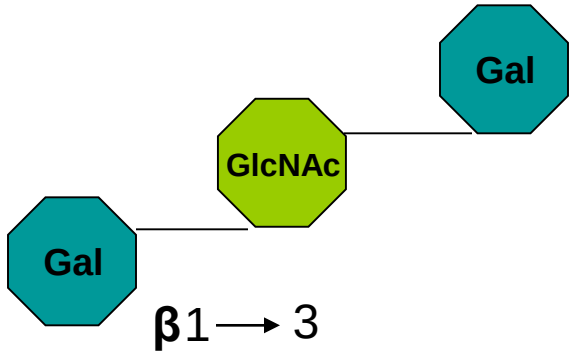
Chromosome 19

Red Cell Membrane showing Antigen-bearing Glycosylation of Proteins and Lipids

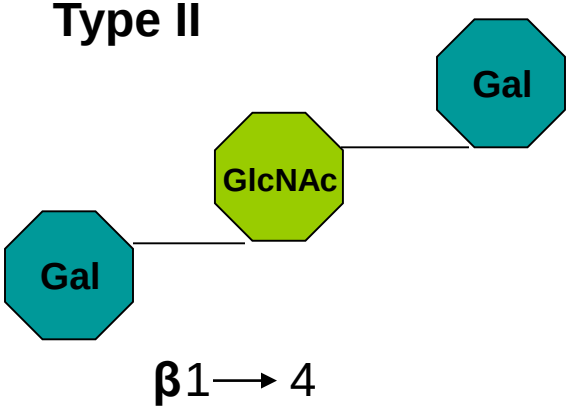


Paraglobоside

Type I



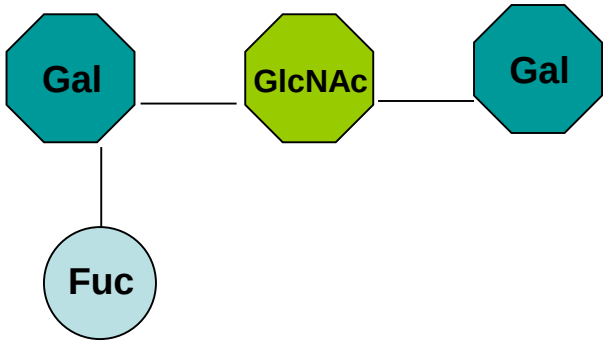
Type II



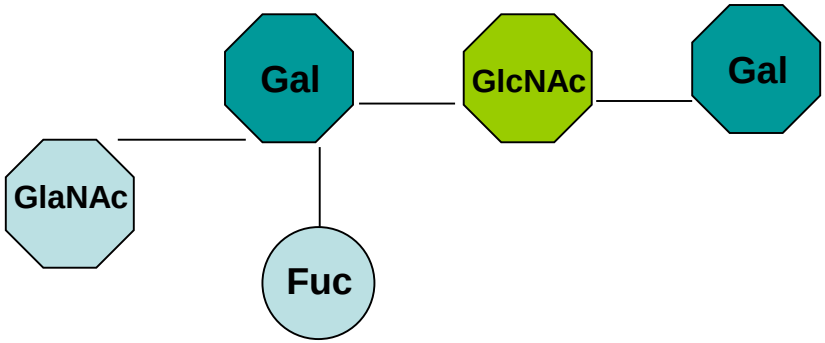
ABH Antigens

Type 2 **H (FucT 1)**

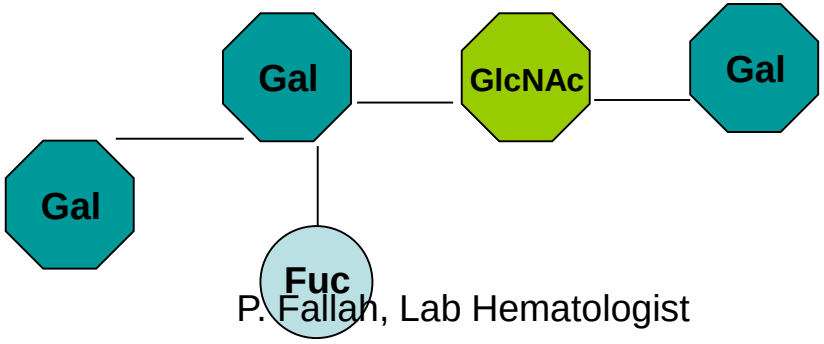
Type 1 **Se (FucT 2)**

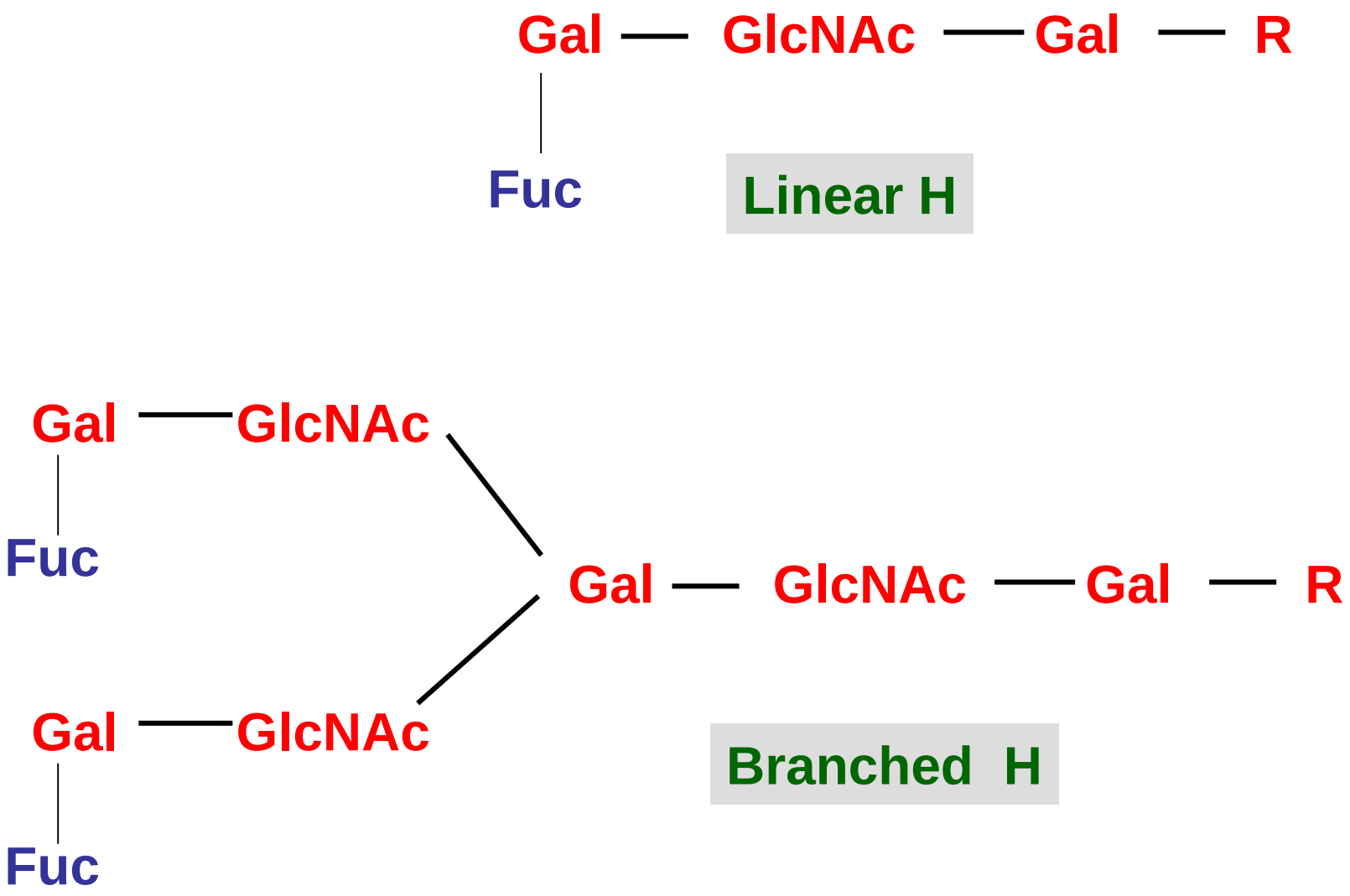


A

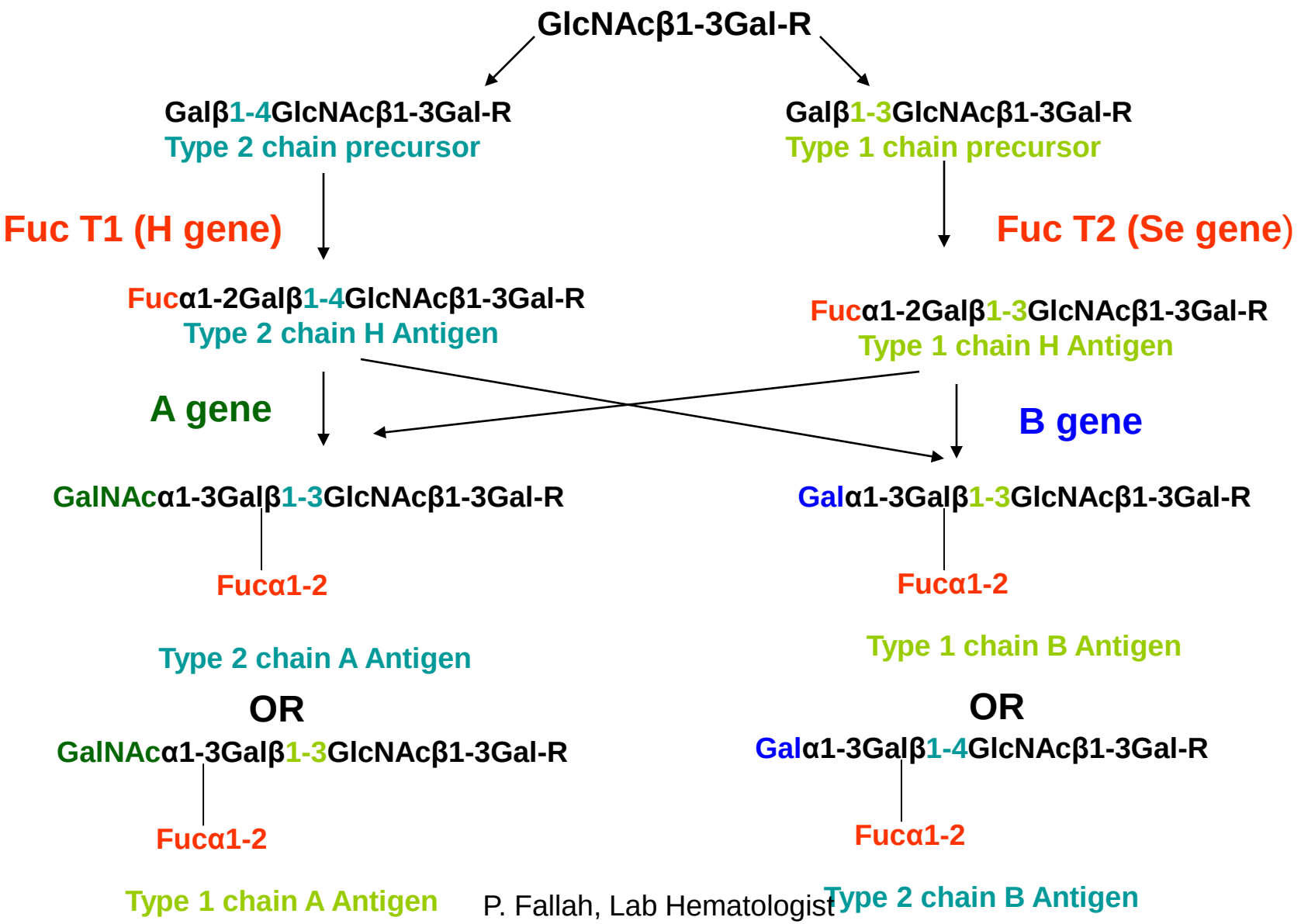


B




Gal
GlcNAc
Gal
R
Fuc
Linear H
Gal
GlcNAc
Fuc
Gal
GlcNAc
Gal
R
Gal
GlcNAc
Fuc
Branched H

ABH Antigen Synthesis



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Amino Asid Substitutions in A and B Transferase

Amino Acid Number

Phenotype	176	235	266	268
A	Arg	Gly	Leu	Gly
B	Gly	Ser	Met	Ala

Mating Phenotype	Matting Genotype	Offspring Possible Phenotypes and Genotypes
A X A	AA X AA	A (AA)
	AA X AO	A (AA, AO)
	AO X AO	A (AA, AO), O (OO)
B X B	BO X BB	B (BB)
	BO X BO	B (BB, BO)
	BO X BO	B (BB, BO), O (OO)
AB x AB	AB X AB	AB (AB), A (AA), B (BB),
O X O	OO X OO	O (OO)
A X B	AA X BB	AB (AB)
	AO X BB	AB (AB), B (BO)
	AO X BO	AB (AB), A (AO)
	AO X BO	AB (AB), A (AO), B (BO), O (OO)
A X O	AA X OO	A (AO)
	AO X OO	A (AO), O (OO)
A X AB	AA X AB	AB (AB), A (AA)
	AO X AB	AB (AB), A (AA,AO), B (BO)
B X O	BB X OO	B (BO)
	BO X OO	B (BO), O (OO)
B X AB	BB X AB	AB (AB), B (BB)
	BO X AB	AB (AB), B (BB, BO), A (AO)
AB X O	AB X OO	A (AO),B (BO)

ABO Typing

Reaction of cells (Forward)			Reaction of serum (Revers)			Interpretation	Incidence
Anti-A	Anti-B	Anti-AB	A_1 Cells	B Cells	O Cells	Blood Group	
0	0	0	+	+	0	O	31%-45%
+	0	+	0	+	0	A	20%-37%
0	+	+	+	0	0	B	14%-32%
+	+	+	0	0	0	AB	5%-10%
0	0	0	+	+	+	O_h	Rare

Serologic Differentiation of the ABO Groups

Phenotype	Red Cells with Anti-					Serum with Cells			Substances in Saliva or Secretors	Level of Transferase	Antigen Sites per RBC x10^3
	A	A_1*	B	A,B	H*	A_1	B	O			
A_1	++++	++++	0	++++	0/+	0	++++	0	A,H	Normal (pH 6)	810-1170
A_{int}	++++	++	0	++++	++	0	++++	0	A,H		
A_2	++++	0	0	++++	+++	-/+	++++	0	A,H	Decreased (pH 7)	240-1290
A_3	$++^{mf}$	0	0	$++^{mf}$	+++	-/+	++++	0	A,H	Low	30
A_x	0/+	0	0	++	++++	+	++++	0	H	Very low	4
A_m	0	0	0	0	++++	0	++++	0	A,H	Low	0.2-1.9
B	0	0	++++	++++	++	++++	0	0	B,H	Normal	750
B_3	0	0	$++^{mf}$	$++^{mf}$	+++	++++	0	0	B,H	Low	
O	0	0	0	0	++++	++++	++++	0	H	Normal	1700
O_h	0	0	0	0	0	++++	++++	++ ++	None	Normal	

*Anti-A_1: Dolichos biflorus
Anti-H: Ulex europaeus

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H substance in different groups

$$O > A_2 > A_2B > B > A_1 > A_1B$$

ABO Antibodies

- The most important in transfusion medicine
- Naturally occuring
- Weak or absent in the sera of newborns until 3 to 6 months of age
- Are detected as Room temperature, Salin Agglutinins with optimal reactivity at 4ºC
- Anti-A & Anti-B are IgM
- Anti-A,B in Group O is IgG
- Are a cause of Hemolytic Transfusion Reaction (HTR) & Hemolytic Disease of the Newborn (HDN)
- Are a cause of acute rejection in solid organ transplantation
- In ABO-incompatible bone marrow transplantation can result hemolysis and a deley in erythroid and megakaryocyte engrafment
- Immune ABO antibodies (following transfusion & pregnancy) are predominantly of IgG isotype and are reactive at 4ºC and 37ºC

ABO Antibodies

Specificity	Serum		
	Group	Incidences	Characteristics
Anti-A	B	All	Titer 1:32-2048 Average1:256 Primarily IgM
Anti-B	A	All	Titer 1:8-512 Average1:64 Primarily IgM
Anti-A,B	O,O_h	All	May have higher titer in pregnancy because of immune stimulation Reacts with A_x anb B_x
Anti-A_1	A_2 A_x A_2B	1-8% Most 22-35%	Usually clinically insignificant Rare transfution reaction are reported
Anti-H	O_h A_1,A_1B nonsecretor	All Some	Usually benign cold agglutinin except inO_h phenotype

ABO & Rh Discrepancies

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Discrepancies

- A **discrepancy** occurs when the red cell testing does NOT match the serum testing results
- In other words, the **forward does NOT match the reverse**

ABO Typing

Reaction of cells (Forward)			Reaction of serum (Revers)			Interpretation	Incidence
Anti-A	Anti-B	Anti-AB	A_1 Cells	B Cells	O Cells	Blood Group	
0	0	0	+	+	0	O	31%-45%
+	0	+	0	+	0	A	20%-37%
0	+	+	+	0	0	B	14%-32%
+	+	+	0	0	0	AB	5%-10%
0	0	0	+	+	+	O_h	Rare

ABO Grouping Discrepancies

- **Red cell-mediated**
  - Subgroup of A or B
  - Genetic chimera
  - Artificial chimera
    - Blood transfusion
    - Bone marrow transplantation
  - Polyagglotinatination
    - Tn Activation
    - Aquired B Antigen
  - Substances in plasma or serum
    - Excess blood group substance
    - Dyes
    - Wharton's jelly
  - Positive direct antiglobulin test
  - Reagents

ABO Grouping Discrepancies

- **Serum-mediated**
  - **Subgroup of A or B**
  - **Alloantibodies that incude anti-M, -Le^{a}, -P1**
  - **Autoantibodies that incude anti-I, -IH**
  - **Rouleaux**
  - **Transfusion of non-ABO identical plasma products**
  - **Age**
  - **Disease**
  - **Reagents**

ABO Discrepancies

Anti-A	Anti-B	Anti-AB	A_1Cell	B Cell
4+	0	4+	2+	4+

Subgroup of A

ABO Discrepancies

	Anti-A	Anti-B	Anti-AB	A_1Cell	B Cell	O Cell	Auto
Polyclonal antiserum	**1+**	**1+**	**1+**	**4+**	**4+**	**0**	**0**
Monoclonal antiserum	**0**	**0**	**0**				

Polyagglutination

ABO Discrepancies

Cell Type		Back Type	
Anti A	Anti B	A_1 Cell	B Cell
+1	+4	+4	-

گروه بندی B(A) با آنتی بادی منوکلونال

ABO Discrepancies

Cell Type		Back Type		
Anti A	Anti B	A_1 Cell	B Cell	O Cell
-	-	+4	+4	+4

گروه بندی سلولی و سرمی افراد O بمبئی

ABO Discrepancies

Cell Type		Back Type	
Anti A	Anti B	A_1 Cell	B Cell
+4	+2	-	+4

گروه بندی سلولی و سرمی در پدیده B کاذب

ABO Discrepancies

group	Cell Type		Back Type	
	Anti A	Anti B	A_1 Cell	B Cell
A2	+4	–	+2	+4
A2B	+4	+4	+2	–

گروه A2 و A2B با آنتی A1 سرم

ABO Discrepancies

Cell Type		Back Type	
Anti A	Anti B	A_1 Cell	B Cell
Before +1	–	–	+3
After +3	–	–	+3

گروه بندی بیمار مبتلا به سرطان معده با ترشح زیاد مواد گروه خونی قبل و بعد از شستن

ABO Discrepancies

Cell Type		Back Type		
Anti A	Anti B	A_1 Cell	B Cell	O Cell
Before +3	+3	+4	+4	+4
After –	–	+4	+4	+4
With Replacement -	–	+4	+4	–

گروه بندی یک بیمار مبتلا به Multiple Myeloma قبل و بعد از شستن گلبول های قرمز

ABO Discrepancies

Methods	Cell Type		Back Type			
	Anti A	Anti B	A_1 Cell	B Cell	O Cell	Auto
Slide Method	**+4**	**+4**	**+4**	**+4**	**+4**	**+4**
Tube method: After 2-3 times RBC washing with 37°C Saline	**+4**	**–**	**+4**	**+4**	**+4**	**+4**
Tube method: After 2-3 times RBC washing with 37°C Saline and Auto Ab Absorption	**+4**	**–**	**+1**	**+4**	**+1**	**+1**

گروه بندی یک بیمار ۷۰ ساله با لنفوم سلول های B و آگلوتینین سرد قوی

ABO Discrepancies

Cell Type		Back Type	
Anti A	Anti B	A_1 Cell	B Cell
Before Washing +4	+4	No Done	
After Washing +4	+4	No Done	

گروه بندی نوزاد با خون آلوده به ژله وارتی (گروه مادر O است)

ABO Discrepancies

تناقض در گروه بندی سلولی و سرمی به علت فقدان یا کاهش عیار آنتی بادی های مورد انتظار

ABO Discrepancies

	Cell Type		Back Type			Blood Group
	Anti A	Anti B	A_1 Cell	B Cell	O Cell	
1st Newborn	+4	–	–		–	A
2nd Newborn	–	–	+1	+1	–	O
3th Newborn	–	–	–	–	–	O

گروه بندی سه نوزداد

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ABO Discrepancies

	Cell Type		Back Type			
	Anti A	Anti B	A_1 Cell	B Cell	O Cell	Auto Control
Room Temperature	–	–	+1	+1	–	–
Back type in refrigerator temperature	–	–	+2	+3	–	–

گروه بندی پیرمرد ۸۰ ساله در دمای اتاق و دمای یخچال

ABO Discrepancies

Cell Type		Back Type	
Anti A	Anti B	A_1 Cell	B Cell
+4	–	–	–

گروه خون بیماری با سندرم ویسکوت آلدریچ

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ABO Discrepancies

	Cell Type		Back Type	
	Anti A	Anti B	A_1 Cell	B Cell
Before	+4	–	–	+4
After	+2 MF	–	–	+2

گروه بندی شخصی در یکسال پیش از ابتلا به لوسمی حاد مایلوبلاستیک

ABO Discrepancies

Cell Type		Back Type	
Anti A	Anti B	A_1 Cell	B Cell
+2 MF	–	–	+2 MF

گروه بندی بیماری با گروه A که ۴ ماه پیش پیوند از شخص O گرفته است

ABO Discrepancies

Cell Type		Back Type				Blood Group
Anti A	Anti B	A_1 Cell	B Cell	O Cell	Auto Control	
–	–	+4	+4	+4	–	بمبئی یا پارابمبئی
+4	–	–	+4	+2	–	A1 با آنتی H
+4	–	+4	+4	+4	+4	A با اتو انتی بادی سرد یا رولکس
+4	–	+4	+4	+4	–	گروه A با آلو آنتی بادی
–	+4	+4	+2		–	گروه B که تزریق کیسه هی پلاکت با گروه O داشته است
–	+4	–	–	–	–	گروه B در نوزاد، هایپوگاما گلوبولینمیا، ویسکوت آلدریچ، افراد پیر، مصرف سرکوبگر های ایمنی
+4	+2	–	+4	–	–	گروه A با B کاذب
+4	+3	+2	–	–	–	گروه A2B با آنتی A1

Let’s practice !

Case study 1

Anti-A	Anti-B	Anti-D	A_1 Cells	B Cells
0	0	4+	+3	0

Initial hypothesis:

Forward: Group O Rh Positive

Reverse: Group B

ABO discrepancy is an unexpected antibody reaction with AI cells.

Resolution:

All tubes are reverified for positive identification. No errors are found.

A new sample is requested to investigate the potential of a mislabeled sample.

All testing is repeated. The results are the same.

History:

She was crossmatched 5 years ago and was typed as B positive.

She was the recipient of a bone marrowtransplant due to aplastic anemia.

The donor was a group 0, D-positive sibling.

Case study 2

Anti-A	Anti-B	Anti-D	A_1 Cells	B Cells
4+	0	4+	0	0

Initial hypothesis:

Forward: Group A Rh Positive

Reverse: Group AB

ABO discrepancy is a missing antibody.

Resolution:

All tubes are reverified for positive identification. No errors are found.

A new sample is requested to investigate the potential of a mislabeled sample.

All testing is repeated. The results are the same.

History:

Patient is 95 years old and has decreased production of anti-B due to her age.

To prove this theory, room temperature incubation at 4° C for 10 minutes is performed testing the patient's serum against A1 and B cells, an auto control, and antibody screening cells.

The negative auto control indicates that no autoantibody is present at room temperature, or 4° C. The negative screening cells indicate that no cold-reactive alloantibody is present in the sample.

The patient's ABO type is group A.

Case study 3

Anti-A	Anti-B	Anti-D	Anti-A_1	Anti-B
4+	4+	4+	0	0

Initial hypothesis:

Forward: Group AB Rh Positive

Reverse: Group AB

No ABO discrepancy

Because the manufacturer's insert requires the performance of a direct antiglobulin test (DAT) whenever the AB typing is determined, a DAT is performed.

DAT Results

Polyspecific Antihuman Globulin	Anti-IgG	Anti-C3d	Anti-C3d (5 min incubation)	Saline Control
4+	4+	0	1+	0

After treating Cells with EDTA glycine acid,
to attempt to remove antibody coating cells.

Anti-A	Anti-B	Anti-D	A_1 Cells	B Cells
4+	4+	0	0	0

Case study 4

Anti-A	Anti-B	Anti-D	A_1 Cells	B Cells
4+	0	3+	2+	2+

Cell	Immediate Spin	LISS/37° C	LISS/AHG
Cell I	2+	0	0
Cell II	2+	0	0
Cell III	2+	0	0
Auto control	2+	0	0

Initial hypothesis:

Forward: Group A Rh Positive

Reverse: Group O

ABO discrepancy is an unexpected antibody reaction with A1 cells and a positive antibody screen.

Resolution:

Because the auto control is positive, a direct antiglobulin test is performed. The DAT is nonreactive, indicating that the patient's red cells are not coated with antibody in vivo.

The antibody screen is repeated at immediate spin and examined under the microscope.
The reactivity observed in the tubes is rouleaux.

A saline replacement is performed at immediate spin and the type and screen repeated.

Anti-A	Anti-B	Anti-D	A_1 Cells	B Cells
4+	0	3+	0	2+

Cell	Immediate Spin
Cell I	0
Cell II	0
Cell III	0
Auto	0

Case study 5

Anti-A	Anti-B	Anti-D	A_1 Cells	B Cells
4+	w+	0	0	4+

Initial hypothesis:

Forward: Group A Rh Negative

Reverse: Group A

ABO discrepancy: additional antigen

Resolution:

The patient's samples are verified. No discrepancies are note.

He has not been transfused in the past. Surgery is scheduled to investigate a potential bowel obstruction.

The antibody screen, including an auto control, is nonreactive.

A1 lectin typing is performed on the patient's red cells.This patient types as A1 positive. An additional source of monoclonal anti-B is used to type the patient's red cells. It is nonreactive with this source. The auto control is negative. This appears to be an example of acquired B antigen, possibly caused by the bacteria from the obstruction entering the patient's blood stream and deacetylating the patient's group A terminal antigen sugar.

Rh System

P. Fallah, Lab Hematologist

HISTORY

HDN was first described by a French midwife in 1609 in a set of twins, of whom one was hydropic and stillborn, and the other was jaundiced and died of kernicterus.

In 1939, Levine and Stetson described a woman who delivered a stillborn fetus and suffered a severe hemolytic reaction when transfused with blood from her husband. Her serum agglutinated the RBCs of her husband and 80 of 104 ABO-compatible donors.

In 1941, Levine and colleagues correctly concluded that the mother had been immunized by the fetus, which carried an antigen inherited from the father, and suggested that the cause of the *erythroblastosis fetalis was maternal* antibody in the fetal circulation.

HISTORY

Landsteiner and Wiener, in an effort to discover additional blood groups, injected rabbits and guinea pigs with rhesus monkey RBCs. The antiserum agglutinated not only rhesus cells but also the RBCs of 85% of a group of white subjects from New York, whom the researchers called *Rh positive; the remaining 15% were Rh negative.' Because* the *anti-Rhesus appeared to have reactivity indistinguishable* from the maternal antibody reported by Levine and Stetson, the antigen responsible for HDN was named *Rh.*

Later it was realized that the rabbit antiserum was not recognizing the same antigen but was detecting an antigen found in greater amounts on Rh-positive than on Rh-negative RBCs.This antigen was named LW for Landsteiner and Wiener, I and the original human specificity became known as anti-D.

The Rh blood group system is one of the most complex genetic polymorphisms in humans

The D antigen is the most clinically important antigen in the Rh blood group system

Antibody	Reaction	Reasult
Anti-D	+	Rh Positive
Anti-D	-	Rh Negative

Nomenclatures for Antigens of the Rh Blood Group system

WEINER	FISHER-RACE	ROSENFIELD
Rh_o	D	Rh1
rh′	C	Rh2
rh″	E	Rh3
hr′	c	Rh4
hr″	e	Rh5

Immunogenicity of Rh Antigens

D, c, E , C, e

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Weiner's Designation for Eight Common Rh Gene Complexes

Gene	Agglotinogen	Blood factor
r	rh	hr′, hr″
r′	rh′	rh′, hr″
r″	rh″	rh″, hr″
r^y	rh^y	rh′, rh″
R^0	Rh_0	Rh_0, hr′,hr″
R^1	Rh_1	Rh_0 rh′,hr″
R^2	Rh_2	Rh_0, rh″, hr′
R^z	Rh_z	Rh_0, rh′, rh″

Fisher-Race Genes and Antigens

Antibodies	Genes and Antigenes							
	CDe	cDE	cde	cDe	cdE	Cde	CDE	CdE
Anti-C	+	-	-	-	-	+	+	+
Anti-D	+	+	-	-	-	-	+	-
Anti-E	-	+	-	-	+	-	+	+
Anti-c	-	+	+	+	+	-	-	-
Anti-e	+	-	+	+	-	+	-	-

Numerical Term	ISBT Symbol
Rh1	D
Rh2	C
Rh3	E
Rh4	c
Rh5	e
Rh6	ce or f
Rh7	Ce
Rh8	C^w
Rh9	C^x
Rh10	V
Rh11	E^w
Rh12*	G
Rh17	Hr_0†
Rh18	Hr
Rh19	hr^s
Rh20	VS
Rh21	C^G
Rh22	CE
Rh23*	D^w
Rh26	c-like
Rh27	cE
Rh28	hr^H
Rh29	Rh29
Rh30	Go^a
Rh31	hr^B
Rh32	Rh32‡

Numerical Term	ISBT Symbol
Rh33	Rh33§
Rh34	Hr^B
Rh35	Rh35‖
Rh36	Be^a
Rh37	Evans
Rh39	Rh39
Rh40	Tar
Rh41	Rh41
Rh42	Rh42
Rh43	Crawford
Rh44	Nou
Rh45	Riv
Rh46	Sec
Rh47	Dav
Rh48	JAL
Rh49	STEM
Rh50	FPTT
Rh51	MAR
Rh52	BARC
Rh53	JAHK
Rh54	DAK
Rh55	LOCR
Rh56	CENR

Rosenfield Numerical Terminology
for **Rh Antigens**

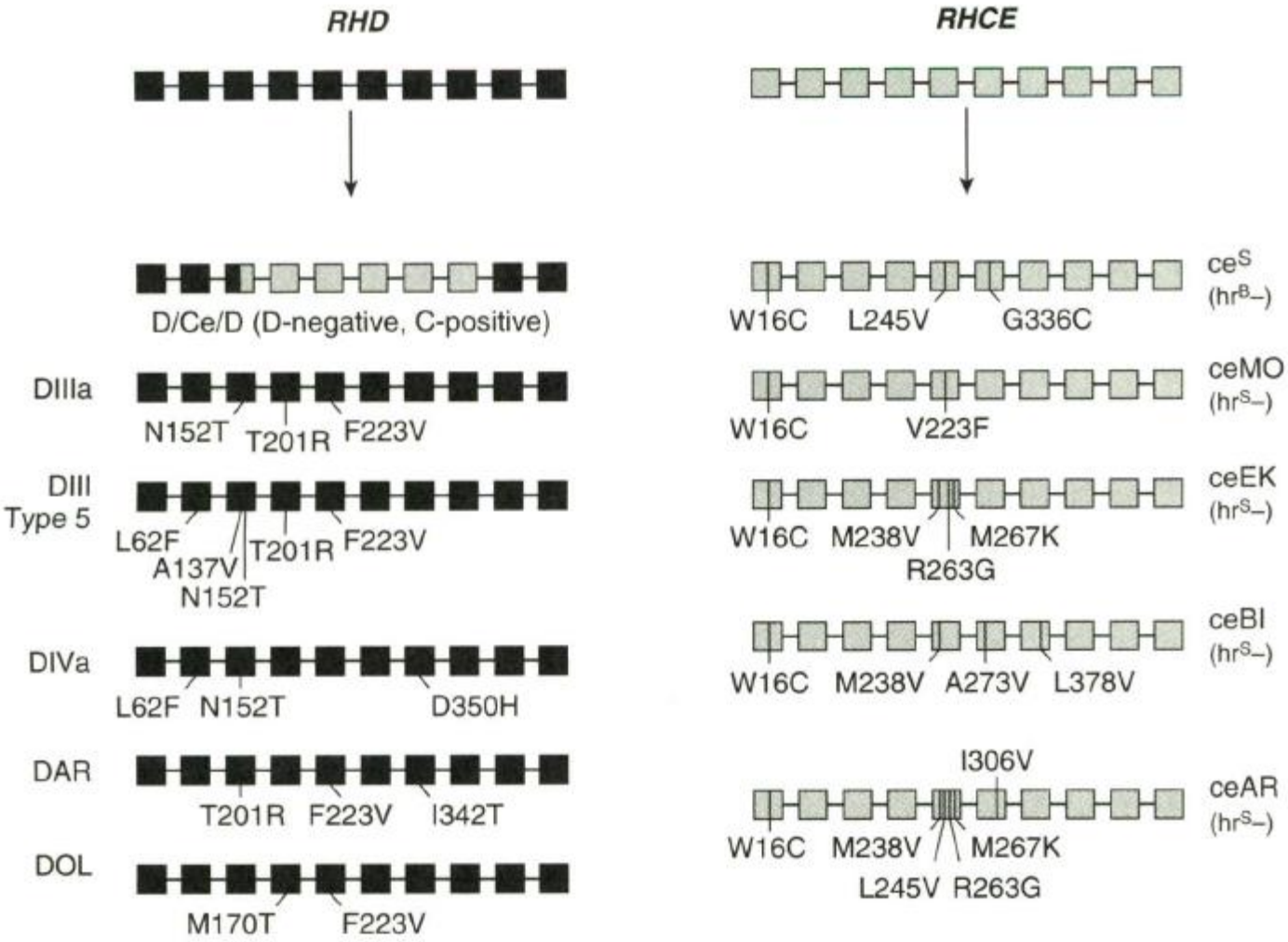
P. Fallah, Lab Hematologist

Molecular Basis of Some Rh Antigens, Partial D, and Unusual Phenotypes

Molecular Basis	Gene	Phenotype/Antigen/Genotype
Single point mutations	*RHD*	Partial D: DMH, D^{VII}, D+G–, DFW, DHR, D^{Va}, D^{HMi}, DNU, D^{II}, DNB, DHO Weak D (previously called D^{u})
	RHCE	C^{X}, C^{W}, Rh–26, E type I, IV, V+,VS+
Multiple mutations (gene conversions)	RHD	Partial D: D^{IIIa}, D^{IVa}, D^{Va}, DFR type I
	RHCE	E type III, IV, V+VS+
Rearranged gene(s) *RHD*	RHD-CE-D	Partial D: D^{IIIb}, D^{IIIc}, D^{IVb}, D^{Va}, D^{VI}, DFR type II, DBT r''^{G}, (Ce)Ce, (C)ce^{s} VS+V–
RHCE	*RHCE-D-CE*	D^{Har}, r^{G}, $\overline{\overline{R}}^{N}$
	RHD-CE	E type II
RHD: RHCE	*RHD-CE: RHCE-D*	DC^{W}–
	RHD: RHCE-D-CE	D– –, D••, Dc–
	RHD: RHD-CE	D••
	RHCE-D: RHD-CE	D••

P. Fallah, Lab Hematologist

Diagram of the *RHO* and *RHCE genes indicating changes*
often found in African backgrounds that complicate transfusion in sickle cell patients.



P. Fallah, Lab Hematologist

Composition (lgM and IgG Clones) and Reactivity of FDA-Licensed Anti-D Reagents withSome Rh Variant RBCs That Can Result in D Typing Discrepancies

Reagent	IgM Monoclonal	IgG	DVI	DBT	DHAR (Whites)	Crawford (Blacks)
Gammaclone	GAMA401	F8D8 monoclonal	Neg/Pos*	Pos	Pos	Pos
Immucor Series 4	MS201	MS26 monoclonal	Neg/Pos	Pos	Pos	Neg
Immucor Series 5	Th28	MS26 monoclonal	Neg/Pos	Pos	Vary/Pos	Neg
Ortho BioClone	MAD2	Polyclonal	Neg/Pos	Neg/Pos	Neg/Neg	Neg
Ortho Gel (ID-MTS)	MS201		Neg	Pos	Pos	Neg
Polyclonal			Neg/Pos	Neg/Pos	Neg/Neg	Neg/Neg

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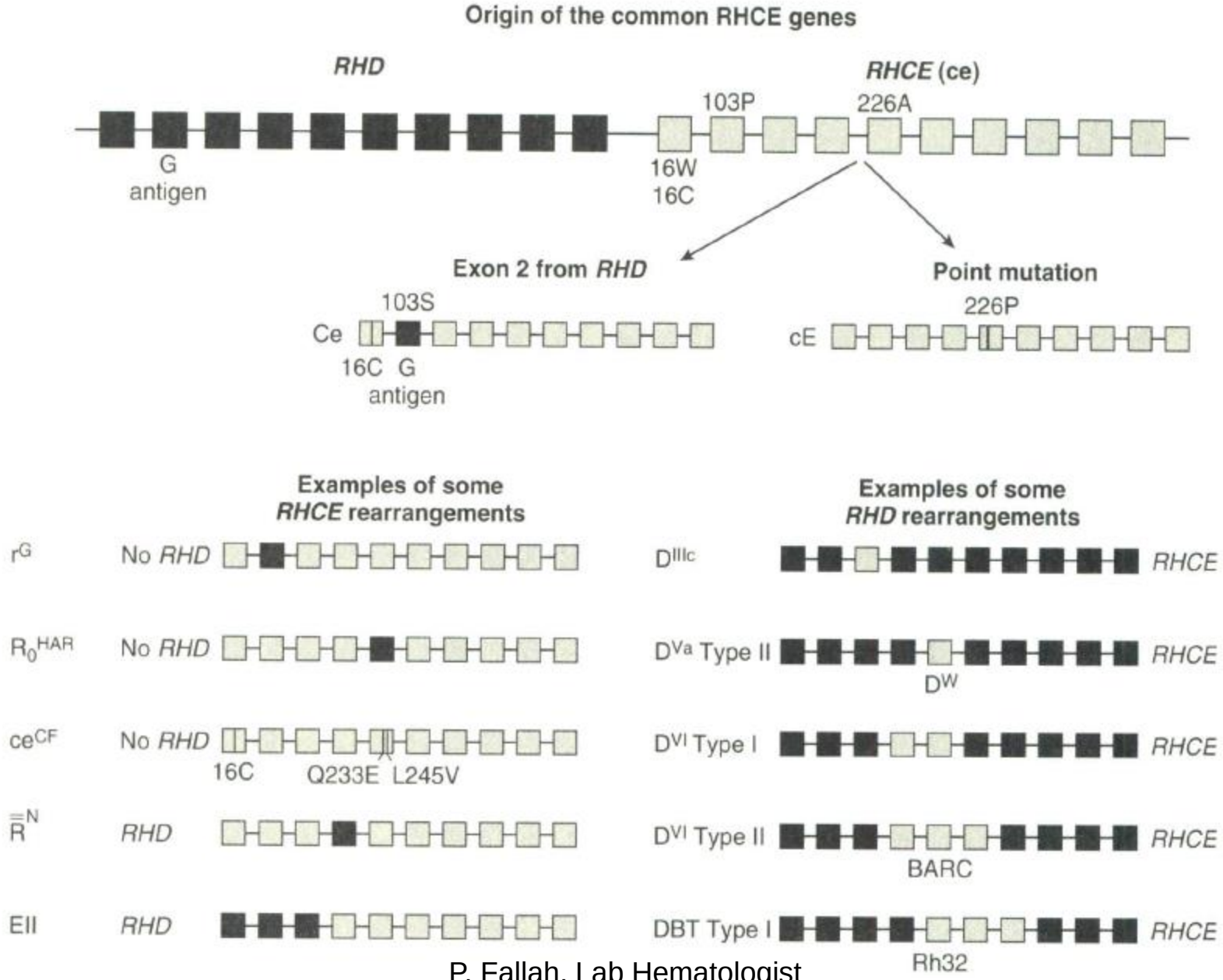

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Rh Genes and eight common Haplotypes Tippett Model

First locus	Second locus	Haplotype	Rh antigen
RHD	RHCe	R_1	D C e
RHD	RHcE	R_2	D c E
RHD	RHCE	R_z	D C E
RHD	RHce	R_0	D c e
----	RHCe	r'	C e
----	RHcE	r''	c E
----	RHCE	r^y	C E
----	RHce	r	c e

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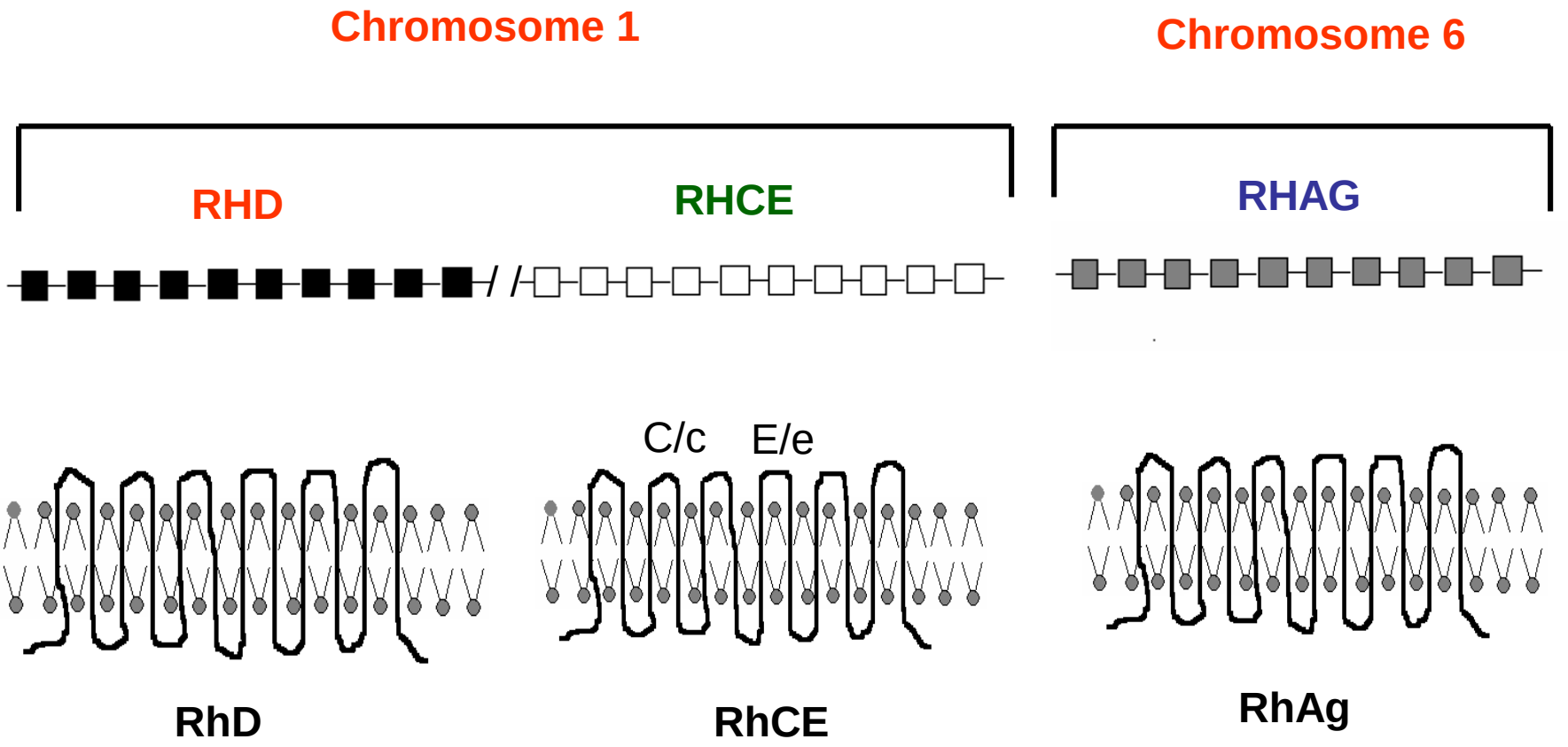

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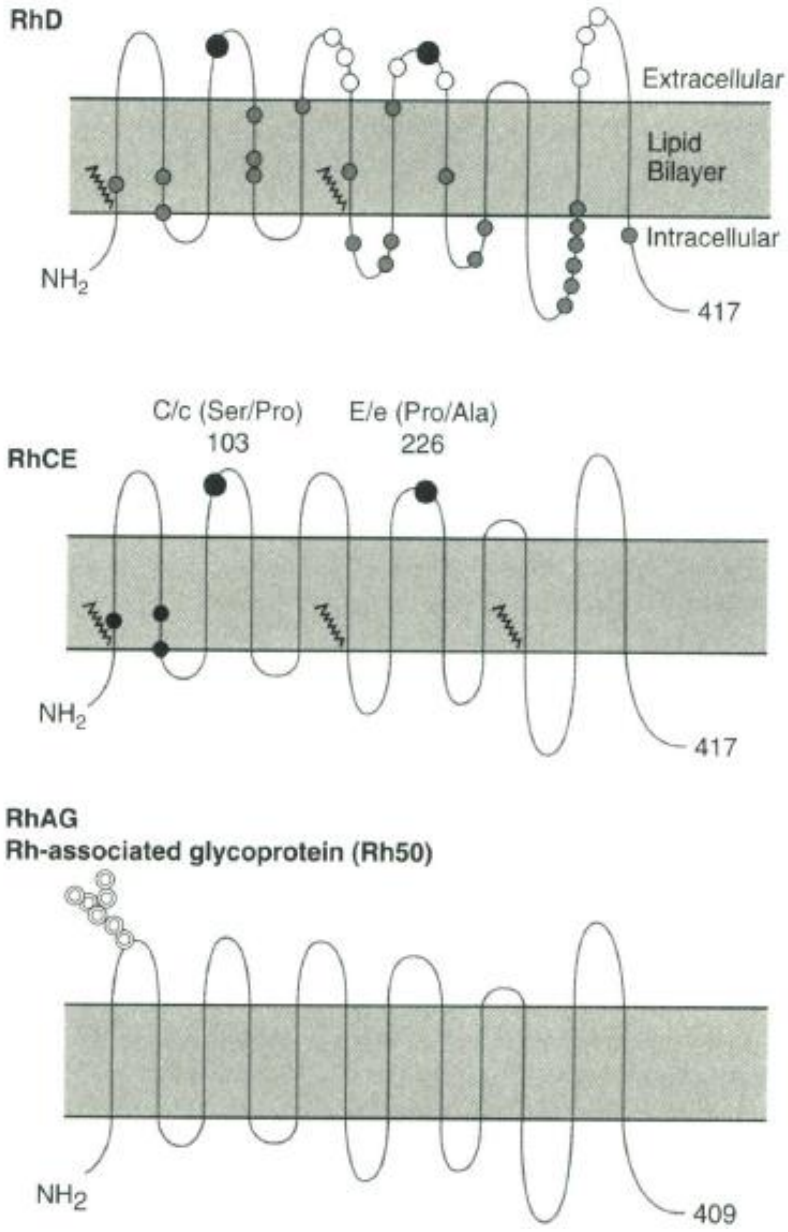

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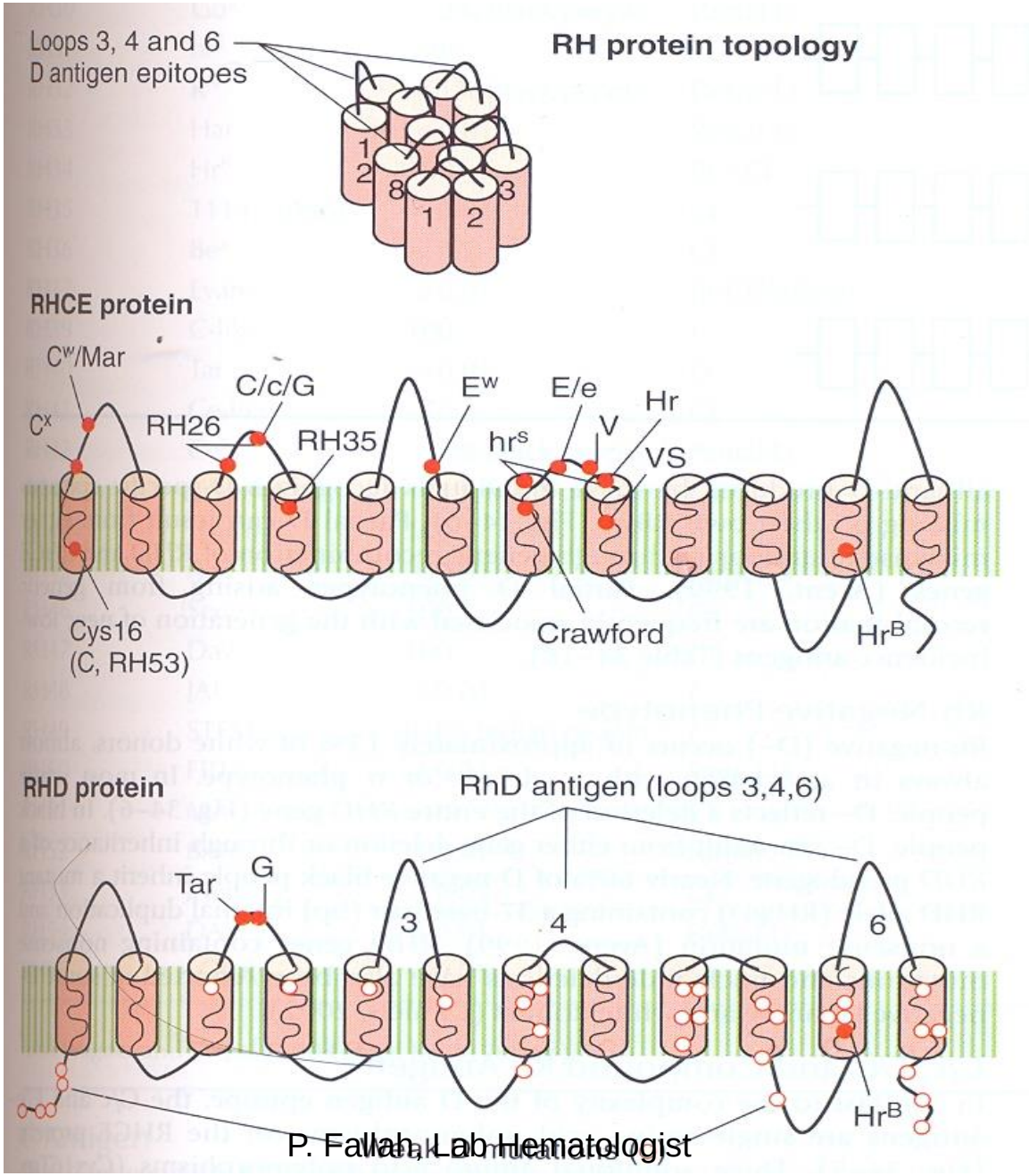


The Rh Haplotypes and their Frequencies

WIENER	FISHER-RACE	Ferequency		
		White	Black	Asian
R^0	Dce	0.04	0.44	0.03
R^1	DCe	0.42	0.17	0.70
R^2	DcE	0.14	0.11	0.21
R^z	DCE	0.00	0.00	0.01
r	dce	0.37	0.26	0.03
r′	dCe	0.02	0.02	0.02
r″	dcE	0.01	0.00	0.00
r^y	dCE	0.00	0.00	0.00

D+, C+, E-, c-, e+

D../D.. or D../...

DC./DC. or DC./.C.

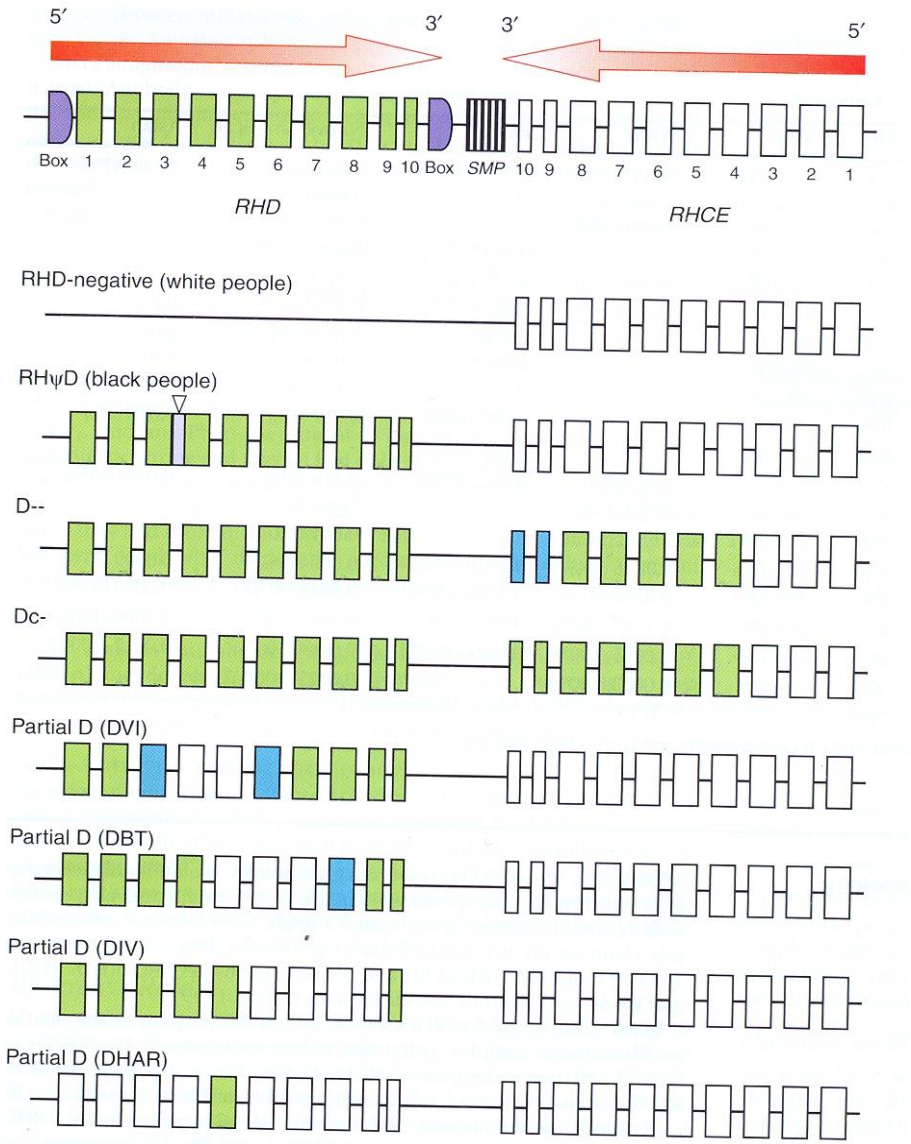
DCe/DCe or DCe/.Ce

R_1R_1 R_1r'

RHDRHCe / RHDRHCe RHDRHCe / ...RHCe

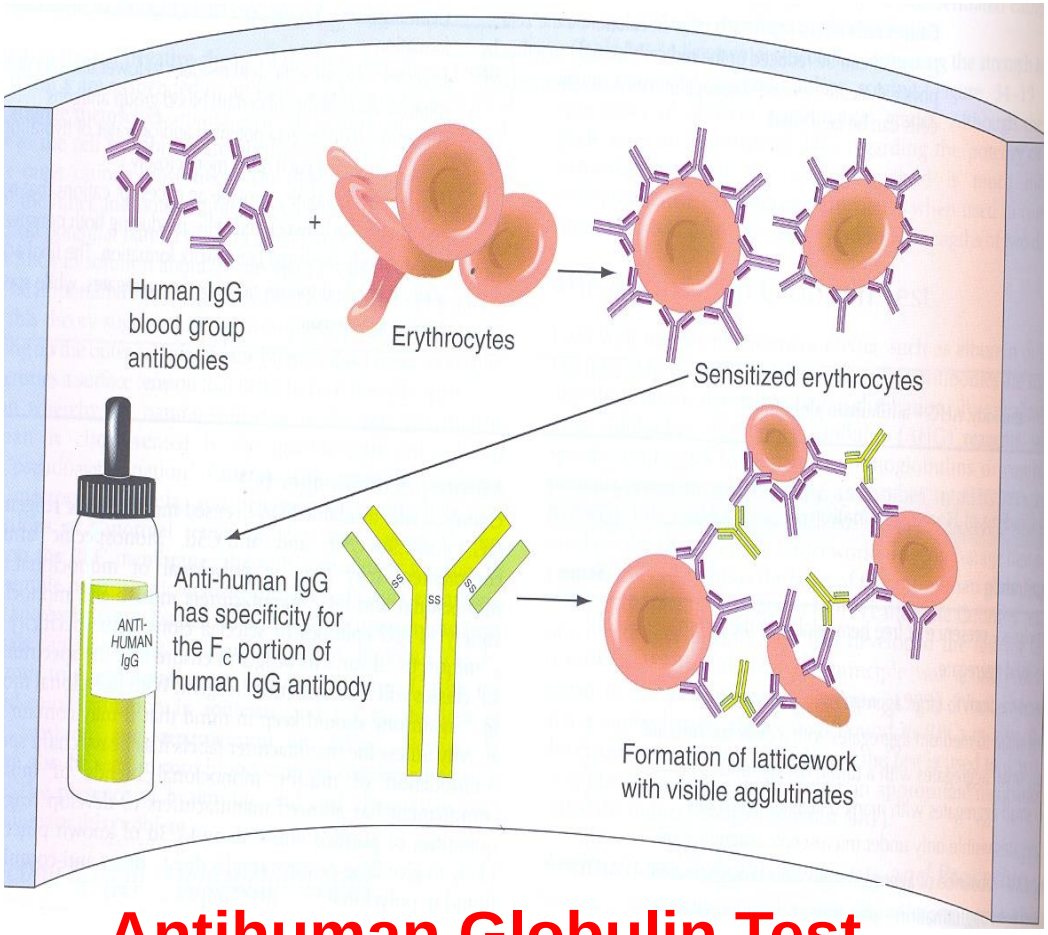
D Antigen

- **Weak D (D^u)**
  - **1% of D positive individuals**
  - **Weak or absent agglutination by anti-D in routine serologic testing, requires antihuman globulin (AHG)**
    - **Partial D**
    - **Autosomal recessive**
    - **dCe in trans with Dce, dCe/Dce**
- **Partial D (D variant; categories of D)**
- **Rh null**
  - **Amorph, Nonsense mutation in the RHCE gene in D-negative people**
  - **Regulator, Mutation in RHAG gene**
- **G antigen**
  - **Present on all D- and C-positive RBCs**
  - **Anti-G alloantibodies have both anti-D and anti-C spesificity**



The Rh Gene cluster and Partial D

P. Fallah, Lab Hematologist



Antihuman Globulin Test

P. Fallah, Lab Hematologist

Rh system antibodies

P. Fallah, Lab Hematologist

Rh system antibodies

- Immune stimulation
- IgG Isotype (IgG_1, IgG_2)
- Anti-C^w and –E can be naturally occuring
- Are reactive at 37ºC and detected in AHG phase
- Clinically importance, Associated with HDN

Thank you for attention

The Antiglobulin Test

Antiglobulin serum (Coombs' Serum) was discovered by Coombs in 1945.

The antiglobulin test can be used to detect red cells sensitized with IgG alloantibodies, IgG autoantibodies or complement components.

Sensitization of red cells can occur in vivo or vitro.

The use of AHG serum to detect sensitization of red cells in vitro can be:

- One stage technique , the direct antiglobulin test (DAT).
- Two stage technique , the indirect antiglobulin test (IAT).

PRINCIPLE

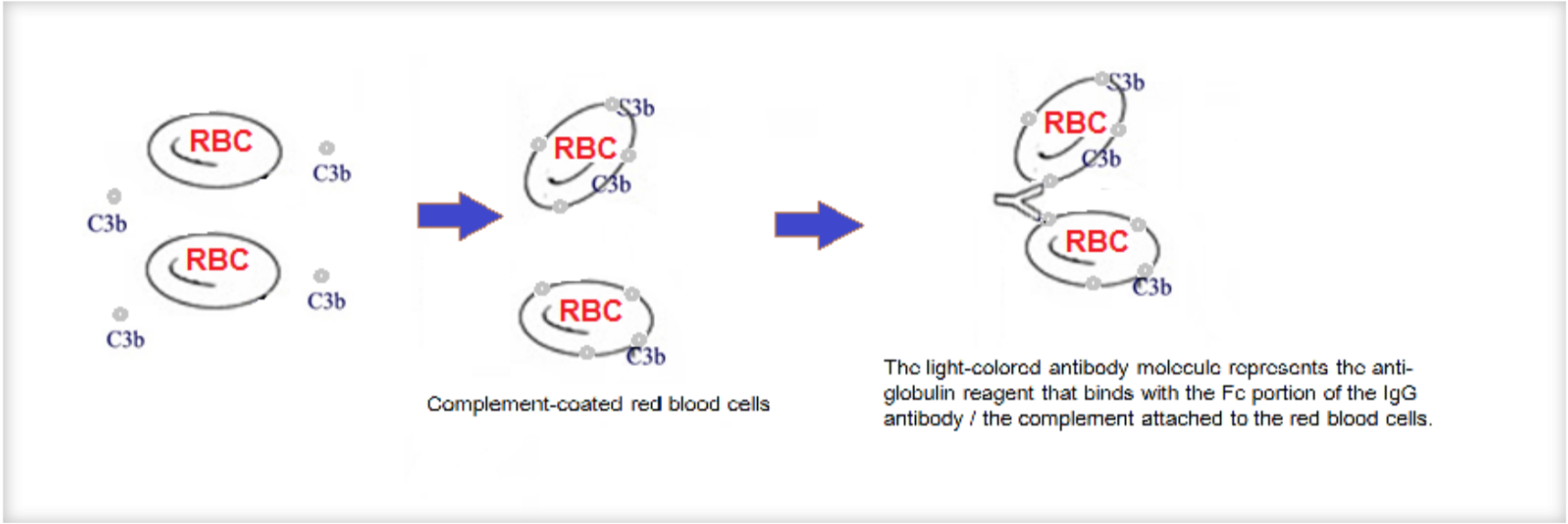
Normal human red blood cells, in presence of antibody directed towards the antigen they possess, may fail to agglutinate when centrifuged and become sensitized. This may be due to the particular nature of the antigen and antibody involved.

Sensitization of RBC's may be with IgG or complement.

In order for agglutination to occur an additional of anti-antibody or anti-complements, which reacts with the Fc portion of the IgG antibody, or with the C3b or C3d component of complement alternatively.

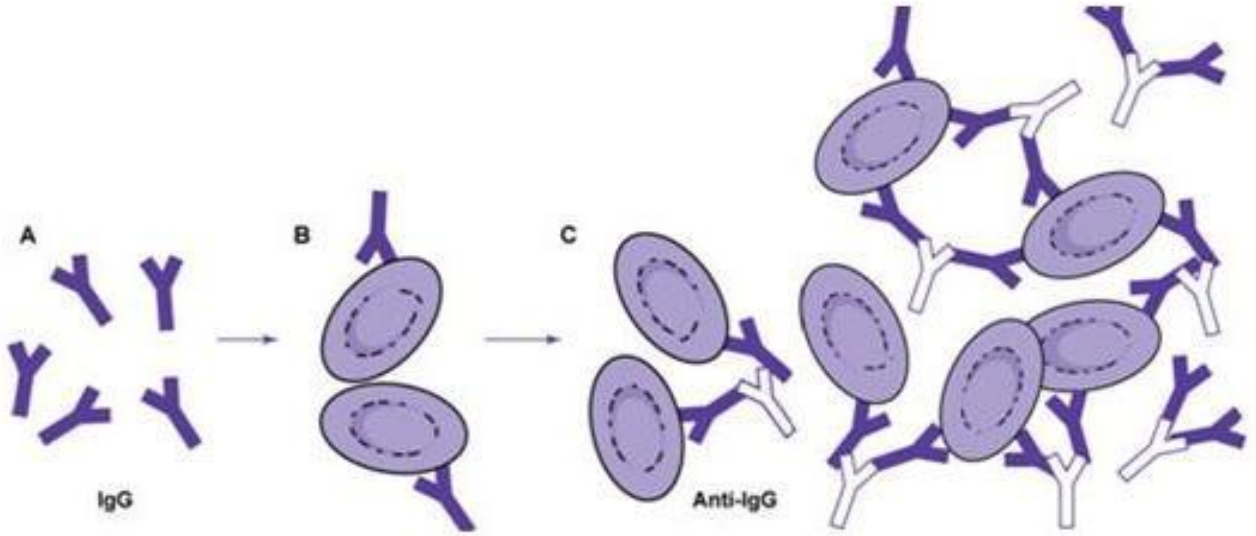
PRINCIPLE

- This will form a "bridge" between the antibodies or complement coating the red cells, causing agglutination.
- The coating (sensitization) of red cells can occur **in vivo or in vitro** following incubation at 37°C with serum containing antibody.



Production Methods of Anti-Human globulin (AHG or Coombs) Reagent

- May be made by injecting rabbits , goats or sheep with purified human IgG or C3, then harvesting the antibodies produced by the rabbit
- Mon
  mon



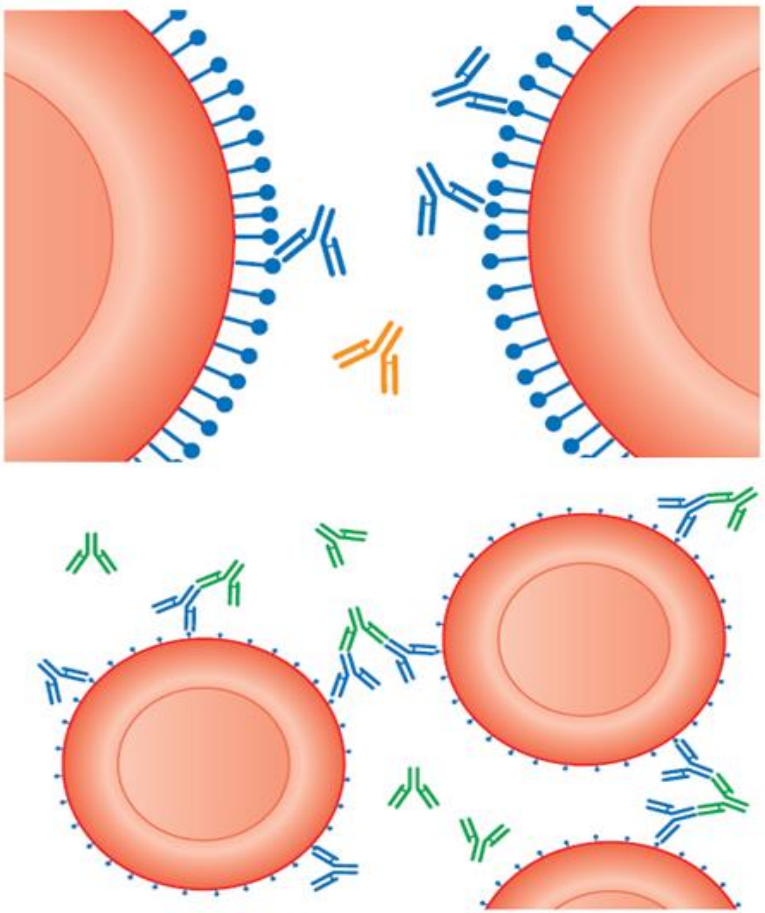
آنتی IgG بین قسمت های FC مولکول های IgG بر سطح گلبول های قرمز پل زده و آنها را آگلوتینه می کند

Types of AHG reagent

- Polyspecific Anti-human Globulin: blend of Anti-IgG and Anti-C3b, -C3d
- Monospecific reagents: Anti-IgG alone or Anti-C3b,-C3d alone

Note: Reagent does not contain antibodies to IgM. Information about IgM coating of cells comes from the presence of C3 coating the cells since IgM is a strong complement activator.

DIRECT ANTIGLOBULIN TEST (DAT)



Cells coated *in vivo*

Washed to remove unbound globulins

Addition of anti-human globulin (AHG) promotes agglutination after centrifugation

DAT

- The direct antiglobulin test (DAT) detects sensitized red cells with IgG and/or complement components C3b and C3d in vivo.
- In vivo coating of red cells with IgG and/or complement may occur in any immune mechanism is attacking the patient's own RBC's.
- These mechanism could be:
  - Autoimmunity
  - Alloimmunity
  - Or a drug-induced immune-mediated mechanism.

Examples of alloimmune hemolysis

- Hemolytic transfusion reaction
- Hemolytic disease of the newborn (also known as HDN or erythroblastosis fetalis)
  - *Rhesus D* hemolytic disease of the newborn (also known as Rh disease)
  - *ABO* hemolytic disease of the newborn (the indirect Coombs test may only be weakly positive)
  - *Anti-Kell* hemolytic disease of the newborn
  - *Rhesus c, E* hemolytic disease of the newborn

Examples of autoimmune hemolysis

- Warm antibody autoimmune hemolytic anemia
  - Idiopathic
  - Systemic lupus erythematosus
  - Evans' syndrome (antiplatelet antibodies and hemolytic antibodies)
- Cold antibody autoimmune hemolytic anemia
  - Idiopathic cold hemagglutinin syndrome
  - Infectious mononucleosis
  - Paroxysmal cold hemoglobinuria (rare)

Drug-induced immune-mediated hemolysis

- Methyldopa (IgG mediated type II hypersensitivity)
- Penicillin (high dose)
- Quinidine (IgM mediated activation of classical complement pathway and Membrane attack complex)

Blood Sample

Whole Blood Sample - It should be as fresh as possible not more than 24 hours old,

otherwise, the sample should be taken in EDTA.

Tak

Wa emove
free wash.
Co

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Mix

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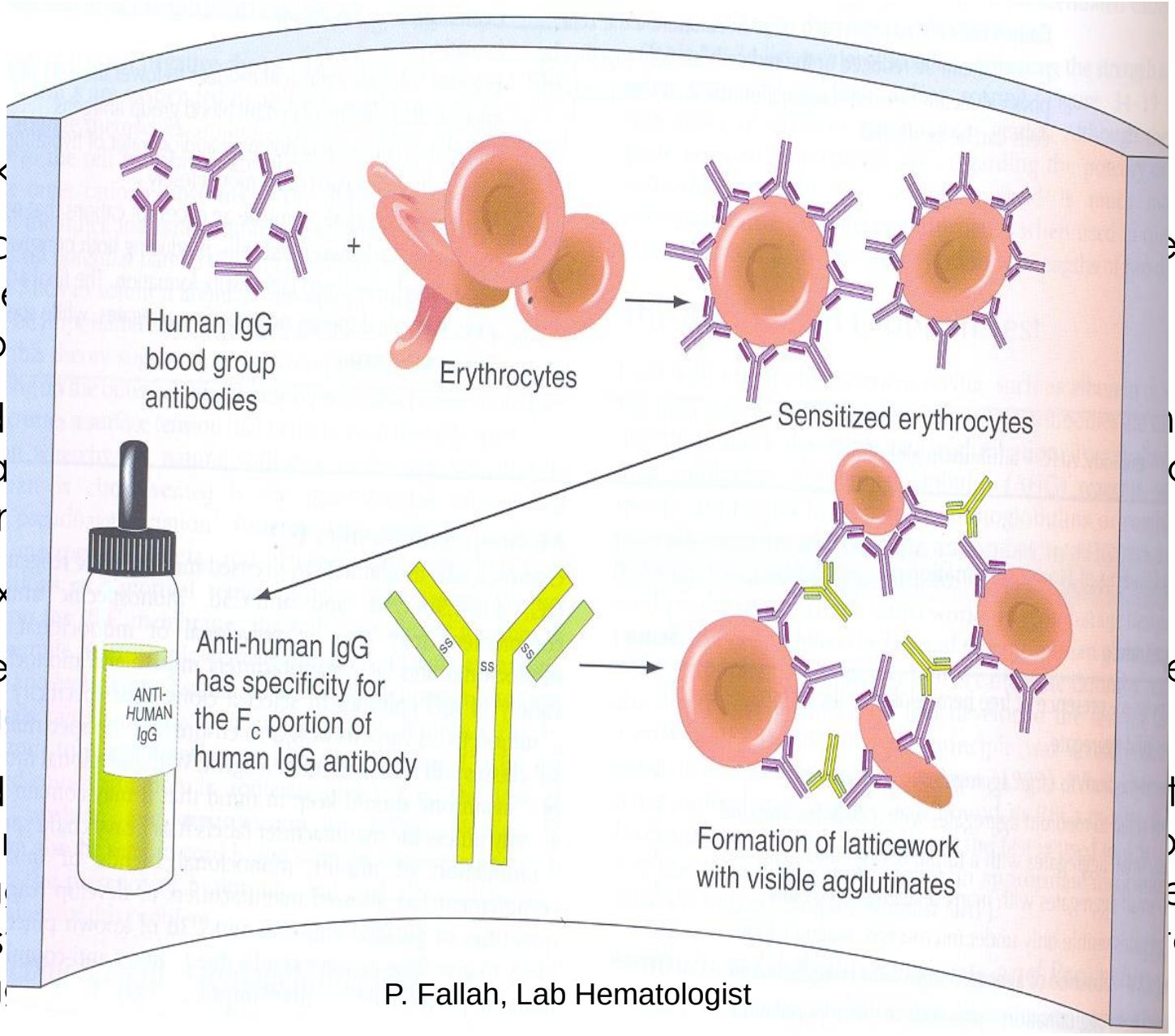

Indirect Antihuman globulin Test (IAT)

- Inc
  The
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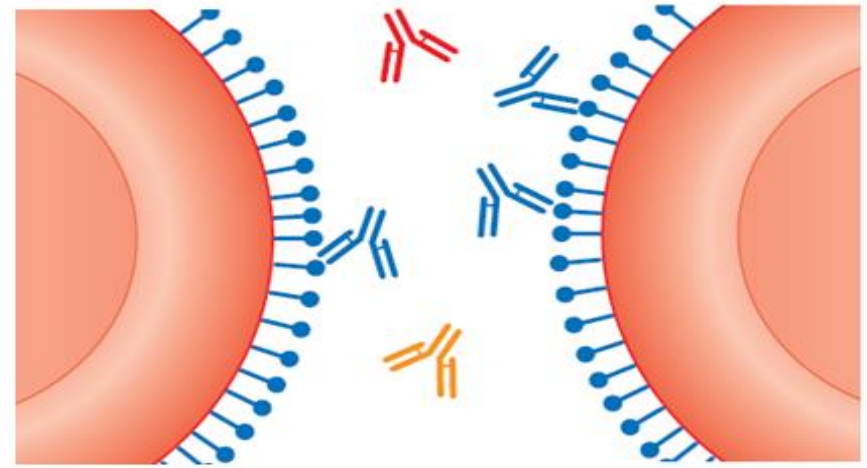
  3.D
  L
  e

	Rh							MNSs				P_1	Lewis		Lutheran		Kell		Duffy		Kidd	
Cell	D	C	E	c	e	f	C^w	M	N	S	s	P_1	Le^a	Le^b	Lu^a	Lu^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b
I R1R1 (56)	+	+	0	0	+	0	0	+	+	0	+	0	+	0	0	+	+	+	+	0	+	+
II R2R2 (89)	+	0	+	+	0	0	0	0	+	+	0	+	0	+	0	+	0	+	0	+	+	0

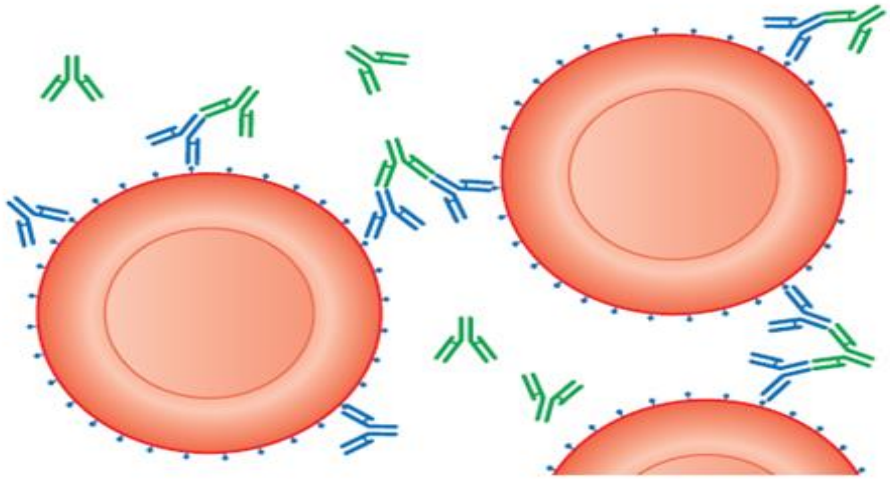
آنتی ژن های شایع و مهم از نظر بالینی در دو تا سه ویال برای غربال کردن (اسکرین) آنتی بادی ها عرضه می گردد . گلبول های معرف اسکرین از گروه O بوده و نوع مرغوب آن دارای آنتی ژن های هموزیگوت برای شناسایی آنتی بادی های با خاصیت دوزاژ است . در تهیه ویال ها تلاش بر آن است که هر ویال از یک اهدا کننده تهیه گردد تا حساسیت آزمایش افزایش یابد . سلول های معرف اسکرین داری نگه دارنده بوده و ممکن است تا سه هفته در ۴ درجه قابل استفاده باشند.

Indirect antiglobulin test



Serum with specific antibody mixed with reagent red cells

Washed x3 after incubation to remove unbound globulins



Anti-human globulin (AHG) added to promote agglutination on centrifugation

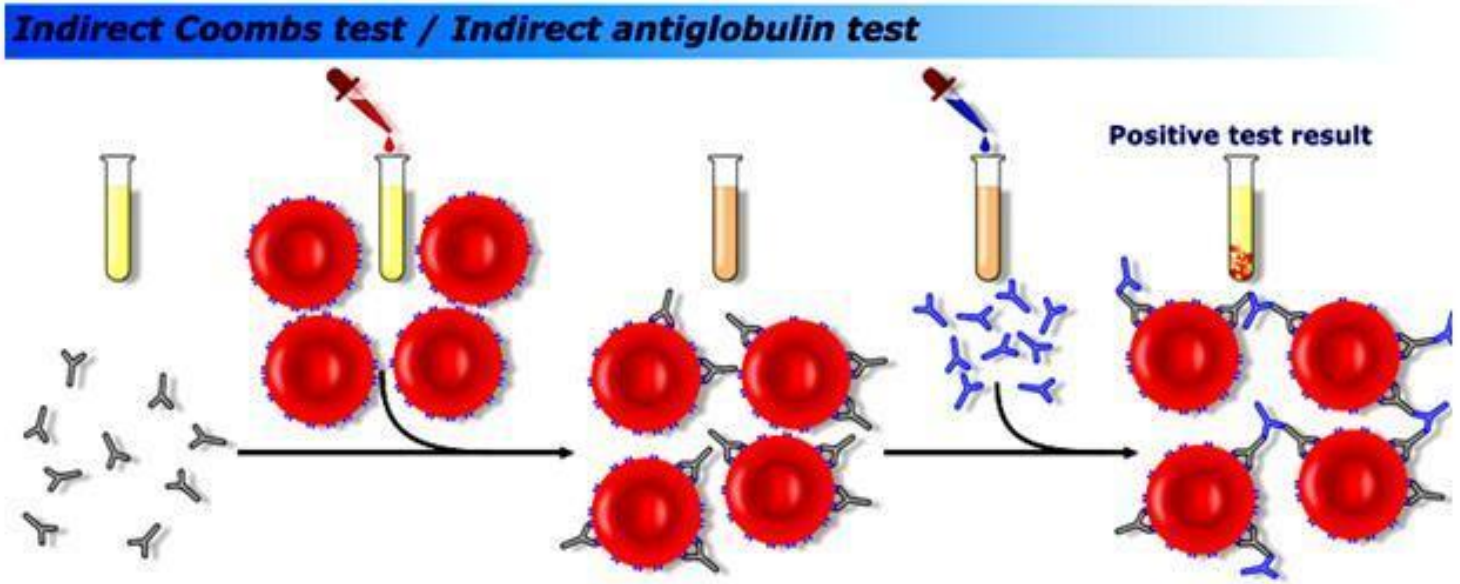
Procedure:

1. Place 2-3 drops of the test serum in a tube. Serum should be fresh for detecting complement components and complement binding antibodies, otherwise, fresh AB serum should be added to it.
2. Add 1 drop of 3-5% suspension of washed O Rh (D) positive red cells to the serum in the tube.
3. Mix and incubate at 37°C for 30-40 minutes.
4. Centrifuge at 1000 rpm for 1 minutes.
5. Examine for hemolysis and/or agglutination. Use optical aid if necessary. Agglutination at this stage indicates the presence of saline (complete) antibodies.
6. If no agglutination is seen, wash cells 3-4 times in large volume of saline. Decant supernatant in each wash as completely as possible.

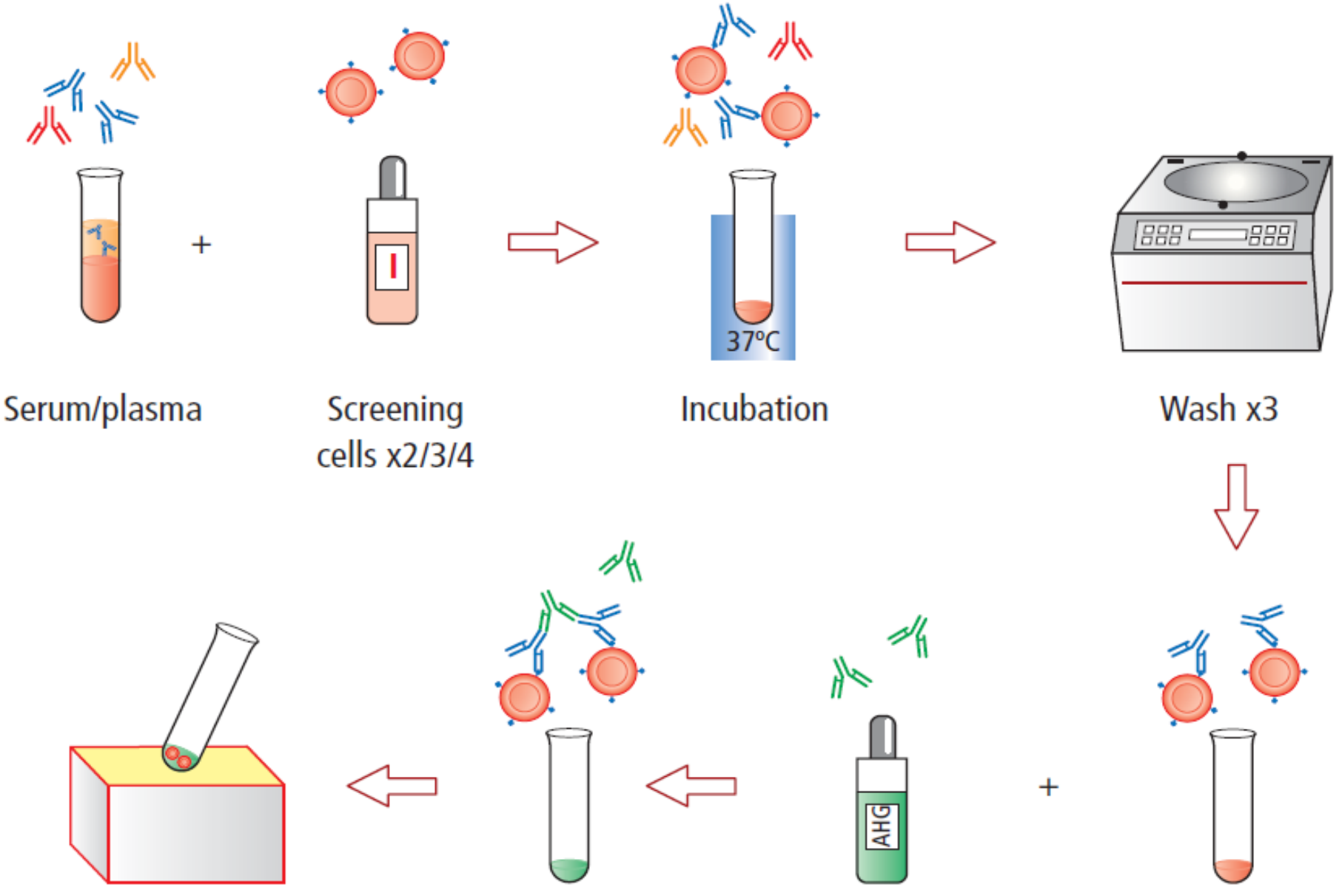
Procedure:

7. Add 2 drops of AHG serum to the cells.
8. Mix and centrifuge at 1000 rpm for 1 minutes immediately.
9. Gently shake the tube to dislodge the button and examine for agglutination, using optical aid. Record the result.
10. Add 1 drop of IgG coated red cells to any test that

11



در آزمایش کومبز غیر مستقیم سرم بیمار با گلبول های قرمز اسکرین مجاور شده و واکنش آنتی ژن – آنتی بادی توسط آنتی هیومن گلوبولین نمایان می گردد.



Serum/plasma

Screening cells x2/3/4

Incubation

Wash x3

Only bound antibody on RBC

Addition of AHG

Centrifugation → agglutination

Resuspend, read over light source

BOVINE ALBUMIN(22%)-IAT

One Stage Method - Additive method

- Procedure:
  1. Two drops of albumin 22.5% are added in step (2) of saline-IAT
  2. Mix and incubate for 20-30 minutes at 37°C
  3. Proceed further as in saline-IAT procedure.

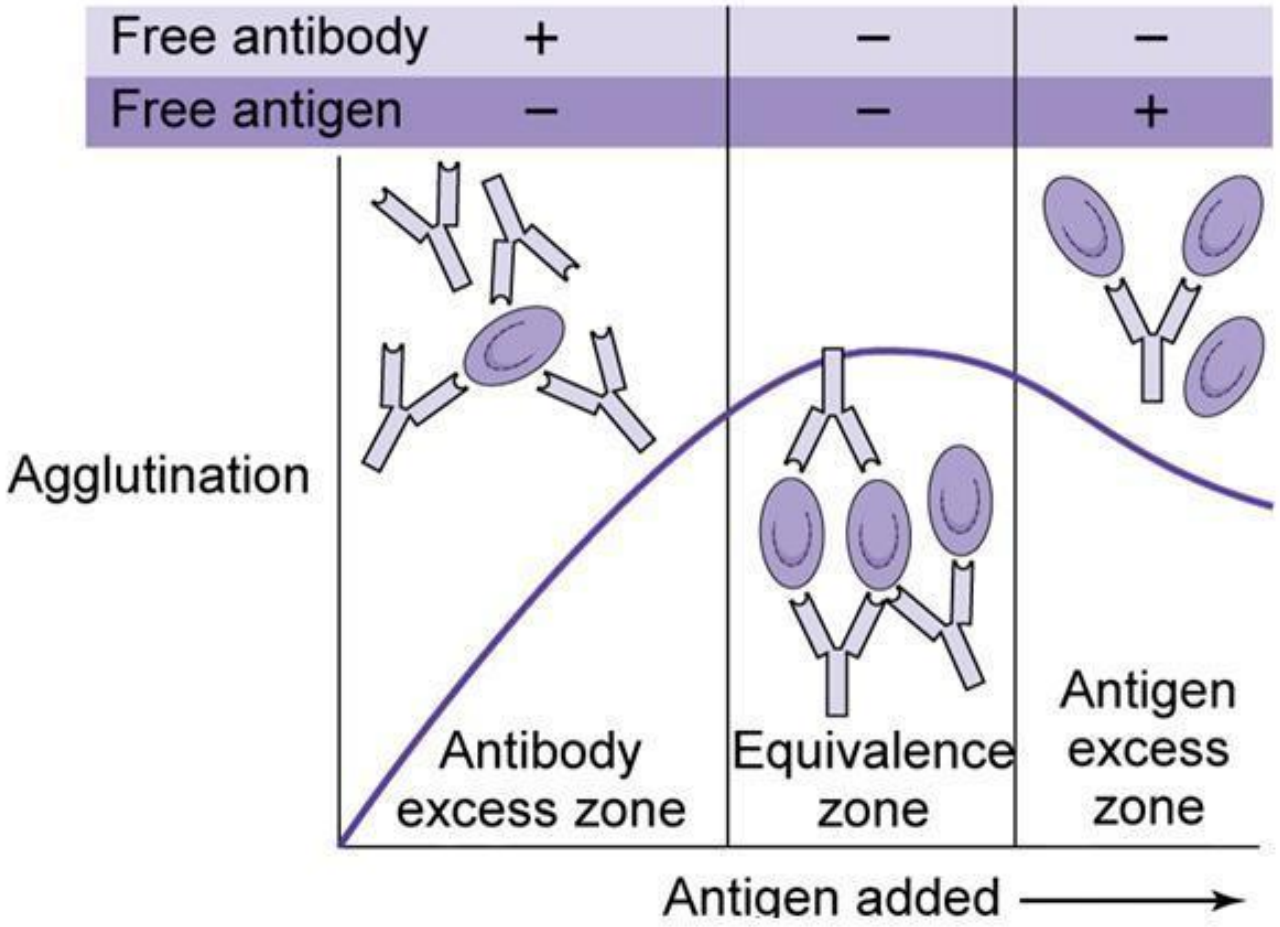
Sources of Error in AHG tests

False negative results: General DAT & IAT

- Failure to wash red blood cells adequately, since globulins not bound to RBCs will neutralize the AHG reagent.
  - The washing process and the addition of AHG reagent must be undertaken as quickly as possible to minimize loss of bound antibodies by elution.
- Improper storage, bacterial contamination and contamination with human serum will impair the AHG reagent activity.
- Not adding the AHG reagent
- Improper centrifugation
- Number of cells present in the test:
  - too many cells give weak reactions
  - too few cells will impair the reading of the agglutination

Antigen-Antibody Ratio

- The optimum ratio is 80 parts antibody to 1 part antigen. There are specific terms for variations in this ratio.
  - **Prozone - antibody excess:** Antibodies saturating all antigen sites; no antibodies forming cross-linkages between cells; no agglutination
  - **Zone of equivalence:** antibodies and antigens present in optimum ratio, agglutination formed
  - **Zone of antigen excess (Post-zone):** too many antigens - any agglutination is hidden by masses of unagglutinated antigens



در آزمایش های سرولوژی بانک خون دو حجم سرم به یک حجم سوسپانسیون ۲ تا ۵٪ گلبول های قرمز ، برای تعادل بهتر واکنش آنتی ژن – آنتی بادی اضافه می گردد.

False negative results

DAT

- All samples negative at the AHG phase should be incubated at room temperature for 5 minutes to achieve maximal sensitivity needed for complement detection.

IAT

- Serum and/or rbcs lose reactivity if improperly stored.
- Plasma used instead of serum can lead to failure to detect antibodies depending on presence of active complement (anti-Jk^a, -Jk^b)
- Temperature and incubation time affect attachment of antibody or complement to cells.
- An optimal proportion of serum to cells should be achieved: usually 2-3 drops serum to one drop of 5% cell suspension.

False positive results:

DAT and IAT ;

- In specimens containing potent cold-reactive antibodies agglutination may occur before adding the AHG reagent.
- Dirty glassware may cause clumping of cells.
- Over centrifugation

DAT

- A positive DAT from a clotted sample should be repeated on an EDTA sample
- Samples collected from infusion lines may have complement present on the cells.

IAT

- Cells with a positive DAT will give a positive result in any indirect antiglobulin procedure.

COOMB'S CELLS

To show that test cells were properly washed and that no neutralization or reagent deterioration has occurred, antibody-coated cells are used as a positive indicator.

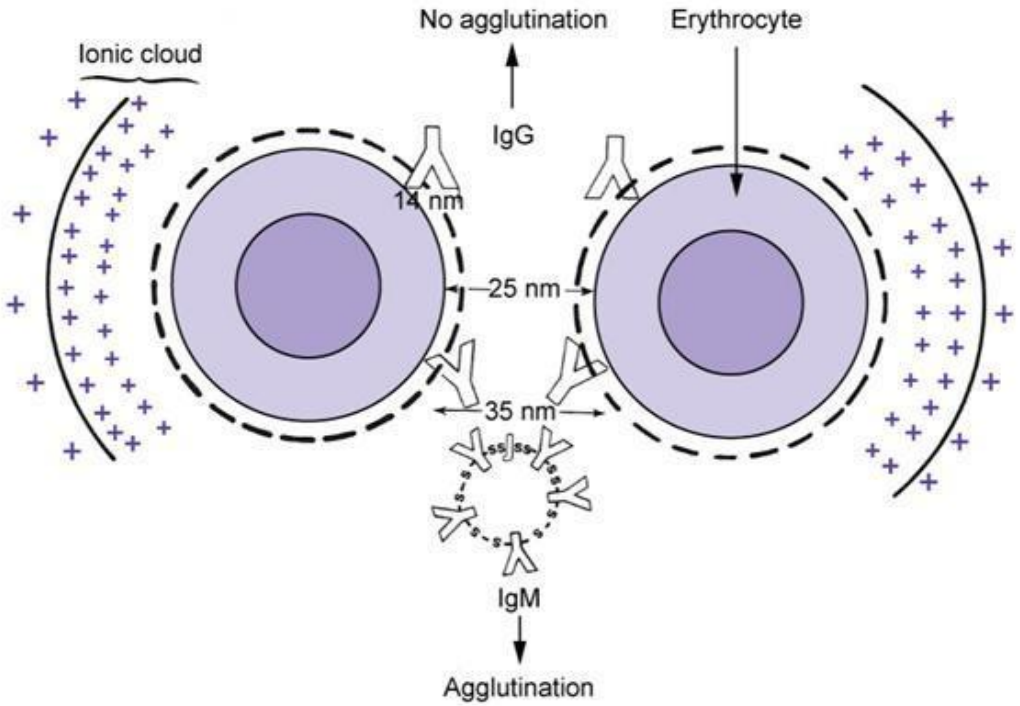
In a negative antiglobulin test the anti-human globulin should remain active and this can be demonstrated by the addition of IgG sensitized cells.

Agglutination of the IgG sensitized cells after mixing and centrifuging confirms that the anti-human globulin was added to the test, that the test cells were properly washed and all free globulin molecules were removed and that the anti-human globulin was active.

Failure of the IgG sensitized cells to agglutinate indicates that the original negative antiglobulin test result is not valid and testing must be repeated.

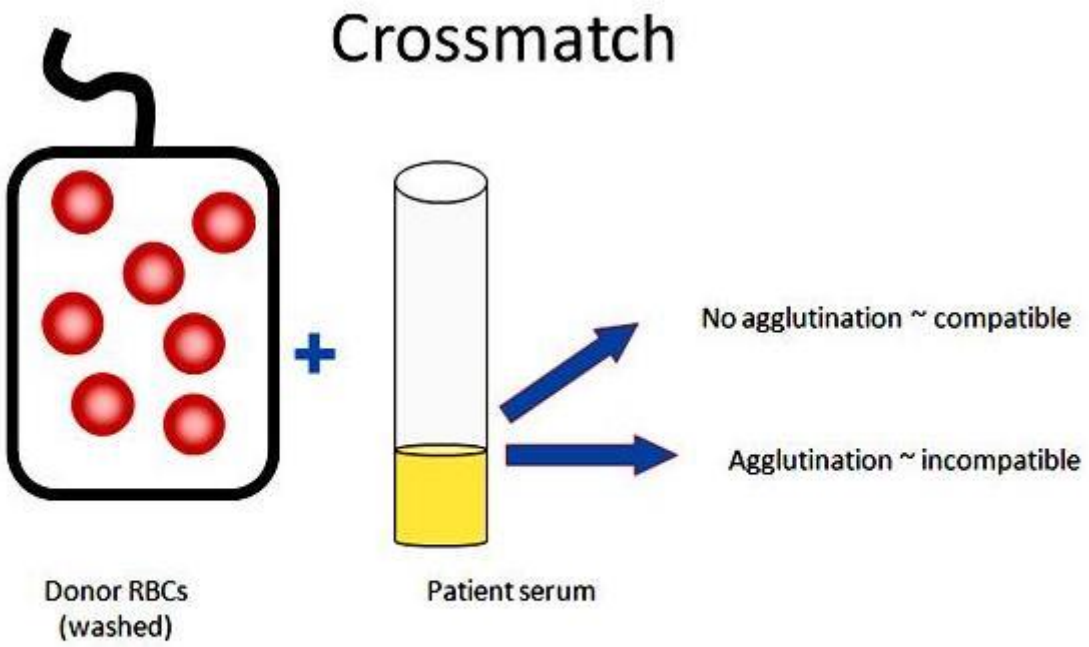
Preparation of Coomb's cells

- Preparing Coombs control cells is very easy. To about 10 drops of washed O Positive red cells add 5-6 drops of anti-D antisera. Incubate at 37C for 15 minutes. Wash 4 times then prepare a 3 to 5% cell suspension.
- To verify reaction, add two drops of AHG into test tube and one drop of newly prepared Coombs cells.
- Centrifuge on High speed for 15 seconds , You should get 1-2 + reaction.



کاهش قدرت یونی با کاهش پتانسیل زتا (نیروی الکترواستاتیک دافعه گلبول های قرمز) موجب برخورد بیشتر آنتی ژن با آنتی بادی می گردد.

P. Fallah, Lab Hematologist



برای شناسایی آنتی بادی های آگلوتینه کننده، همولیز کننده و آغشته کننده احتیاج به مجاورت سرم با گلبول اهدا کننده در حرارت اتاق (RT)، ۳۷ درجه و مرحله آنتی هیومن گلوبولین است.

P. Fallah, Lab Hematologist

مجاورت سرم بیمار با گلبول های معرف اسکرین

Screen Cells	IS	37°C	AHG
Vial I	0	0	0
Vial II	0	0	+2

Direct Coomb´s test: Negative

مجاورت سرم بیمار با گلبول های معرف اسکرین

Screen Cells	IS	37°C	AHG
Vial I	0	0	+3
Vial II	0	+2	+3

Direct Coomb´s test: Negative

مجاورت سرم بیمار با گلبول های معرف اسکرین

Screen Cells	IS	37°C	AHG
Vial I	+1	0	0
Vial II	+2	0	0

Direct Coomb´s test: Negative

مجاورت سرم بیمار با گلبول های معرف اسکرین

Screen Cells	IS	37°C	AHG
Vial I	+1	0	0
Vial II	+1	0	0

Direct Coomb´s test: +1

مجاورت سرم بیمار با گلبول های معرف اسکرین

Screen Cells	IS	37°C	AHG
Vial I	0	0	+2
Vial II	0	0	+2

Direct Coomb´s test: +2