SAPIENZA
UNIVERSITÀ DI ROMA

The ABO and Rh system

Transfusion Medicine

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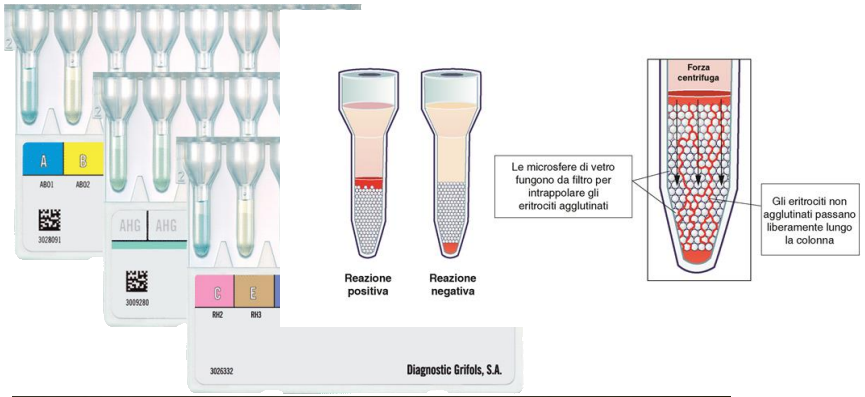
10 November 2023

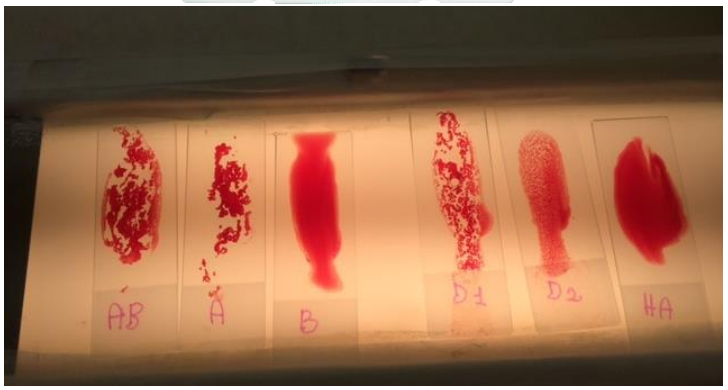
Main learning endpoints!

- ✓ **Chemical structure**
- ✓ **Inheritance pathways**
- ✓ **AB0 and Rh antibodies and their importance in transfusion**
- ✓ **Principles of AB0 and Rh typing**

Immunohematology and Transfusion Medicine- Lab



HEMAGGLUTINATION IS THE PROCESS BY WHICH RED BLOOD CELLS AGGLUTINATE, MEANING CLUMP OR CLOG. THE AGGLUTIN INVOLVED IN HEMAGGLUTINATION IS CALLED HEMAGGLUTININ.



HEMOLYSIS IS THE RUPTURING (LYSIS) OF RED BLOOD CELLS AND THE RELEASE OF THEIR CONTENTS (HB) INTO THE PLASMA.

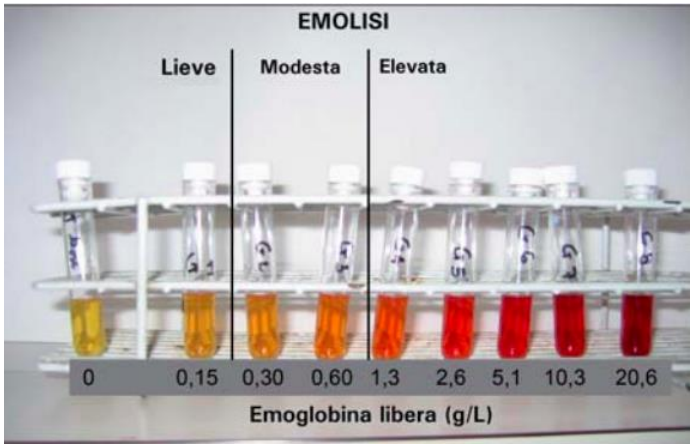


TABLE OF BLOOD GROUP ANTIGENS

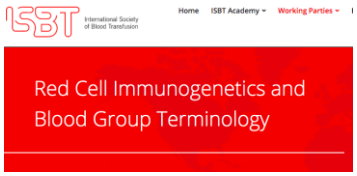
The red cell membrane contains many anchored surface proteins and proteins that cross the lipid bilayer. Many of these proteins are polymorphic and carry different blood groups.

Today, 38 registered blood group systems, with more than 300 antigens

The ABO, the first blood group discovered system, still represent the most important

System		Antigen number												Total
		001	002	003	004	005	006	007	008	009	010	011	012	
001	ABO	A	B	A,B	A1	...								4
002	MNS	M	N	S	s	U	He	Mi^a	M^c	Vw	Mur	M^g	Vr	49
003	P1PK	P1	...	P^k	NOR									3
004	RH	D	C	E	c	e	f	Ce	C^w	C^x	V	E^w	G	55
005	LU	Lu^a	Lu^b	Lu3	Lu4	Lu5	Lu6	Lu7	Lu8	Lu9	...	Lu11	Lu12	27
006	KEL	K	k	Kp^a	Kp^b									
007	LE	Le^a	Le^b	Le^{ab}	Le^{bH}									
008	FY	Fy^a	Fy^b	Fy3	...									
009	JK	Jk^a	Jk^b	Jk3										
010	DI	Di^a	Di^b	Wr^a	Wr^b									
011	YT	Yt^a	Yt^b	YTEG	YTLI									
012	XG	Xg^a	CD99											
013	SC	Sc1	Sc2	Sc3	Rd									
014	DO	Do^a	Do^b	Gy^a	Hy									
015	CO	Co^a	Co^b	Co3	Co4									
016	LW	...	...	...	...									
017	CH/RG	Ch1	Ch2	Ch3	Ch4									
018	H	H												
019	XK	Kx												

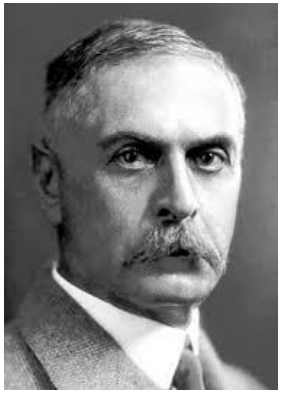
System		Antigen number												Total in system
		001	002	003	004	005	006	007	008	009	010	011	012	
020	GE	...	Ge2	Ge3	Ge4	Wb	Ls^a	An^a	Dh^a	GEIS	GEPL	GEAT	GETI	11
021	CROM	Cr^a	Tc^a	Tc^b	Tc^c	Dr^a	Es^a	IFC	WES^a	WES^b	UMC	GUTI	SERF	20
022	KN	Kn^a	Kn^b	McC^a	Sl1	Yk^a	McC^b	Sl2	Sl3	KCAM	KDAS			10
023	IN	In^a	In^b	INFI	INJA	INRA	INSL							6
024	OK	Ok^a	OKGV	OKVM										3
025	RAPH	MER2												1
026	JMH	JMH	JMHK	JMHL	JMHG	JMHM	JMHQ	JMHN						7
027	I	I												1
028	GLOB	P			PX2									2
029	GIL	GIL												1
030	RHAG	Duclos	Ol^a	DSLK†	... §									3
031	FORS	FORS1												1
032	JR	Jr^a												1
033	LAN	Lan												1
034	VEL	Vel												1
035	CD59	CD59.1												1
036	AUG	AUG1	At^a	ATML	ATAM									4
037	KANNO	KANNO1												1
038	SID	Sd^a												1

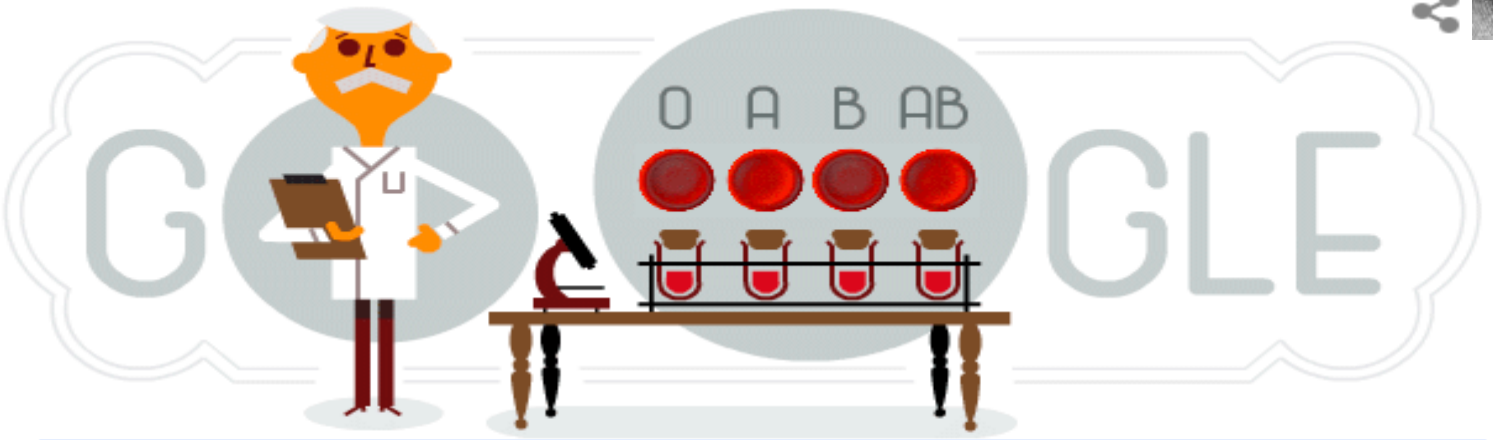


History

- **1900** Discovery of the AB0 system (K. Landsteiner)
- **1907** the first successful blood transfusion was performed by Reuben Ottenberg at Mount Sinai Hospital in New York
- **1915** The use of sodium citrate as blood anti- coagulant (R. Lewisohn)
- **1917** First Blood Bank
- **1921** «First blood donor Service» (O. Percy ,London)
- **1943** ACD became the standard anticoagulant (J.F. Loutit, P.L. Mollison)
- **1950** The use of the plastic blood container (C.Walter,W.P. Murphy)
- **1951** First use of a cell separator (E. Cohn)

14th June birthday of Karl Landsteiner





The discovery of the ABO blood group, over 100 years ago, caused great excitement. Until then, all blood had been assumed to be the same, and the often tragic consequences of blood transfusions were not understood.

The discovery of Blood group

In 1901, Karl Landsteiner discovered the AB0 blood group antigens.
By systematically mixing the RBC from a number of individuals (his colleagues) with the sera from others, he found that the RBCs from some individuals were agglutinated by the sera from others.

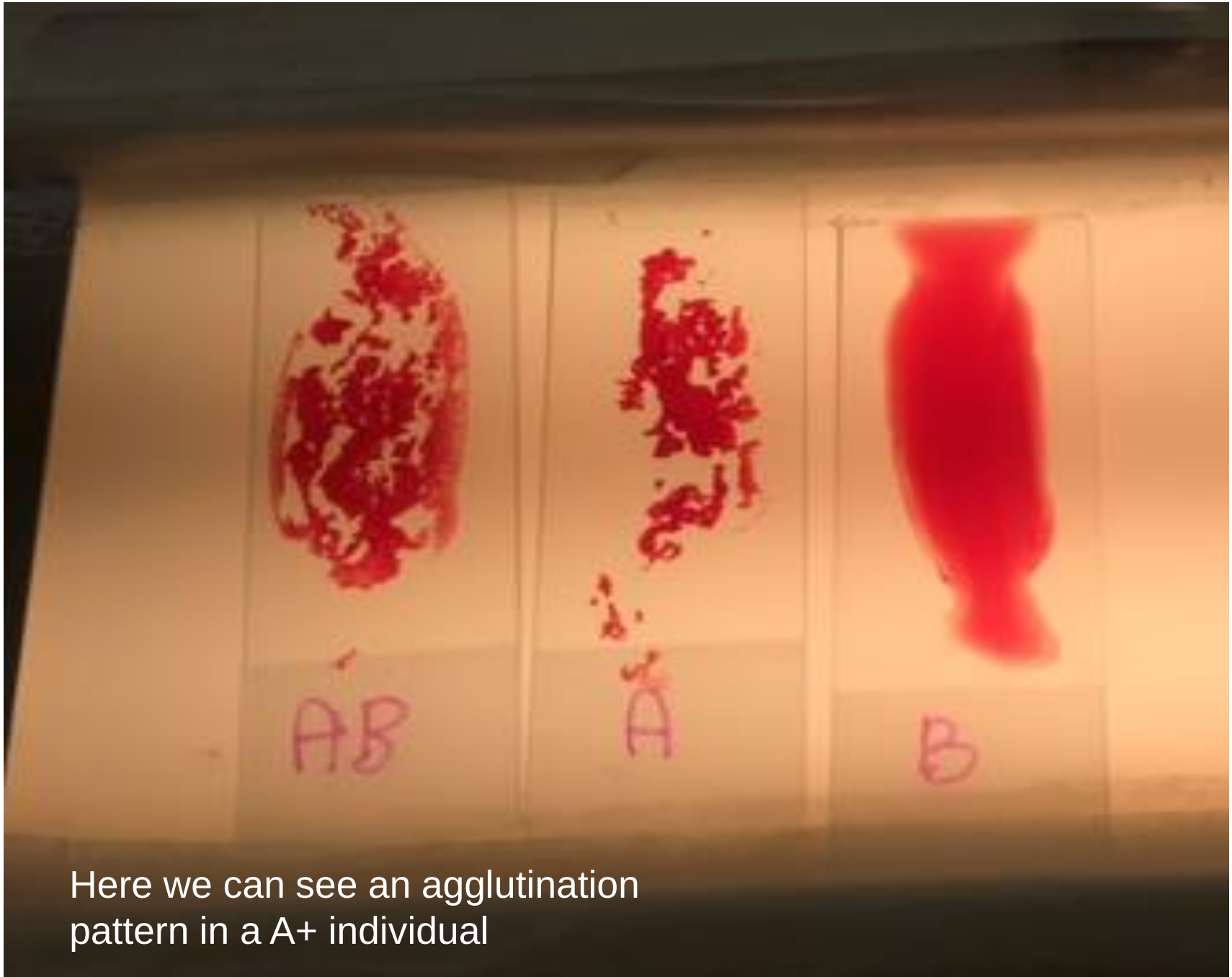
He called the antigens A and B.
A third blood group without the agglutination properties of A and B, was later called O.
One year later, the fourth blood group, AB, was added to the ABO blood group system (discovered by De Castello and Sturli)

A pattern of four major groups emerged: A, B, AB, or 0. Individuals have either A or B antigen on their cells, a combination of A&B, or neither (group 0).


SCIENCE

DR. KARL LANDSTEINER

This discovery earned Landsteiner a Nobel Prize

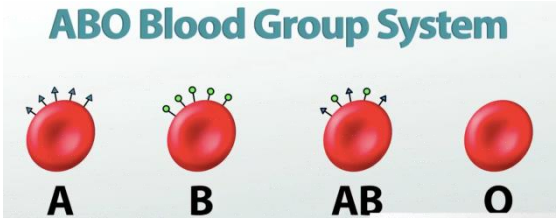


Here we can see an agglutination pattern in a A+ individual

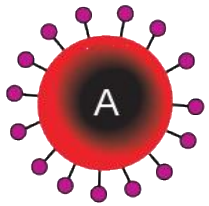
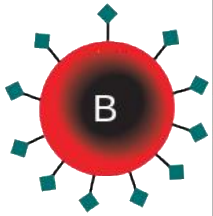
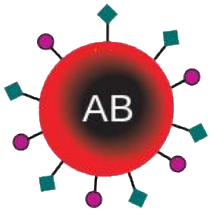
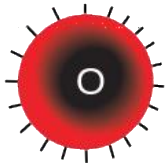
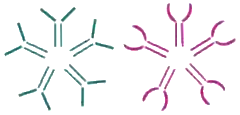
The AB0 System and the Landsteiner's Law

- ✓ **Two antigens (A and B) found on RBCs, tissue cells, fluid and secretions**
- ✓ **Four different groups: A, B, AB, 0**
- ✓ **… whichever ABO antigens are lacking in a given person's RBCs, that person will always have the corresponding antibody or isohemagglutinin**

	Group A	Group B	Group AB	Group O
Red blood cell type	A	B	AB	O
Antibodies in Plasma	Anti-B	Anti-A	None	Anti-A and Anti-B
Antigens in Red Blood Cell	A antigen	B antigen	A and B antigens	None



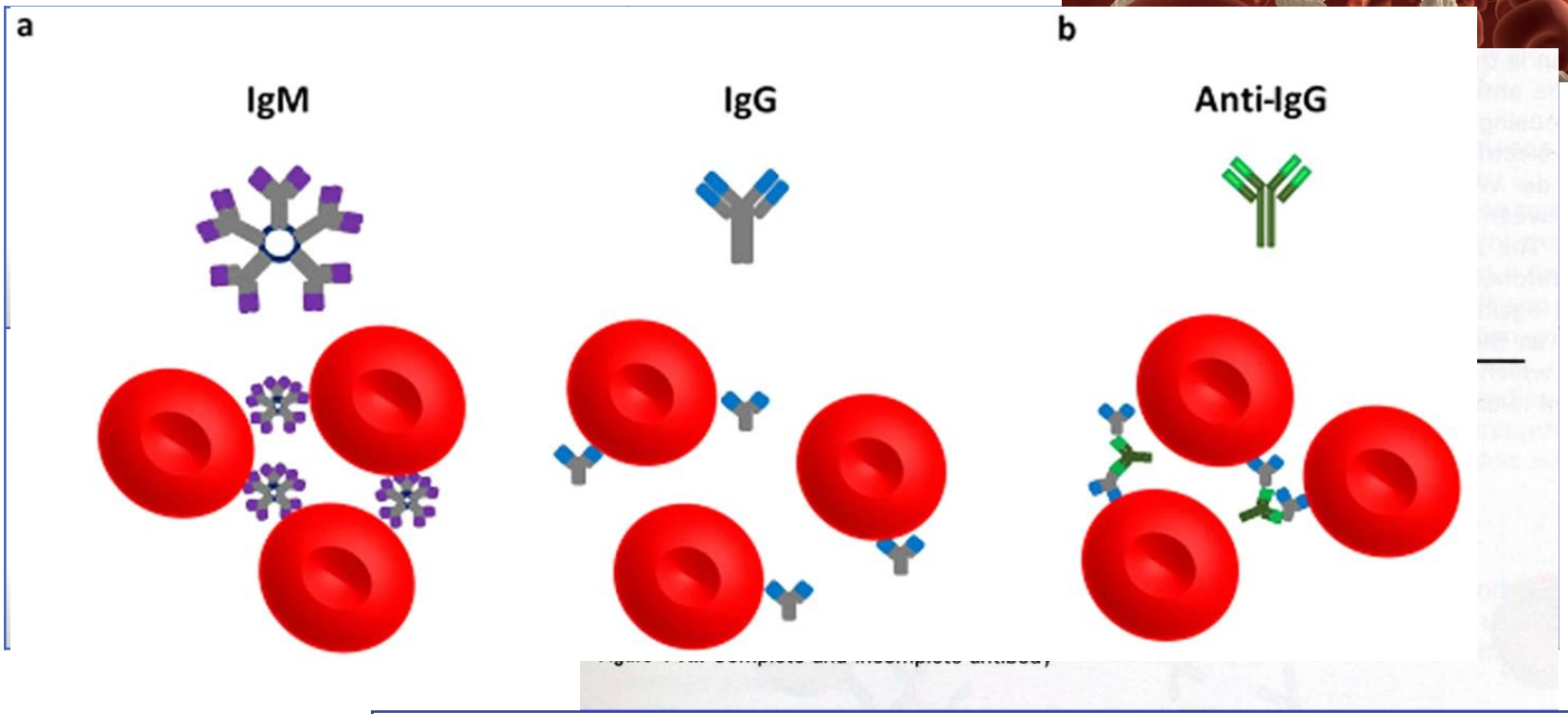
The AB0 System and the Landsteiner's Law

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in Plasma	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens in Red Blood Cell	 A antigen	 B antigen	 A and B antigens	None

- ✓ **Group A individuals always have anti-B in their plasma**
- ✓ **Group B individuals always have anti-A in their plasma**
- ✓ **Group O individuals always have anti-A and anti-B and anti-AB**
- ✓ **Group AB individuals don't have any isohemoagglutinins**

ABO ANTIBODIES

… following the Landsteiner law



Whether they are IgG or IgM, ABO antibodies can readily activate complement!!!

INCOMPATIBILITIES CAN CAUSE LIFE THREATENING TRANSFUSION REACTIONS!!!

Why do we have natural antibodies?

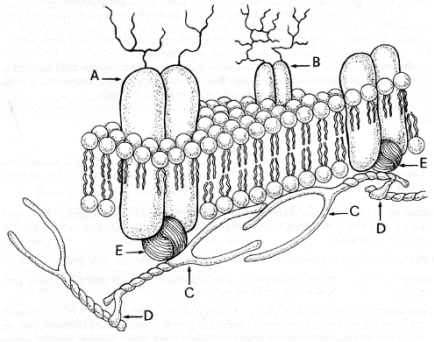
Antibodies are usually not present at birth but are present in most individuals by about 6 months of age.

In 1960s Springer and colleagues showed ABO isohemoagglutinins were produced as a response to bacterial antigens.

Infants are exposed to a variety of microorganisms and foodstuffs which have antigenic determinants that are cross reactive with the blood group substances and which can thus provide the stimulation for isoantibody formation (ie E. coli has type B like Ag).

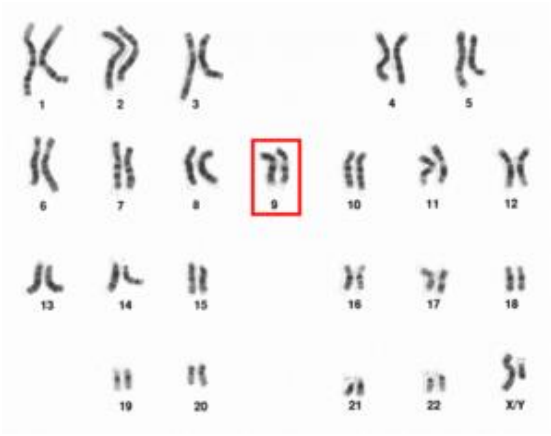
These cross reacting Ags induce formation of Abs in individuals lacking these antigens because epitopes are too similar to self and a state of self tolerance to these epitopes should exist.

The AB0 Genes

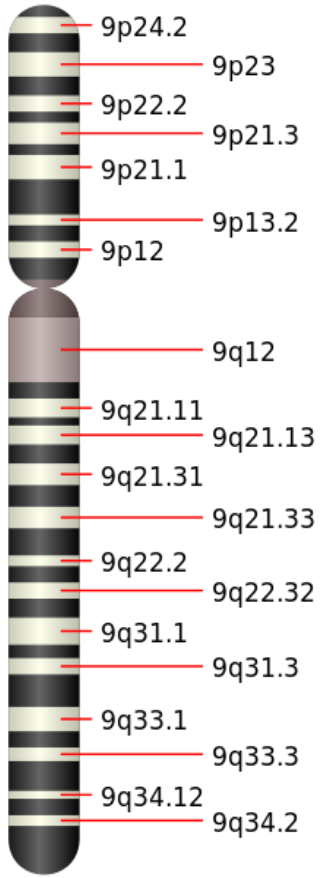


- ✓ DO NOT ENCODE A AND B ANTIGENS DIRECTLY
- ✓ They encode TRANSFERASE ENZYMES which catalyse the addition of specific monosaccharides to oligosaccharide precursor chain having a terminal galactose
- ✓ H antigen form a precursor oligosaccharide necessary to form A antigen and B antigen

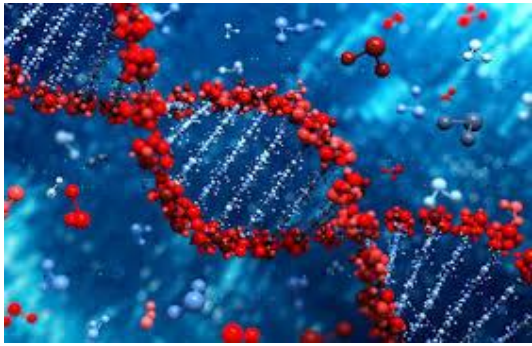
AB0 genetics



- Chromosome 9q34.1 – q34.2
- There are three main allelic forms: A, B and O
- A and B co-dominant
- 0 is the recessive form encoding a non-functional enzyme
- Each individual has a pair of chromosomes so has two genes for the AB0 group

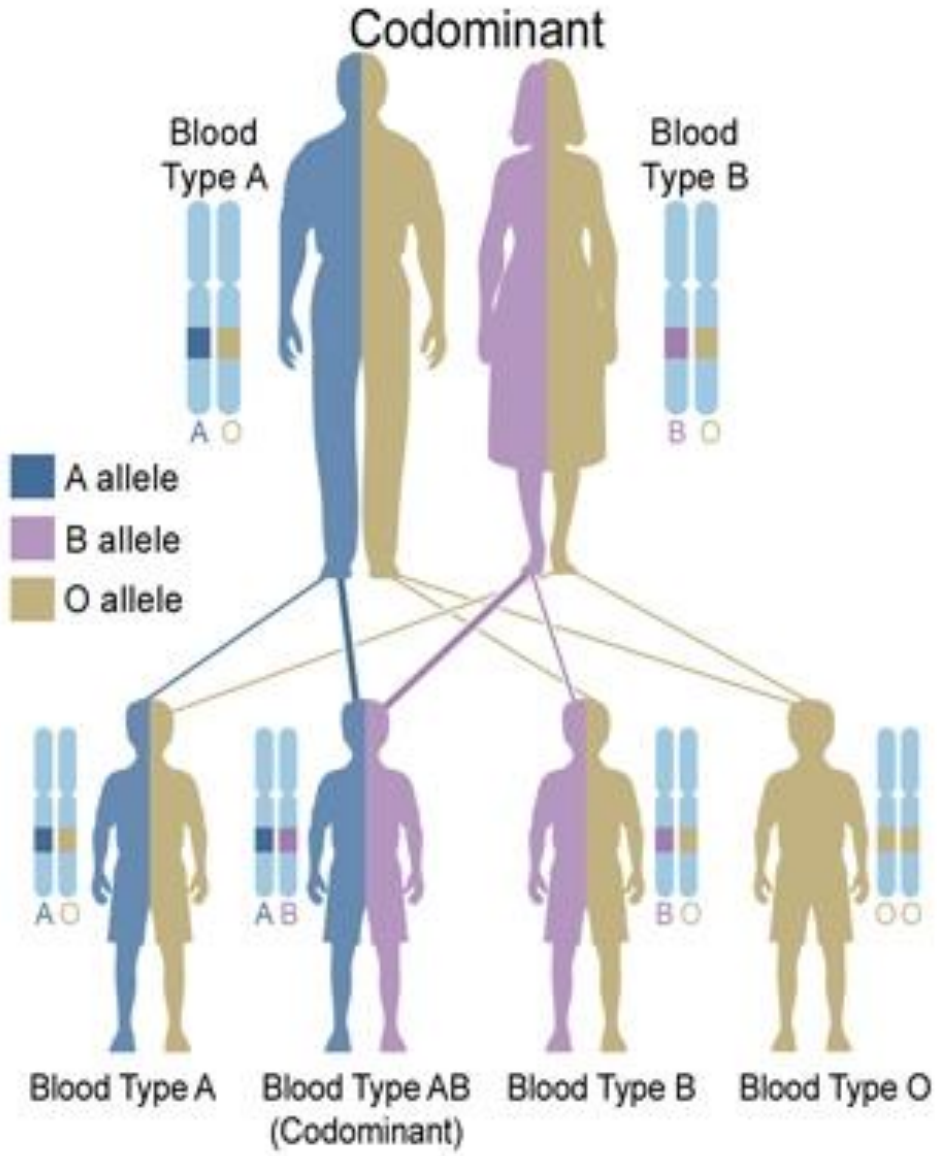


Phenotype vs genotype

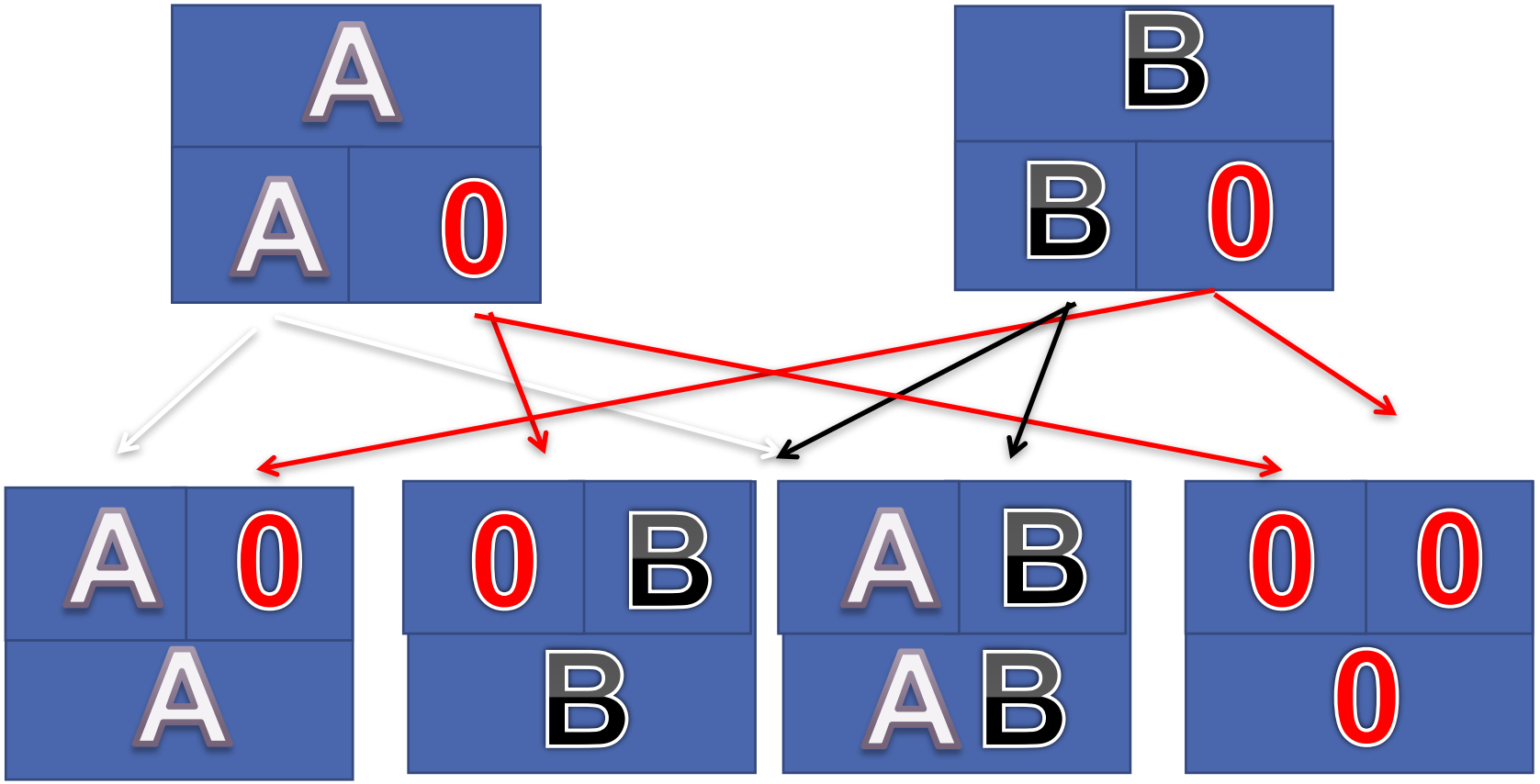


Two chromosomes → Two genes

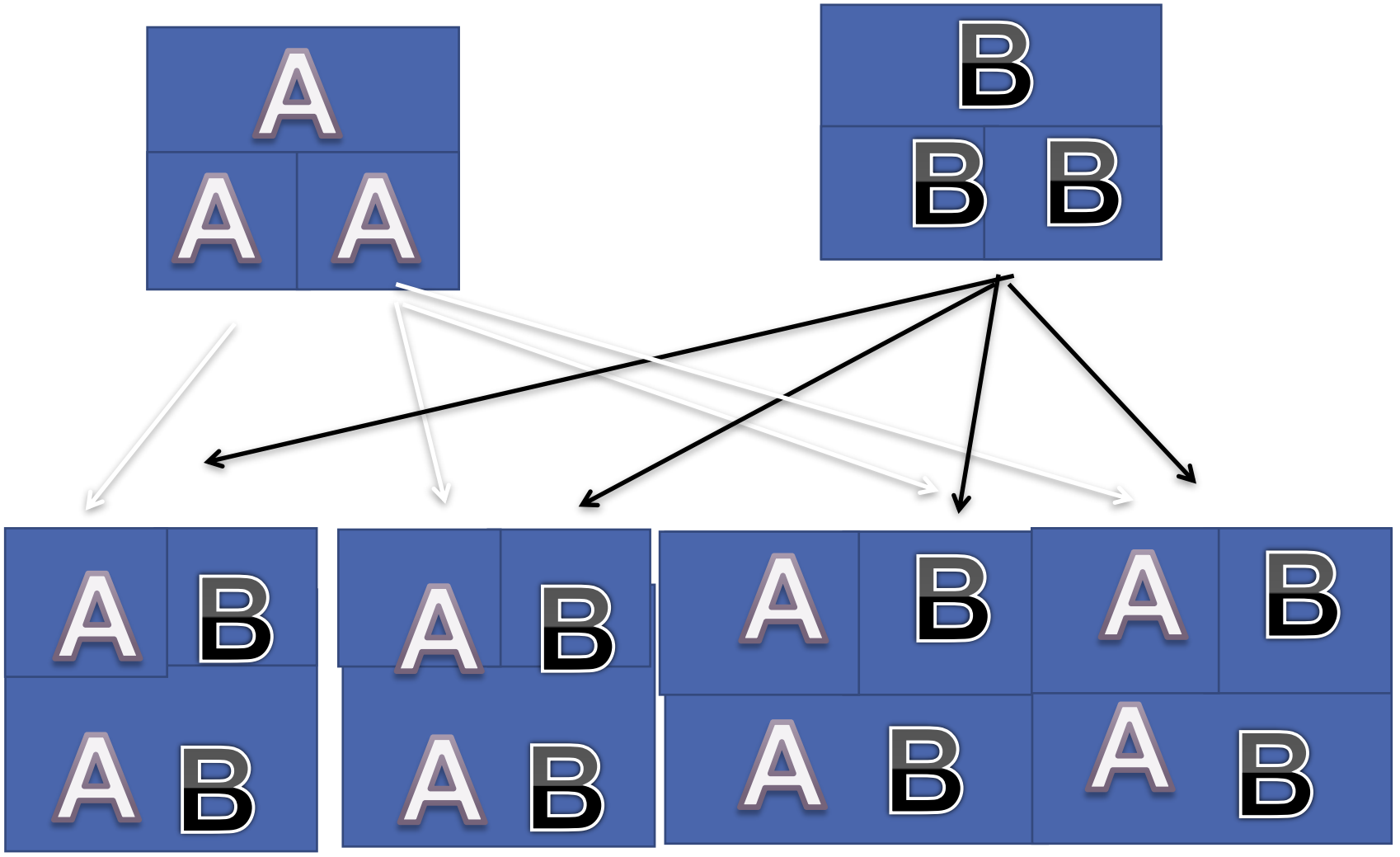
GENOTYPE		PHENOTYPE
A	B	AB
A	0	A
A	A	A
B	0	B
B	B	B
0	0	0


Codominant
Blood Type A
Blood Type B
A O
B O
A allele
B allele
O allele
A O
A B
B O
O O
Blood Type A
Blood Type AB (Codominant)
Blood Type B
Blood Type O
U.S. National Library of Medicine

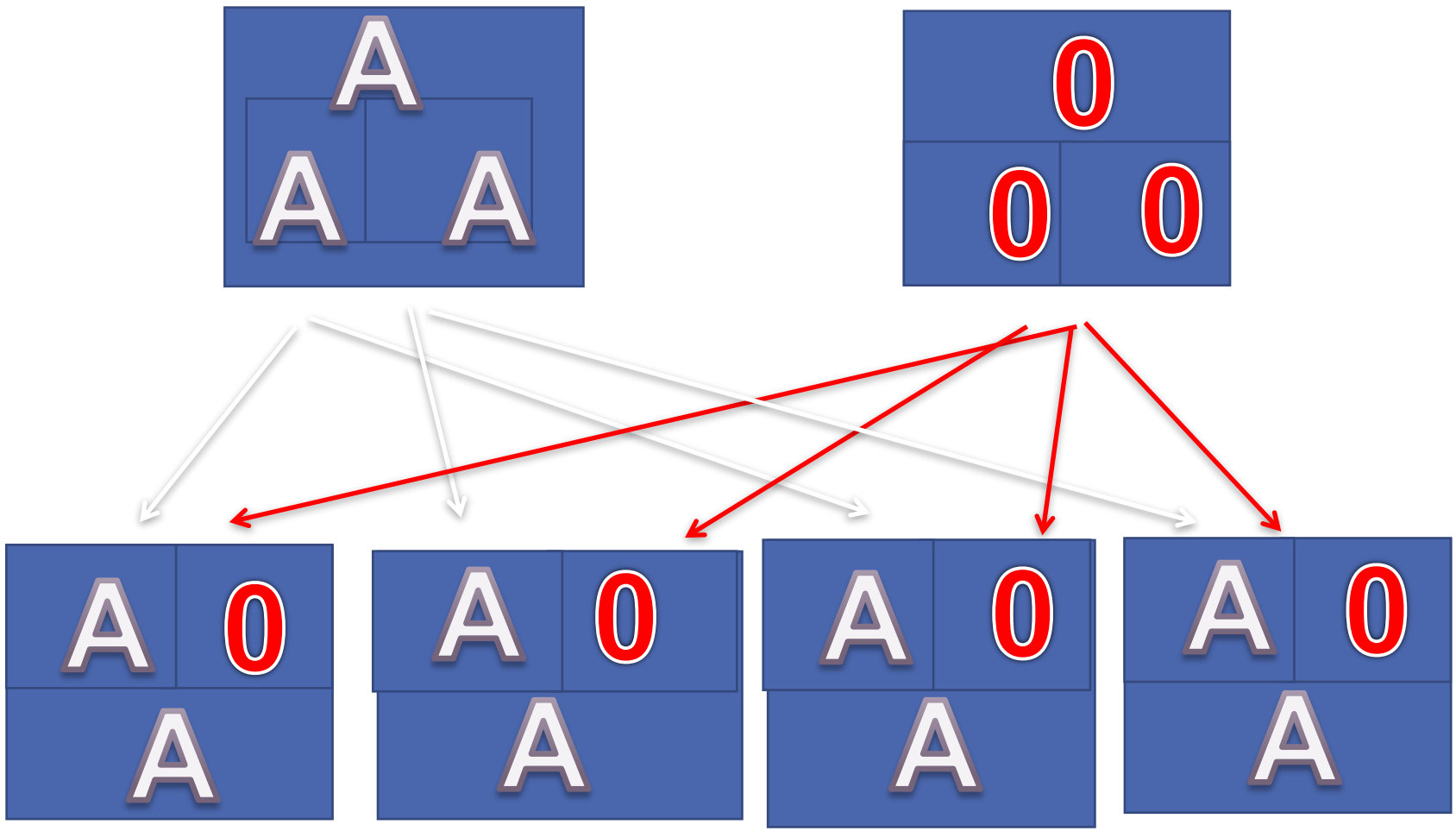
Phenotype vs genotype



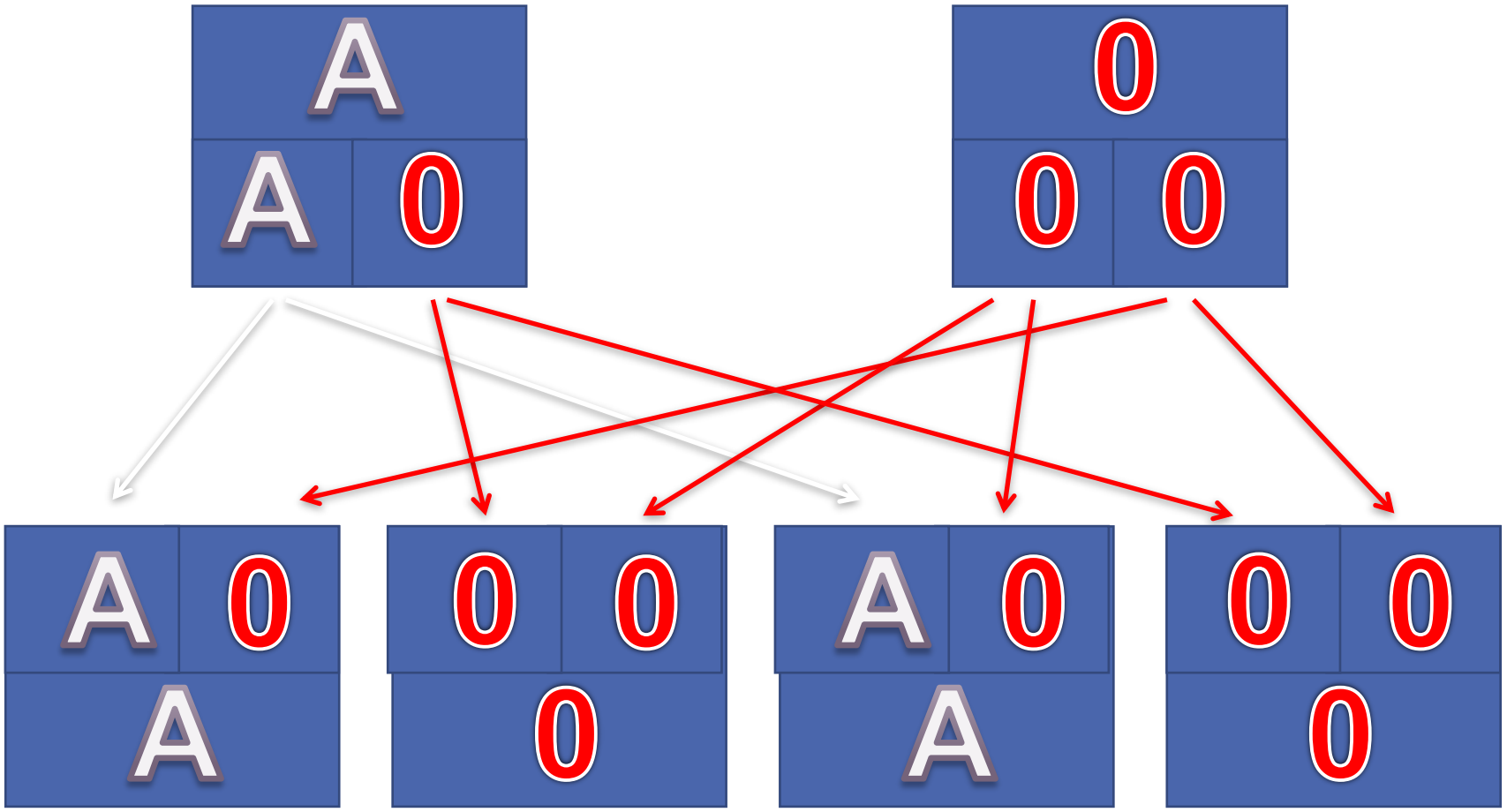
Phenotype vs genotype



Phenotype vs genotype



Phenotype vs genotype

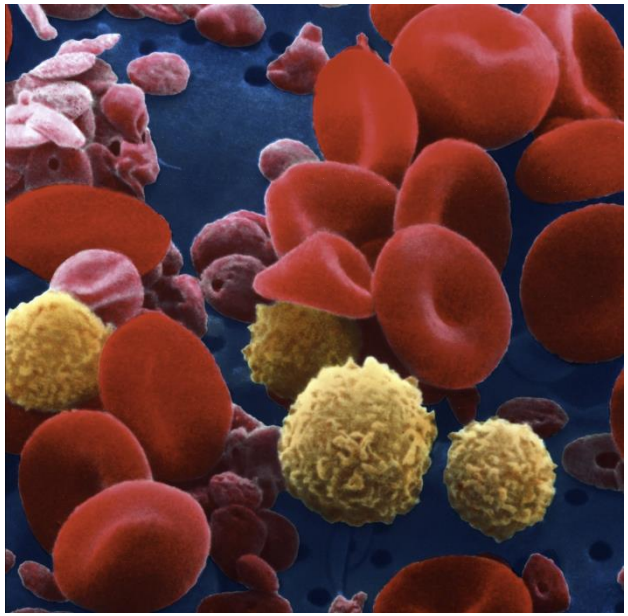


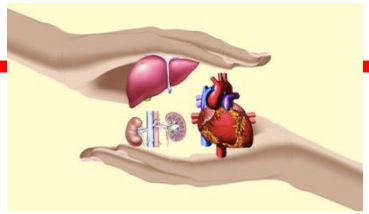
A and B antigens

- ✓ Not fully developed at birth (few copies of antigens on the cells)
- ✓ Antigens detectable as early as 5 weeks after conception
- ✓ Complete expression at 6 months

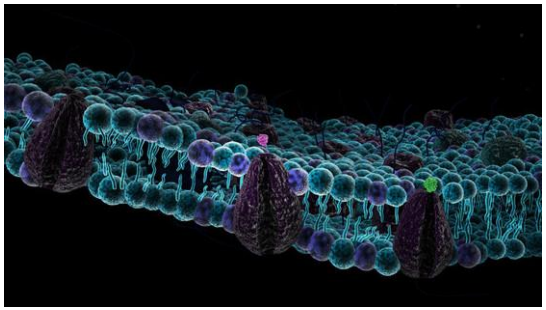
Other cells holding A, B substances

- ✓Plasma
- ✓Leucocytes
- ✓Platelets
- ✓Epithelial cells
- ✓Amniotic liquid cells
- ✓Sperms
- ✓Cells of the endhothelium of: capillaries, veins, arteries





The H gene



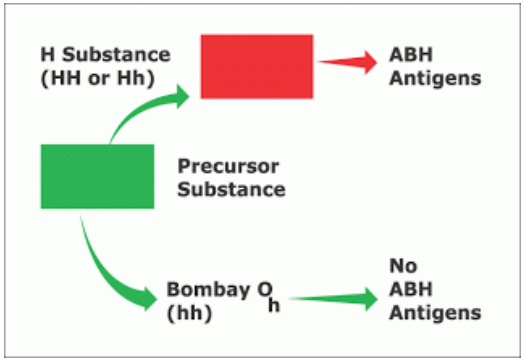
- The H locus is found on chromosome 19
- Why is therefore included in the AB0 blood group system?

Although H is encoded by a gene on a different chromosome from ABO, the H blood group system is considered in this chapter because H is a precursor of A and B.

ABH System Hh genes

- Two alleles: **H** and **h**
- Located on **Chromosome 19**
- **H gene**, dominant, has a higher frequency (> 99,9%)
- **h gene** is called “amorphous gene”; homozygosity (hh) is extremely rare

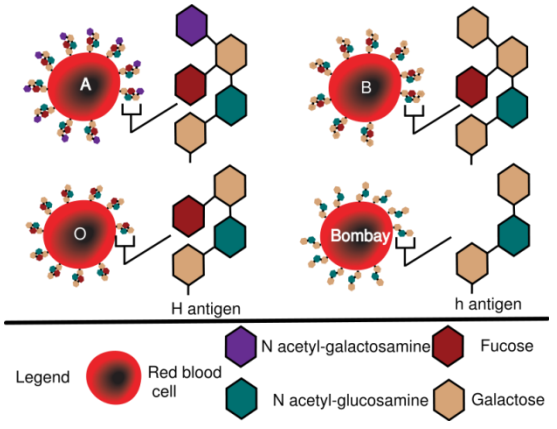
The product of H gene is a α-L-fucosiltransferase, that adds a **L-fucose molecule** on the common substance, **with the consequent formation of H substance**
This is a crucial step for developing A and B specifities

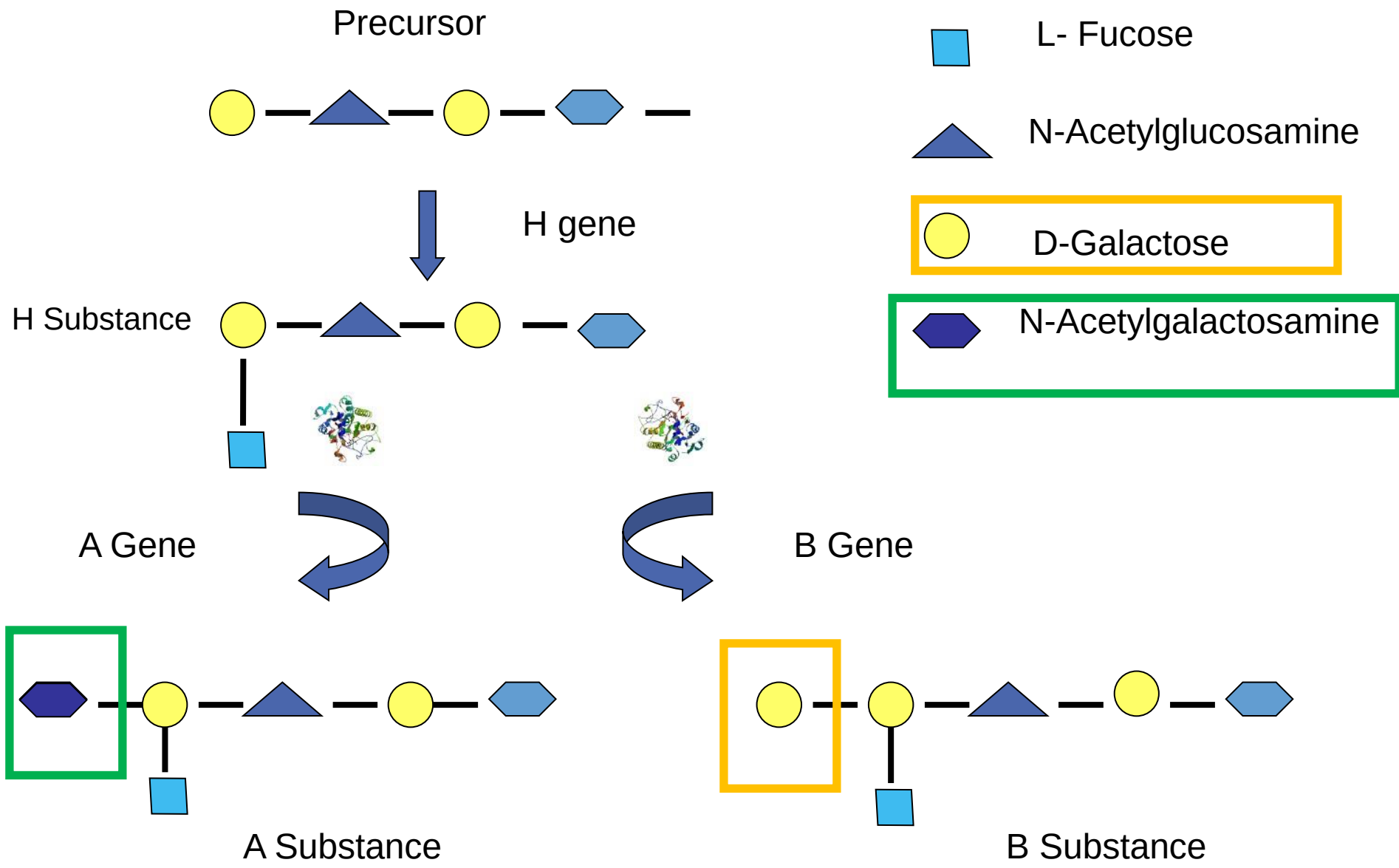


Chemical Structure

All normal individuals synthesize a common core glycan called the **H Ag** that is attached to a polypeptide backbone (precursor).

- ✓ **A INDIVIDUALS POSSESS AN A GENE , GTA, A GLYCOSYLTRANSFERASE THAT ADDS A TERMINAL N-ACETYLGALACTOSAMINE TO THEIR H AGS**
- ✓ **THE B ALLELE ENZYME GTB, A DIFFERENT GLYCOSYLTRANSFERASE ADDS A TERMINAL GALACTOSE TO THE H AGS.**
- ✓ **TYPE 0 BLOOD HAVE ONLY H SUBSTANCE BECAUSE iT HAVE A NON-FUNCTIONAL GENE.**

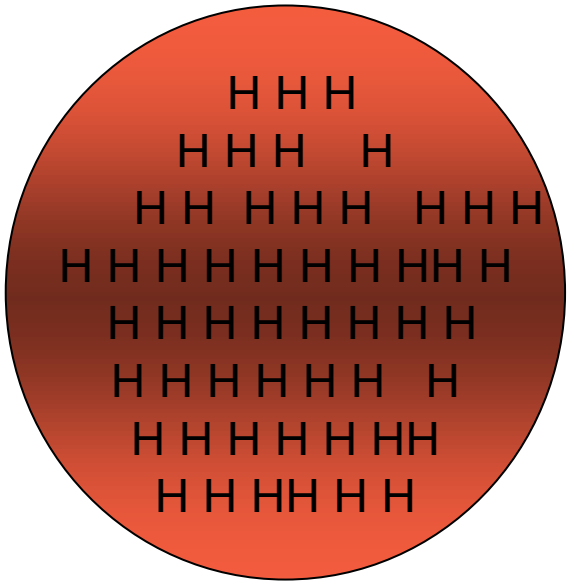


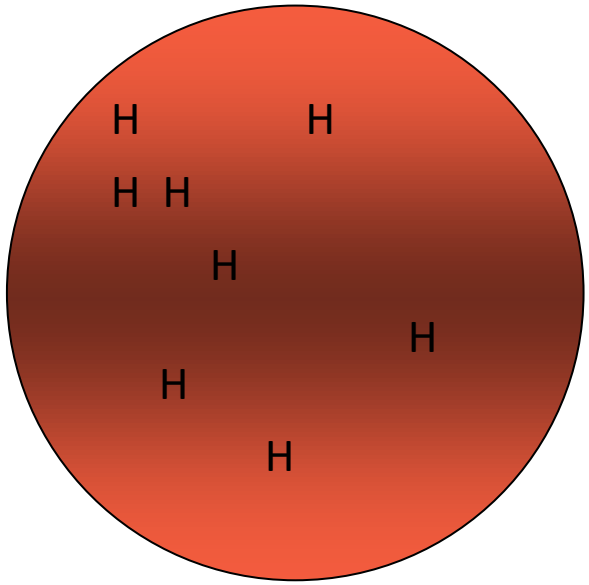


✓ GTA and GTB are almost identical, with only four aminoacid changes.

H ANTIGEN CONCENTRATION IN RED BLOOD CELLS

0 > B > A > AB





Bombay Phenotype: a dangerous recipient

Homozygosity for genes h (Oh)

Detected in Bombay by Bhende et al., 1952

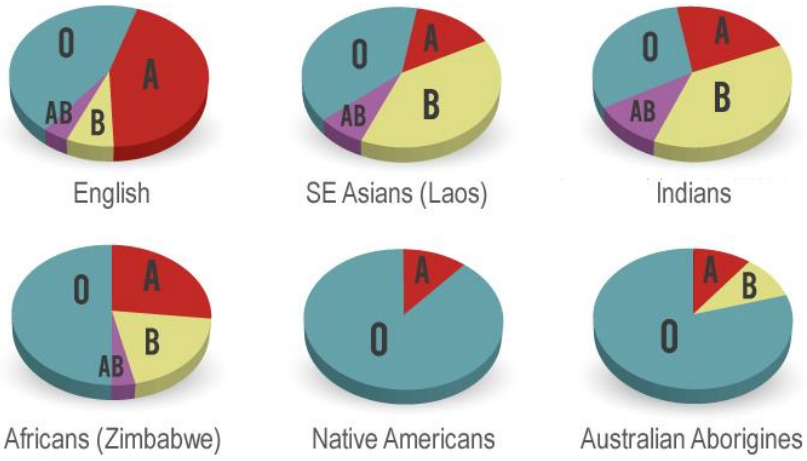
- Absence of H substance -> lack of A and/or B substance
- Presence in serum of anti-A,-B,-H
- Definition of “apparent 0” o “0_h”
- It is recognized if the serum is tested against group 0 cells causing strong agglutination.
  - Have anti-A, -B, -A,B and –H
  - Can only be transfused with Bombay blood <0.01%

ABO frequency

IT'S FASCINATING TO OBSERVE HOW FREQUENCIES DIFFER IN SELECTED POPULATIONS AND ETHNIC GROUPS DUE TO THE GEOGRAPHICAL SPREAD AND A CONTINUOUS PROCESS OF NATURAL SELECTION AGAINST ENVIRONMENTAL FACTORS SUCH AS DISEASES, CLIMATE, HUMIDITY, ALTITUDE, ETC. WILL CONTINUE.

- Frequencies differ in selected populations and ethnic groups
- Group B is higher in African and Asian populations
- Frequency in Caucasian population:

✓ group 0 45%
✓ group A 40%
✓ group B 11%
✓ group AB 4%.



ABO frequency

Beyond immunohaematology:
the role of the ABO blood group in human diseases

Giancarlo Maria Liumbruno[1], Massimo Franchini[2]

[1]Immunohaematology, Transfusion Medicine and Clinical Pathology Units, "San Giovanni Calibita" Fatebenefratelli Hospital, AFAR, Rome; [2]Department of Transfusion Medicine and Haematology, "Carlo Poma" Hospital, Mantua, Italy

Many authors tried identifying the role of blood antigens in developing diseases. One of the most important examples is represented by malaria

Individual of blood group O seems to be proteced against malaria…

Frequencies differ in selected populations and ethnic groups as a consequence of the geographical spread and a continuos process of natural selection against environmental factors such as diseases, climate, humidity, altitude…

The ABO system is important because the original allele, encoding glycosylation with the A sugar, acts as an adhesion ligand with infected red blood cells thus promoting rosette formation with uninfected red blood cells and adhesion to vascular endothelium, which cause vaso-occlusion and severe disease. The least rosette formation is observed in individual with blood group O, thereby explaining the prevalence of this blood group in areas in which malaria is endemic

Blood Type Biochemistry and Human Disease

Blood Type Biochemistry and Human Disease

D Rose Ewald and **Susan CJ Sumner***
RTI International, Discovery Sciences, 3040 East Cornwallis Drive, Research Triangle Park, NC, 27709

Over the last years, our knowledge on hundreds of blood groups antigens, classified into 38 blood systems, in terms of structural homology, secondary structure and biological functions (structural proteins, enzymes, transporters, channels, receptors) has increased, but there is still much research to be done …

ASSOCIATIONS BETWEEN BLOOD TYPE AND DISEASE HAVE BEEN STUDIED SINCE THE EARLY 1900S WHEN RESEARCHERS DETERMINED THAT ANTIBODIES AND ANTIGENS ARE INHERITED.

Ewald DR, Sumner SC.. *Wiley Interdiscip Rev Syst Biol Med*. 2016;8(6):517–535.

Blood Type Biochemistry and Human Disease

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RTI International, Discovery Sciences, 3040 East Cornwallis Drive, Research Triangle Park, NC, 27709

Blood antigens can serve as receptors and ligands for microbes, and may play a role…

Although the **exact mechanisms are not yet known** that will explain all of the reported associations **between blood group antigens and disease**, what is known about their structure and functions provides some intriguing clues.

An unexpected number of the antigenic structures found on RBCs act as **cell adhesion molecules (CAMs)**; some contribute to normal RBC development and some play a role in human disease. These antigens can serve as receptors and ligands for microbes…

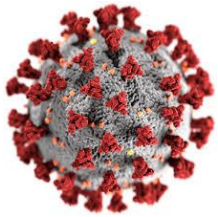
Ewald DR, Sumner SC.. *Wiley Interdiscip Rev Syst Biol Med*. 2016;8(6):517–535.

Blood Type Biochemistry and Human Disease

DISEASE	RISK FACTOR	BLOOD GROUP/ANTIGENS
VASCULAR DISORDERS, VENOUS AND ARTERIAL THROMBOEMBOLISM, CORONARY HEART DISEASE, ISCHEMIC STROKE, MYOCARDIAL INFARCTION	REDUCED CLEARANCE OF VON WILLEBRAND FACTOR AND FVIII	GROUPS A > AB > B
PLAGUE, CHOLERA, TUBERCULOSIS, MUMPS	ANTIGEN PROFILE	GROUP O
GONORRHEA, TUBERCULOSIS, S. PNEUMONIAE, E. COLI, SALMONELLA	ANTIGEN PROFILE	GROUP B
SMALLPOX, E. COLI, SALMONELLA	ANTIGEN PROFILE	GROUP AB
N. MENINGITIDES, H. INFLUENZA, C. ALBICANS, S. PNEUMONIAE, E. COLI URINARY TRACT INFECTIONS, S. PYOGENES, V. CHOLERA	ANTIGEN PROFILE	NON-SECRETORS
H. PYLORI	STRAIN-DEPENDENT	GROUP A; 95% NON-O
PEPTIC ULCERS, GASTRODUODENAL DISEASE	SECRETOR STATUS, H. PYLORI STRAIN	ALL NON-SECRETORS; GROUP O

Disease risk is clearly multifactorial and causation is not implied by association, but blood group antigens may be one of the predisposing factors that contribute to or prevent disease processes.

Ewald DR, Sumner SC.. Wiley Interdiscip Rev Syst Biol Med. 2016;8(6):517–535.



Annals of Hematology
https://doi.org/10.1007/s00277-020-04169-1

ORIGINAL ARTICLE

Check for updates

Blood type and outcomes in patients with COVID-19

Christopher A. Latz[1] · Charles DeCarlo[1] · Laura Boitano[1] · C. Y. Maximilian Png[1] · Rushad Patell[2] · Mark F. Conrad[1] · Matthew Eagleton[1] · Anahita Dua[1]

Received: 21 June 2020 / Accepted: 6 July 2020

A curiosity regarding the association between SARS-COv2 infection and blood type.

In the last months, some authors showed that the ABO polymorphism impacts the COVID-19 infection risk, particularly blood group 0 individuals who have a decreased risk of contracting the infection.

Blood Adv (2020) 4 (20): 4981–4989.

The association of ABO blood group with indices of disease severity and multiorgan dysfunction in COVID-19

Ryan L. Hoiland,[1,2,*] Nicholas A. Fergusson,[3,4,*] Anish R. Mitra,[5] Donald E. G. Griesdale,[1,4-6] Dana V. Devine,[7-9] Sophie Stukas,[7] Jennifer Cooper,[7] Sonny Thiara,[5] Denise Foster,[5] Luke Y. C. Chen,[10] Agnes Y. Y. Lee,[10] Edward M. Conway,[9,10] Cheryl L. Wellington,[7,11-13,†] and Mypinder S. Sekhon[5,†]

Key Points

- COVID-19 patients with blood group A or AB are at increased risk for requiring mechanical ventilation vs those with blood group O or B.
- COVID-19 patients with blood group A or AB appear to exhibit a greater disease severity than patients with blood group O or B.

Blood Adv 2020 Oct 27;4(20):4990-4993.

Reduced prevalence of SARS-CoV-2 infection in ABO blood group O

Mike Bogetofte Barnkob,[1,2] Anton Pottegård,[3] Henrik Støvring,[4] Thure Mors Haunstrup,[5] Keld Homburg,[6] Rune Larsen,[6] Morten Bagge Hansen,[7] Kjell Titlestad,[1] Bitten Aagaard,[5] Bjarne Kuno Møller,[8] and Torben Barington[1,2]

Key Points

- Blood group O is associated with a decreased risk for contracting SARS-CoV-2 infection.

COVID-19?

This author explained this issue taking into account that SARS-COV 2 is encapsulated with the host cell membrane, where spike proteins are expressed with A or B glycan antigens, reflecting the ABO phenotype of the cells where viruses are produced.

So the SARS-COV 2 viruses produced in individuals of group A, B, AB or O express .... antigens and none in o individuals.

VoxSanguinis
The International Journal of Transfusion Medicine
ISBT International Society of Blood Transfusion
Vox Sanguinis (2020)

COMMENTARY

DOI: 10.1111/vox.13004

Blood group ABO polymorphism inhibits SARS-CoV-2 infection and affects COVID-19 progression

Fumiichiro Yamamoto,[1] Miyako Yamamoto[1] & Eduardo Muñiz-Diaz[2]
[1]*Laboratory of Immunohematology and Glycobiology, Josep Carreras Leukaemia Research Institute, Badalona, Spain*
[2]*Department of Immunohematology, Banc de Sang i Teixits – BST, Barcelona, Spain*

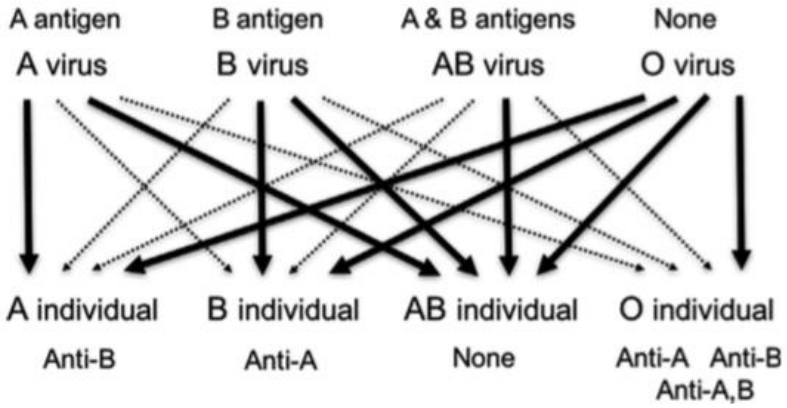
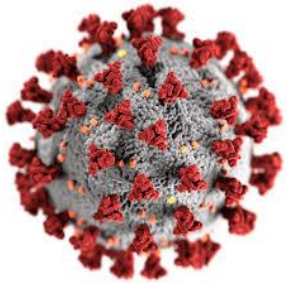


Fig. 1 Differential inhibition of infection between SARS-CoV-2 viruses exhibiting different ABO phenotypes and individuals of groups A, B, AB and O.

ABO antibodies will inhibit interpersonal infection between individuals with different blood antigens



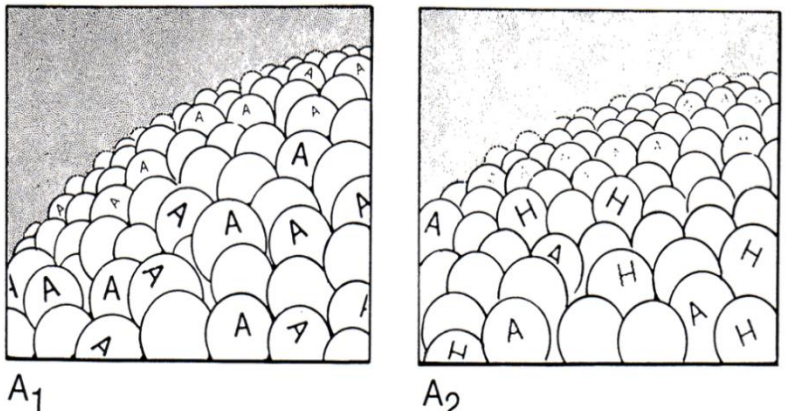
...The new studies that are coming will help us to better clarify this and many other aspects of the ABO involvement in the SARS-CoV-2 infection and the COVID-19 progression. ..

Subgroups of A (A^1 and A^2)

- Subgroups of A are phenotypes that differ from others of the same ABO group with respect to the amount of A antigen carried on RBCs
- Variant gene produces a weaker than normal red cell antigen

Subgroups of A (A^1 and A^2)

- Different levels of expression of A on RBCs are classified into ***subgroups***
  - 80% of group A individuals are A1
  - Approximately 20% are A2
- **Transferase produced by A2 gene differs from that produced by A1, less efficient in converting H chains to A**



Difference between A^1 and A^2

- **A^1 has more A and less H antigen on the cell.**
- **A^2 has less A and more H antigen**
- Cannot be detected serologically
- **A^2 can produce anti- A^1**
- In most cases, anti-A1 is of no clinical significance, reacting well below body temperature, and is merely a laboratory nuisance causing ABO discrepancies.
- Anti-A1 is considered clinically significant when it reacts at 37 °C.
- The presence of anti-A1 may cause discrepancies in forward and reverse grouping.

Subgroups of A

- Subgroups of A weaker than A2 (**Ael, Aint, A3, Ax, Am, etc**) are seen only infrequently (**less then 1%**) and are characterized by decreasing numbers of A antigens

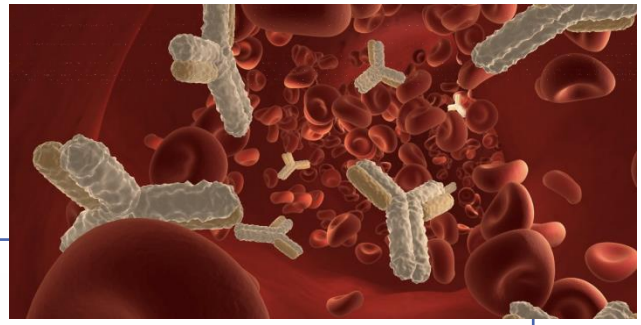
Subgroups of B

- Less common than subgroups of A
- Criteria resembles that used for A subgroups

AB's have a wide variety, as they can inherit all the possibilities of the A group

ABO ANTIBODIES

… following the Landsteiner law

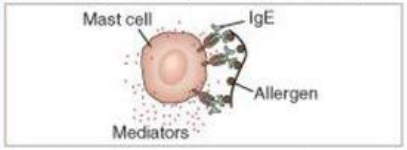
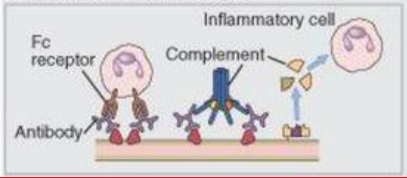
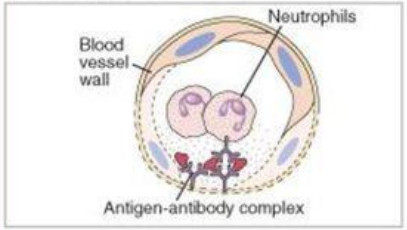
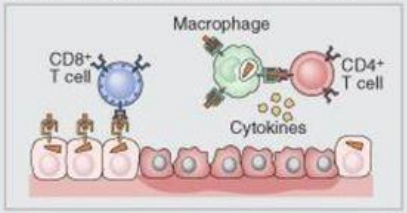


NATURAL		**IgM** **“COLD” (REACT AT 20-24°C)**
IMMUNE		**IgG** **“WARM” (REACT AT 37°C)**

Whether they are IgG or IgM, ABO antibodies can activate complement readily !!!
INCOMPATIBILITIES CAN CAUSE LIFE THREATENING TRANSFUSION REACTIONS!!!

HYPERSENSITIVITY

Transfusion reactions are an example of type II hypersensitivity in which red blood cells are rapidly destroyed by specific preformed antibodies (anti-ABO or -Rh) and complement

Type of hypersensitivity	Pathologic immune mechanisms	Mechanisms of tissue injury and disease
Immediate hypersensitivity (Type I)	T_H2 cells, IgE antibody, mast cells, eosinophils 	Mast cell–derived mediators (vasoactive amines, lipid mediators, cytokines) Cytokine-mediated inflammation (eosinophils, neutrophils)
Antibody-mediated diseases (Type II)	IgM, IgG antibodies against cell surface or extracellular matrix antigens 	Complement- and Fc receptor-mediated recruitment and activation of leukocytes (neutrophils, macrophages) Opsonization and phagocytosis of cells Abnormalities in cellular function, e.g., hormone receptor signaling
Immune complex–mediated diseases (Type III)	Immune complexes of circulating antigens and IgM or IgG antibodies deposited in vascular basement membrane 	Complement and Fc receptor-mediated recruitment and activation of leukocytes
T cell–mediated diseases (Type IV)	1. $CD4^+$ T cells (delayed-type hypersensitivity) 2. $CD8^+$ CTLs (T cell–mediated cytolysis) 	1. Macrophage activation, cytokine-mediated inflammation 2. Direct target cell lysis, cytokine-mediated inflammation

Types of hypersensitivity diseases.

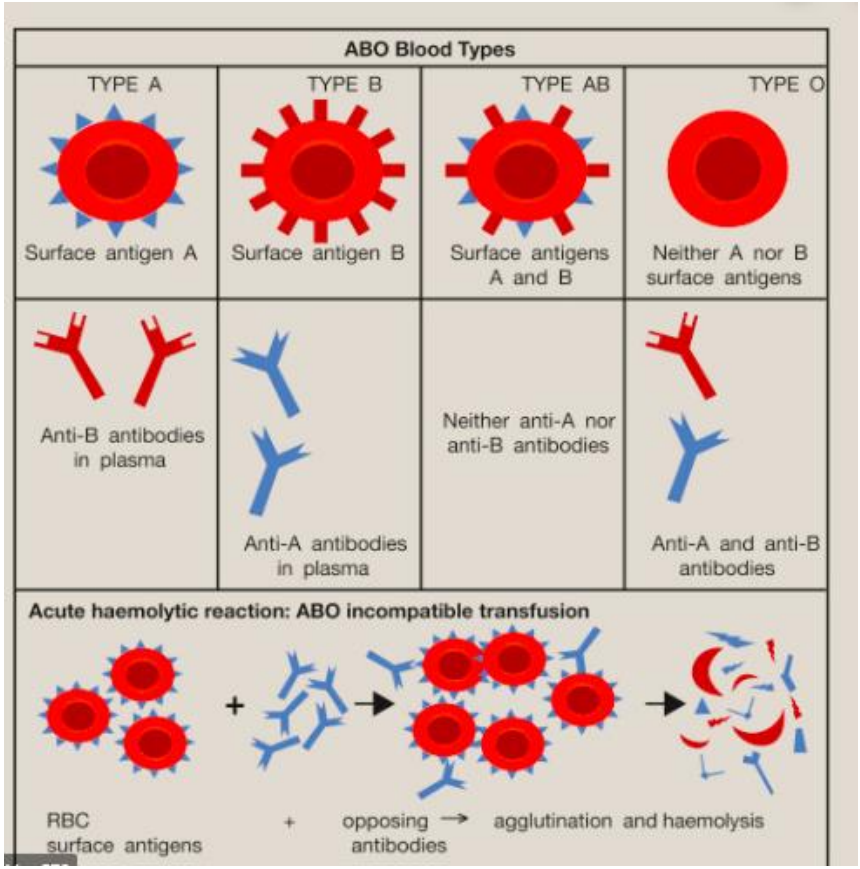
In the four major types of hypersensitivity reactions, different immune effector mechanisms cause tissue injury and disease

HYPERSENSITIVITY

TYPE II HYPERSENSITIVITY (CYTOTOXIC) IS MEDIATED BY ANTIBODIES DIRECTED TOWARD ANTIGENS PRESENT ON THE SURFACE OF CELLS OR OTHER TISSUE COMPONENTS.

- ✓ **Type II Hypersensitivity**
- ✓ Type II hypersensitivity is an antibody-dependent process in which specific antibodies bind to antigens, resulting in tissue damage or destruction.
- ✓ If the antigen is present on cell surfaces, antibody binding can result in cell lysis through the in situ fixation of complement.
- ✓ IgM antibodies (multimeric) are often more effective in fixing complement than are than IgG antibodies (monomeric).
- ✓ **Type II hypersensitivity is typified by a transfusion reaction in which mismatched red blood cells are rapidly destroyed by specific preformed antibodies (anti-ABO or -Rh) and complement.**
- ✓ Although fixation of complement can result in direct cell lysis, opsonization and recruitment of inflammatory cells is often a more important cause of cell injury.

Hemolytic Transfusion Reaction

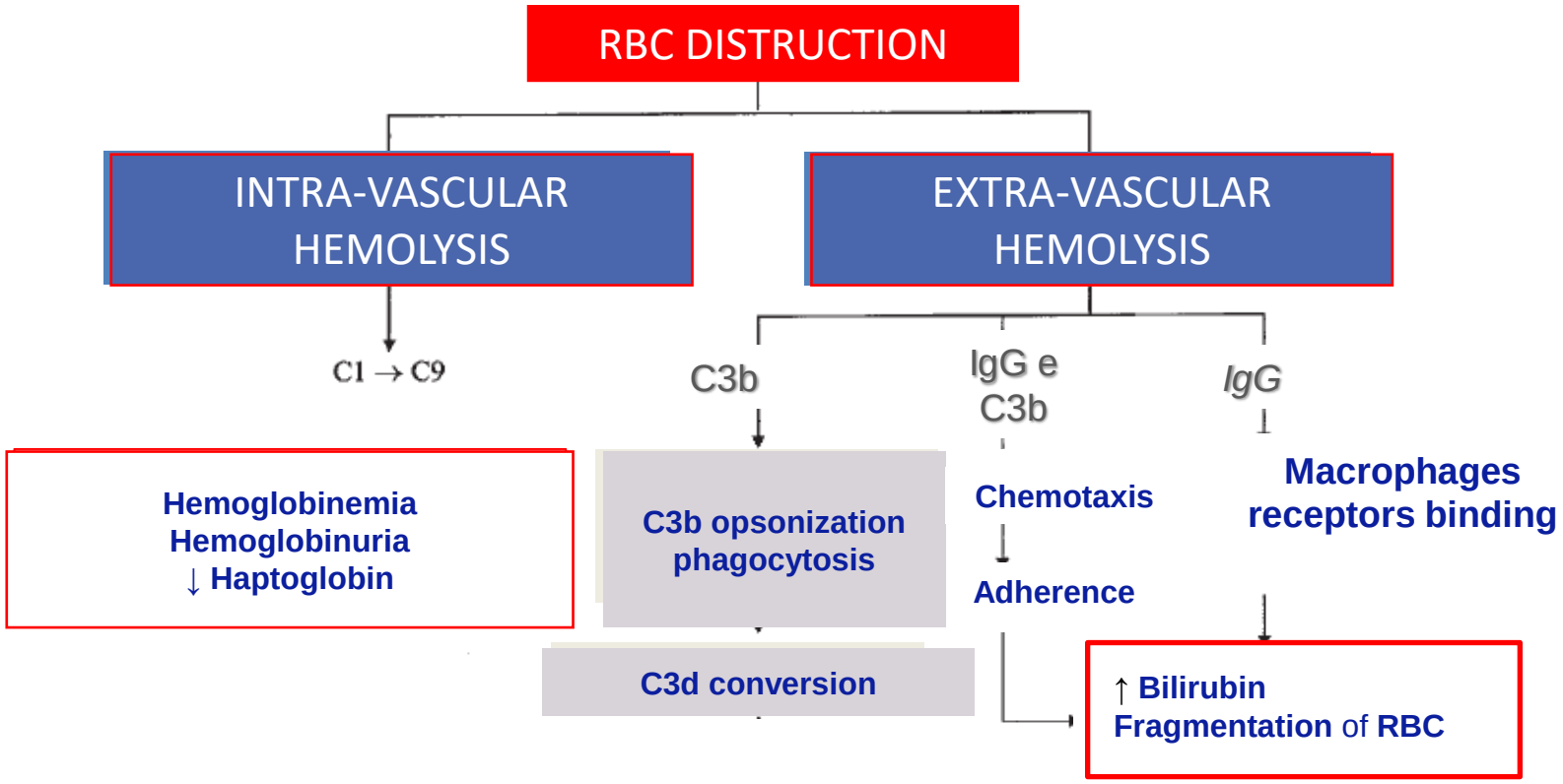


Immunologic incompatibility between donor and recipient cell types is the most common cause of clinically significant hemolytic transfusion reactions.

Acute reactions (i.e., those occurring within 24 hours after transfusion) develop in response to red cells transfused in patients with preexisting antibodies.

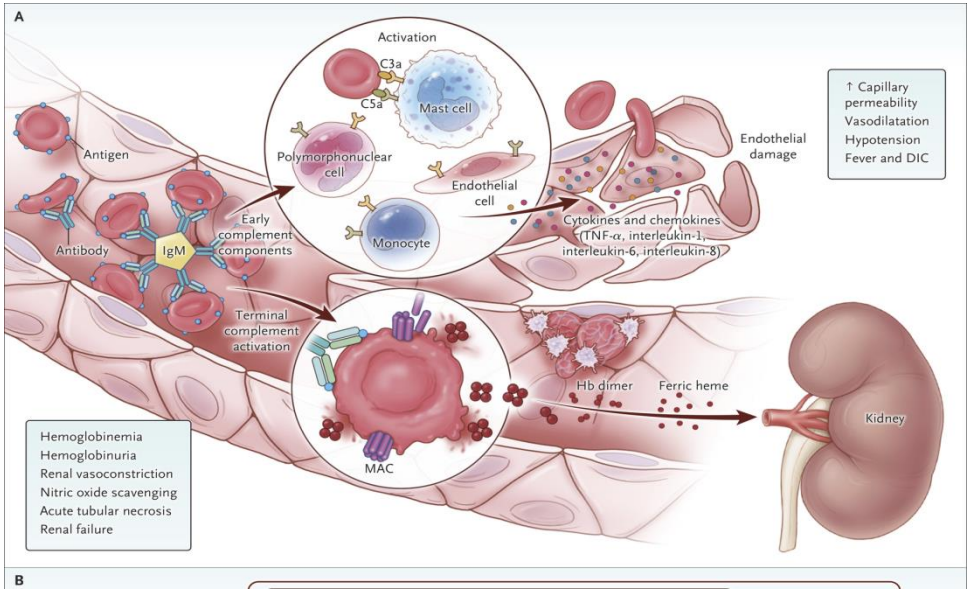
Incompatible A and B blood-group antigens interact with preexisting IgM antibodies and less commonly with hemolytic **IgG antibodies, both of which fix and activate complement.**

N Engl J Med 2019;381:150-62.



AABB, Technical Manual,

Hemolytic Transfusion Reactions I - Intravascular Hemolysis



Pathophysiological features of

ACUTE HEMOLYTIC TRANSFUSION REACTIONS.

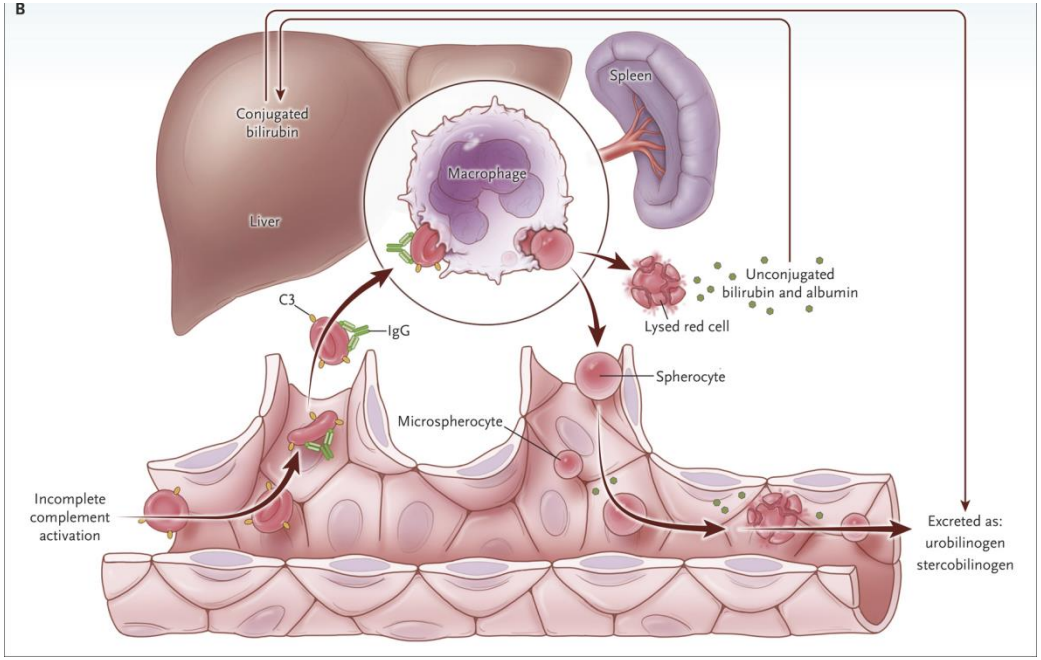
Foreign blood-group antigen recognition and binding by **circulating IgM** → activation of **TERMINAL COMPLEMENT** → formation of the **membrane attack complex (MAC).**

The **MAC destroys red-cell membranes,** releasing **free hemoglobin (Hb**) into the intravascular space → **end-organ damage (acute tubular necrosis and renal failure)**

Early complement components → endothelial damage, increased capillary permeability through activation of mast cells, polymorphonuclear cells, monocytes, and endothelial cells → release of cytokines and interleukins , DIC (**disseminated intravascular coagulation**) and ↑ TNF-α tumor necrosis factor α.

N Engl J Med 2019;381:150-62.

Hemolytic Transfusion Reactions II- Extravascular Hemolysis



Pathophysiological features of

DELAYED HEMOLYTIC TRANSFUSION REACTIONS.

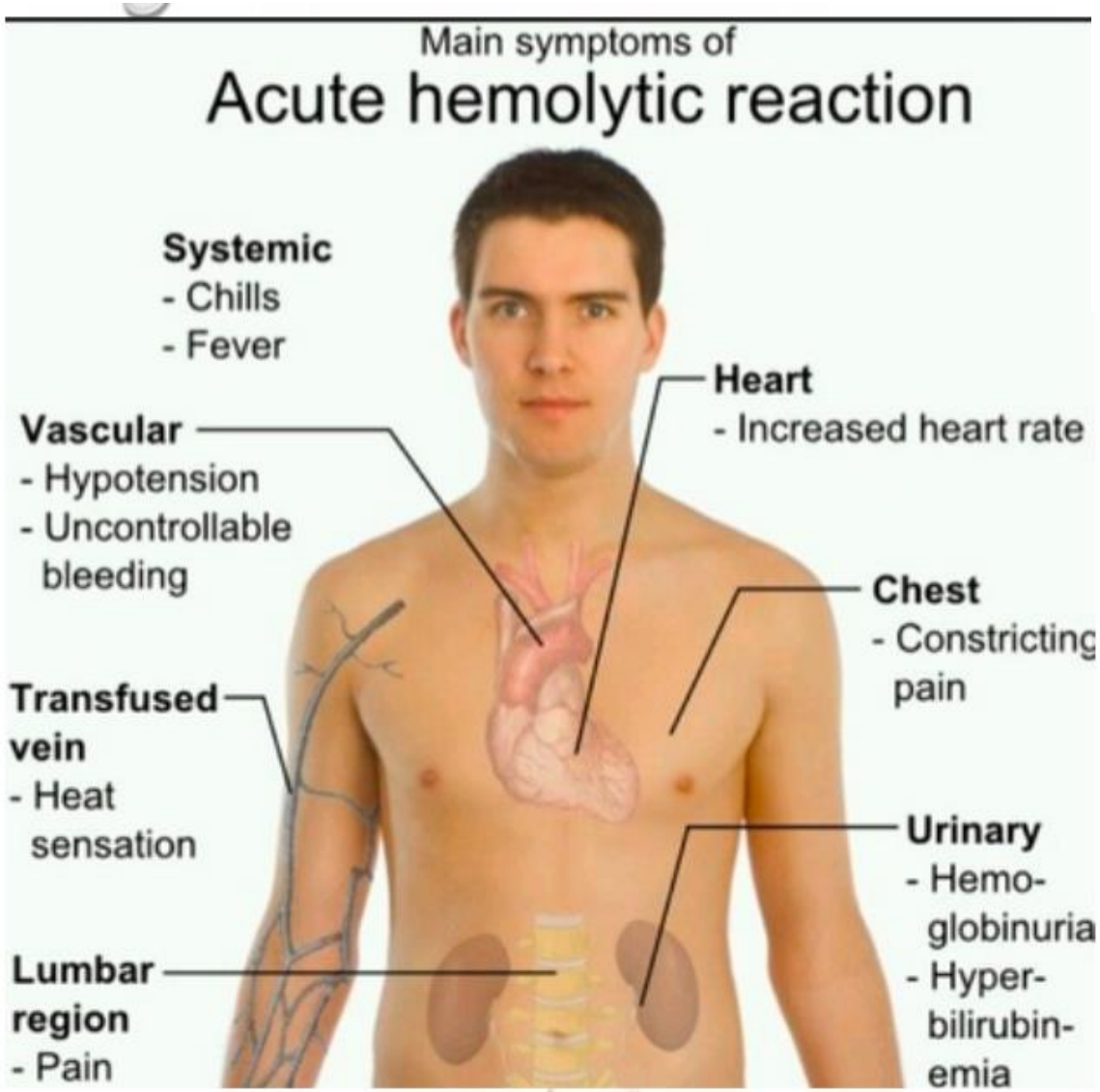
Incomplete complement activation (IgG and C3b opsonization) → splenic and hepatic erythrophagocytosis, resulting in **spherocytes and microspherocytes**.

Lysis of red cells → unconjugated bilirubin, which is **transported to the liver**.
Hepatic conjugated bilirubin is excreted as urobilinogen and stercobilinogen.

Anemia from red-cell destruction and **jaundice** from excess unconjugated and conjugated bilirubin are the primary clinical **manifestations of delayed hemolytic transfusion reactions.**

N Engl J Med 2019;381:150-62.

Hemolytic Transfusion Reactions

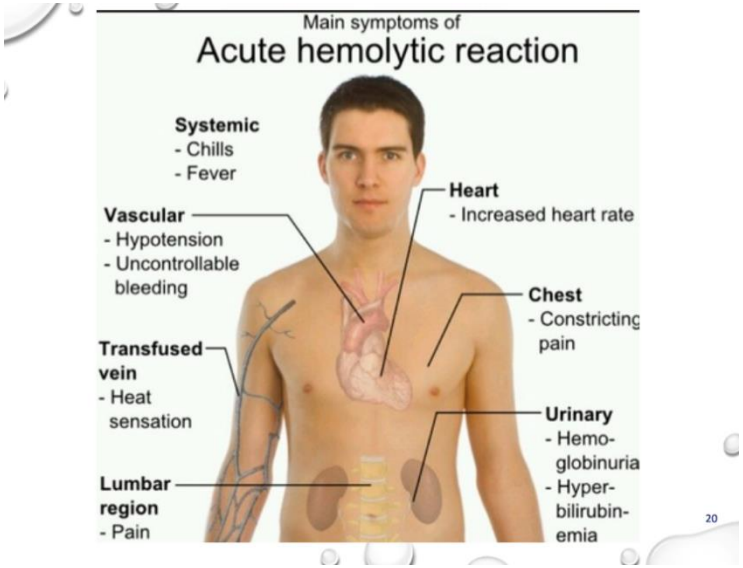


Hemolytic Transfusion Reactions

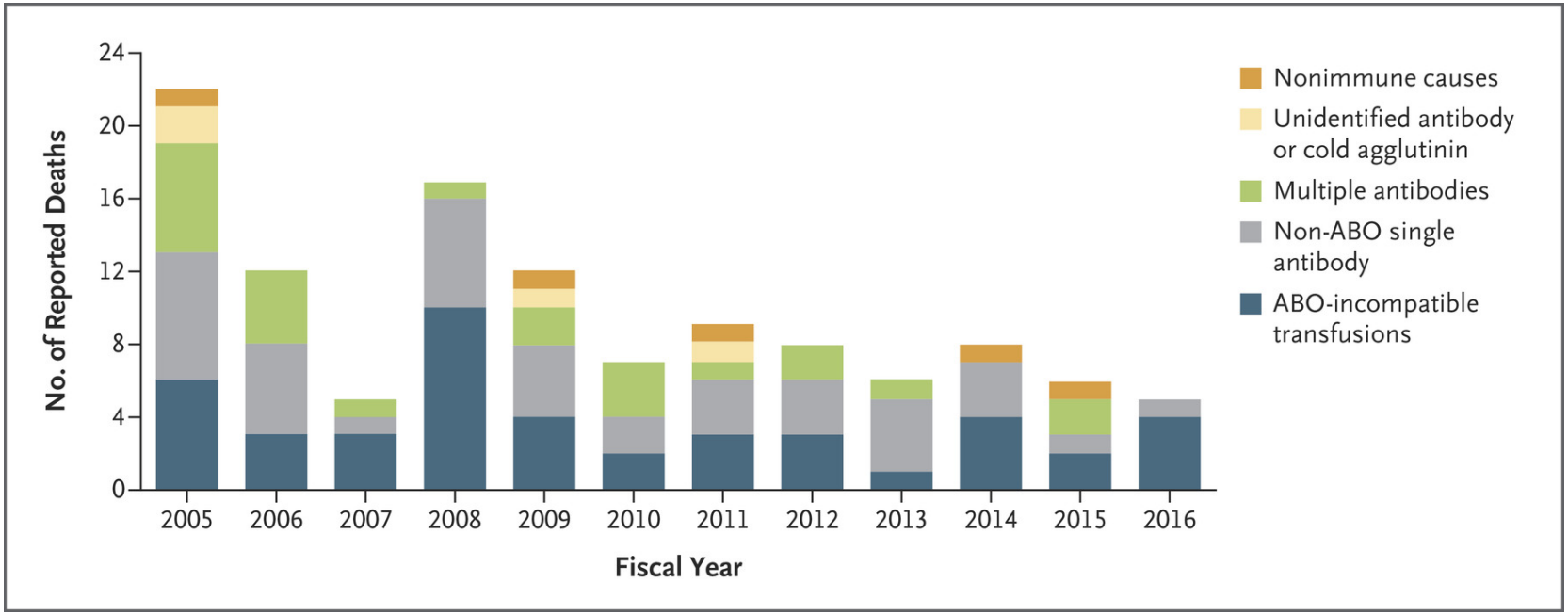
The earliest description of an incompatible hemolytic transfusion reaction dates to the experimental start of transfusion therapy in the mid-17th century.

Jean-Baptiste Denis described what has become the classic reaction:

The patient was transfused with 5-6 ounces of calves' blood. During the procedure, the patient complained that the vein in his right arm became quite painful. The procedure was repeated 2 days later; a larger transfusion was given. Following the transfusion, however, *the patient complained of pain in the arm vein; his pulse rose, he vomited, and he had a severe nosebleed, pain over the kidney, and an "oppressive sensation in the chest." The next day, he "made a great glass of urine with a color as black as if it had been mixed with the soot of a chimney."*



Hemolytic Transfusion Reactions



Annual Reported Deaths in the United States from Hemolytic Transfusion Reactions.
The data, reported by the Food and Drug Administration for fiscal years 2005 through 2016, show **an overall decline in deaths related to hemolytic transfusion reactions, with persistently low numbers of reported deaths in more recent years.**

Hemolytic Transfusion Reactions

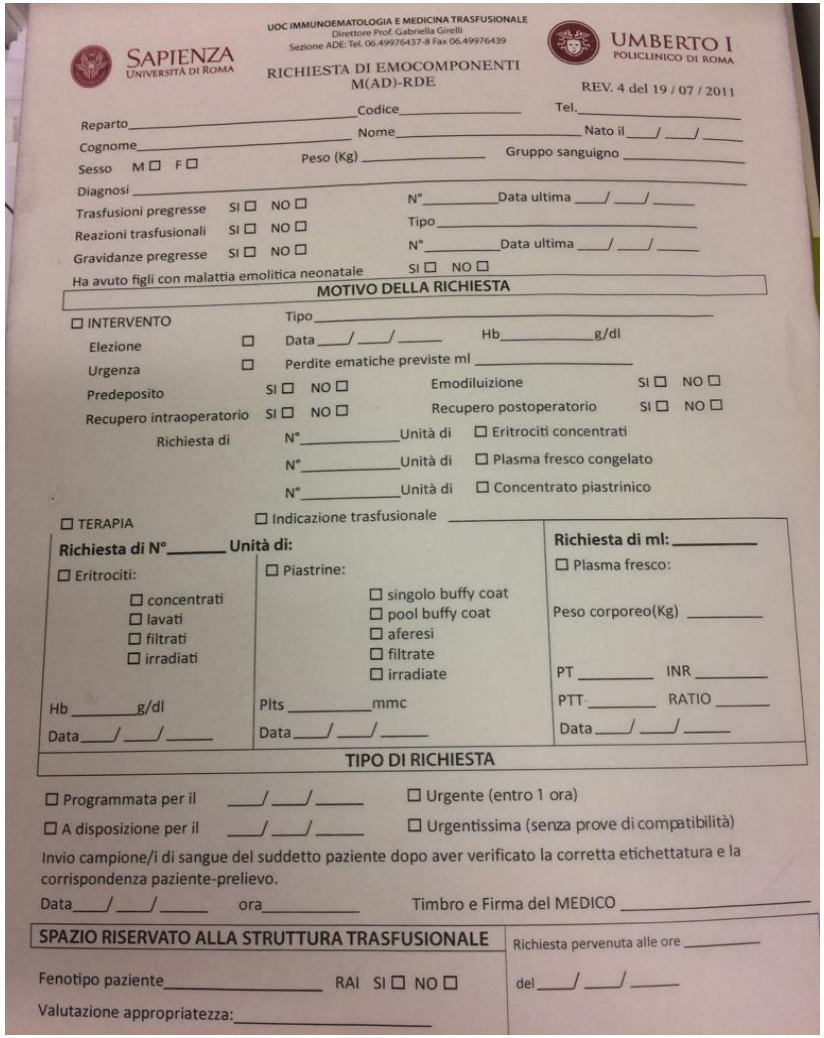
- If an ABO-incompatible transfusion occurs or is suspected, the transfusion should be stopped immediately, the venous line should be kept open with normal saline, and supportive care administered as needed.
- Close surveillance of the patient's vital signs for the first 30 minutes of transfusion should help identify most incompatible transfusions early.

Death occurs in 15% of cases of ABO incompatibility

and may result from as little as 30 mL of transfused ABO incompatible blood

Janatpour KA, Kalmin ND, Jensen HM, et al. Clinical outcomes of ABO-incompatible RBC transfusions. Am J Clin Pathol 2008;129:276–81.

BLOOD TRANSFUSION REQUEST



UOC IMMUNOEMATOLOGIA E MEDICINA TRASFUSIONALE
Direttore Prof. Gabriella Girelli
Sezione ADE: Tel. 06.49976437-8 Fax 06.49976439

SAPIENZA UNIVERSITÀ DI ROMA

UMBERTO I POLICLINICO DI ROMA

RICHIESTA DI EMOCOMPONENTI M(AD)-RDE

REV. 4 del 19 / 07 / 2011

Reparto_____ Codice_____ Tel._____

Cognome_____ Nome_____ Nato il ___/___/____

Sesso M☐ F☐ Peso (Kg) _____ Gruppo sanguigno _____

Diagnosi _____

Trasfusioni pregresse SI☐ NO☐ N°_____Data ultima ___/___/____

Reazioni trasfusionali SI☐ NO☐ Tipo _____

Gravidanze pregresse SI☐ NO☐ N°_____Data ultima ___/___/____

Ha avuto figli con malattia emolitica neonatale SI☐ NO☐

MOTIVO DELLA RICHIESTA

☐ INTERVENTO Tipo _____

Elezione ☐ Data ___/___/____ Hb_____g/dl

Urgenza ☐ Perdite ematiche previste ml _____

Predeposito SI☐ NO☐ Emodiluizione SI☐ NO☐

Recupero intraoperatorio SI☐ NO☐ Recupero postoperatorio SI☐ NO☐

Richiesta di N°_____Unità di ☐ Eritrociti concentrati

N°_____Unità di ☐ Plasma fresco congelato

N°_____Unità di ☐ Concentrato piastrinico

☐ TERAPIA ☐ Indicazione trasfusionale _____

Richiesta di N°_____ Unità di:

☐ Eritrociti:
- ☐ concentrati
- ☐ lavati
- ☐ filtrati
- ☐ irradiati

Hb _____g/dl

Data ___/___/____

☐ Piastrine:
- ☐ singolo buffy coat
- ☐ pool buffy coat
- ☐ aferesi
- ☐ filtrate
- ☐ irradiate

Plts _____mmc

Data ___/___/____

Richiesta di ml:_____

☐ Plasma fresco:

Peso corporeo(Kg) _____

PT _____ INR _____

PTT _____ RATIO _____

Data ___/___/____

TIPO DI RICHIESTA

☐ Programmata per il ___/___/____ ☐ Urgente (entro 1 ora)

☐ A disposizione per il ___/___/____ ☐ Urgentissima (senza prove di compatibilità)

Invio campione/i di sangue del suddetto paziente dopo aver verificato la corretta etichettatura e la corrispondenza paziente-prelievo.

Data ___/___/____ ora_____ Timbro e Firma del MEDICO _____

SPAZIO RISERVATO ALLA STRUTTURA TRASFUSIONALE

Fenotipo paziente_____ RAI SI☐ NO☐

Valutazione appropriatezza:_____

Richiesta pervenuta alle ore _____

del ___/___/____

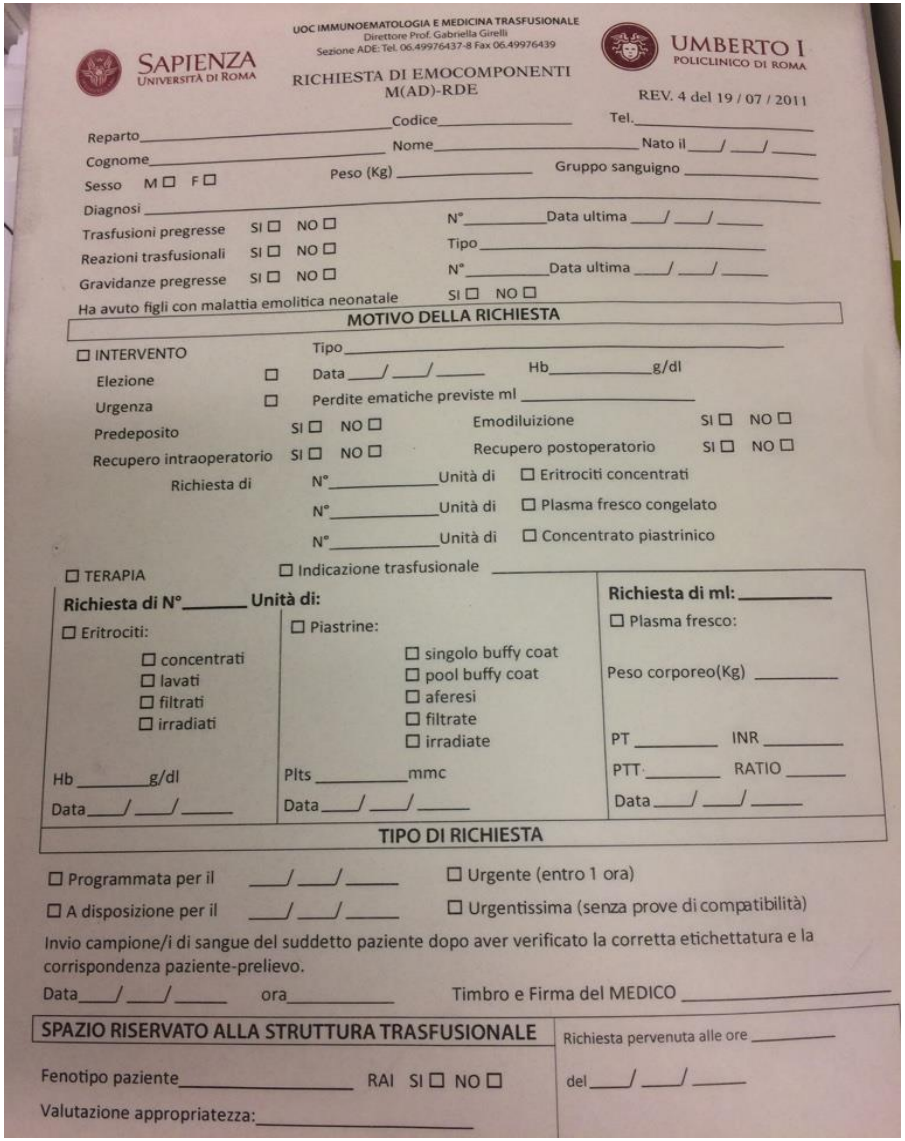
The request for blood products should be in writing form, including:

- Patient identification (full name, age or birth date).
- Diagnosis and indication for transfusion.
- Requested product(s) and number of units.
- Date and time of request and desired delivery.
- Name, signature of the prescribing physician.

The following information must be mentioned:

- ABO blood group and RhD.
- Did the patient receive transfusion earlier?
- If YES when was the last?
- Has the patient been pregnant?
- Are previous transfusion reactions known?
- Have red cell antibodies ever been detected?

BLOOD TRANSFUSION REQUEST



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M(AD)-RDE

REV. 4 del 19 / 07 / 2011

Reparto_____ Codice_____ Tel._____
Cognome_____ Nome_____ Nato il ___/___/____
Sesso M ☐ F ☐ Peso (Kg) _____ Gruppo sanguigno _____
Diagnosi _____
Trasfusioni pregresse SI ☐ NO ☐ N°_____Data ultima ___/___/____
Reazioni trasfusionali SI ☐ NO ☐ Tipo _____
Gravidanze pregresse SI ☐ NO ☐ N°_____Data ultima ___/___/____
Ha avuto figli con malattia emolitica neonatale SI ☐ NO ☐

MOTIVO DELLA RICHIESTA

☐ INTERVENTO Tipo _____
Elezione ☐ Data ___/___/____ Hb_____g/dl
Urgenza ☐ Perdite ematiche previste ml _____
Predeposito SI ☐ NO ☐ Emodiluizione SI ☐ NO ☐
Recupero intraoperatorio SI ☐ NO ☐ Recupero postoperatorio SI ☐ NO ☐
Richiesta di N°_____Unità di ☐ Eritrociti concentrati
N°_____Unità di ☐ Plasma fresco congelato
N°_____Unità di ☐ Concentrato piastrinico

☐ TERAPIA ☐ Indicazione trasfusionale _____

Richiesta di N°_____ Unità di:
☐ Eritrociti:
☐ concentrati
☐ lavati
☐ filtrati
☐ irradiati
Hb _____g/dl
Data ___/___/____

☐ Piastrine:
☐ singolo buffy coat
☐ pool buffy coat
☐ aferesi
☐ filtrate
☐ irradiate
Plts _____mmc
Data ___/___/____

Richiesta di ml:_____
☐ Plasma fresco:
Peso corporeo(Kg) _____
PT _____ INR _____
PTT _____ RATIO _____
Data ___/___/____

TIPO DI RICHIESTA

☐ Programmata per il ___/___/____ ☐ Urgente (entro 1 ora)
☐ A disposizione per il ___/___/____ ☐ Urgentissima (senza prove di compatibilità)

Invio campione/i di sangue del suddetto paziente dopo aver verificato la corretta etichettatura e la corrispondenza paziente-prelievo.
Data ___/___/____ ora_____ Timbro e Firma del MEDICO _____

SPAZIO RISERVATO ALLA STRUTTURA TRASFUSIONALE
Fenotipo paziente_____ RAI SI ☐ NO ☐
Valutazione appropriatezza:_____
Richiesta pervenuta alle ore _____
del ___/___/____

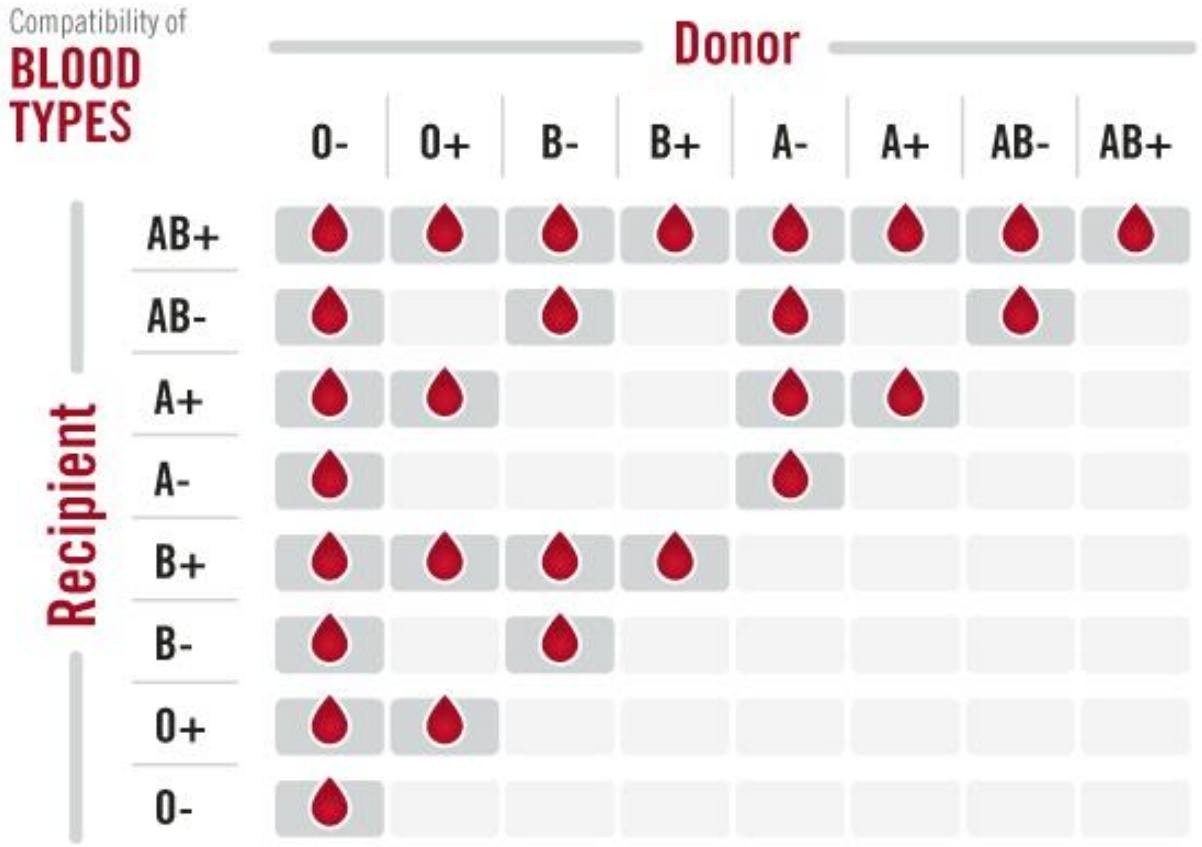
To ensure patient safety, the transfusion laboratory requires the patient to have been tested on more than one occasion prior to deliver red blood cells.

The two separate samples for Group must have been taken at TWO different times

Blood donation

Which blood types are your red blood cells compatible with?

O individuals are universal donors, but they can receive blood only from O individuals, while AB can receive from each ABO group, universal recipients



Blood donation

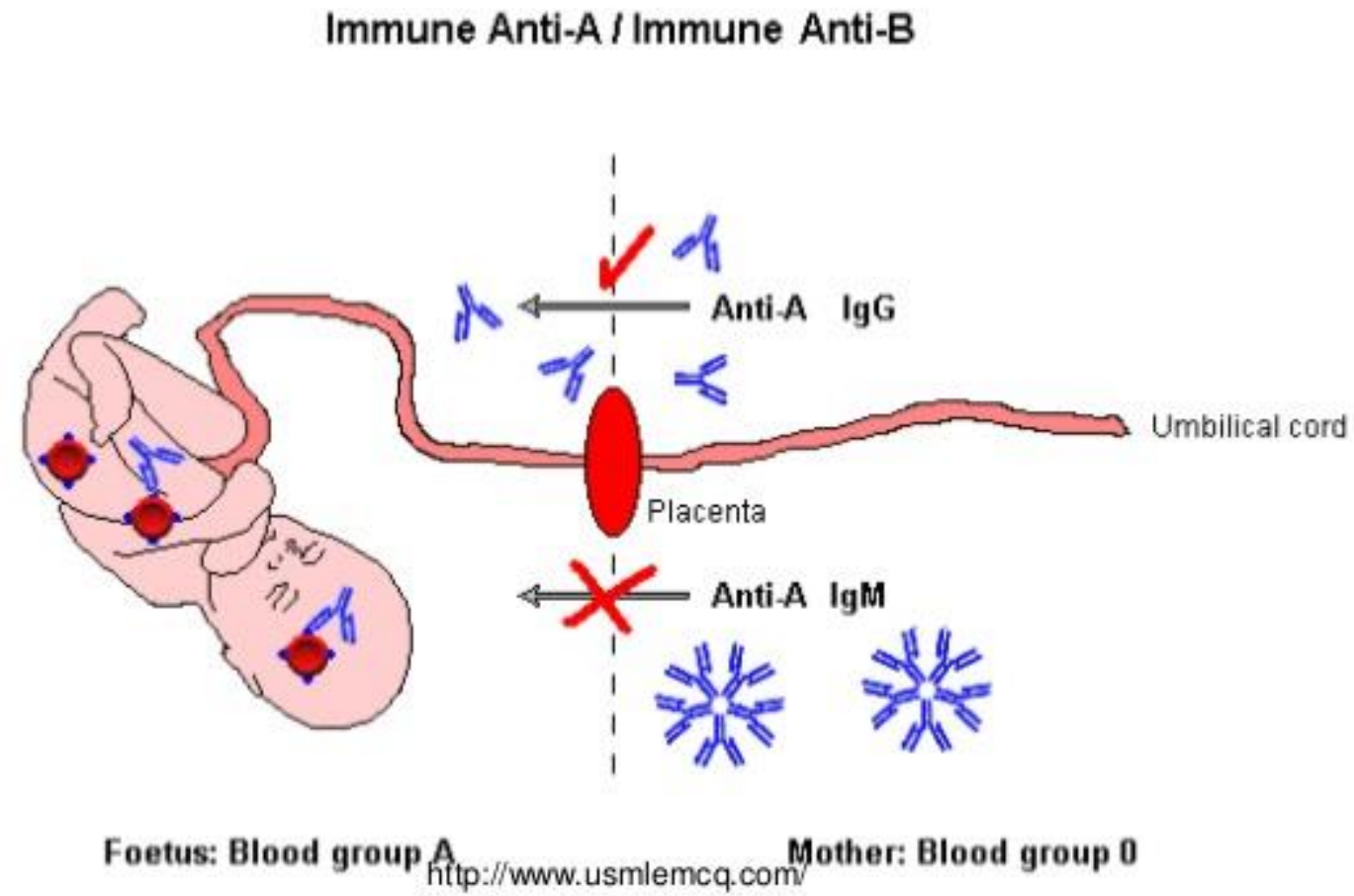
Compatibility Table

	Donor ABO Group		
Recipient ABO Group	RBCs	Plasma	Platelets
UNKNOWN	O	AB	AB
O	O	O, A, B, AB	O, A, B, AB
A	A, O	A, AB	A, AB
B	B, O	B, AB	B, AB
AB	AB, A, B, O	AB	AB

Considering the absence of antibodies AB individuals are universal plasma donor

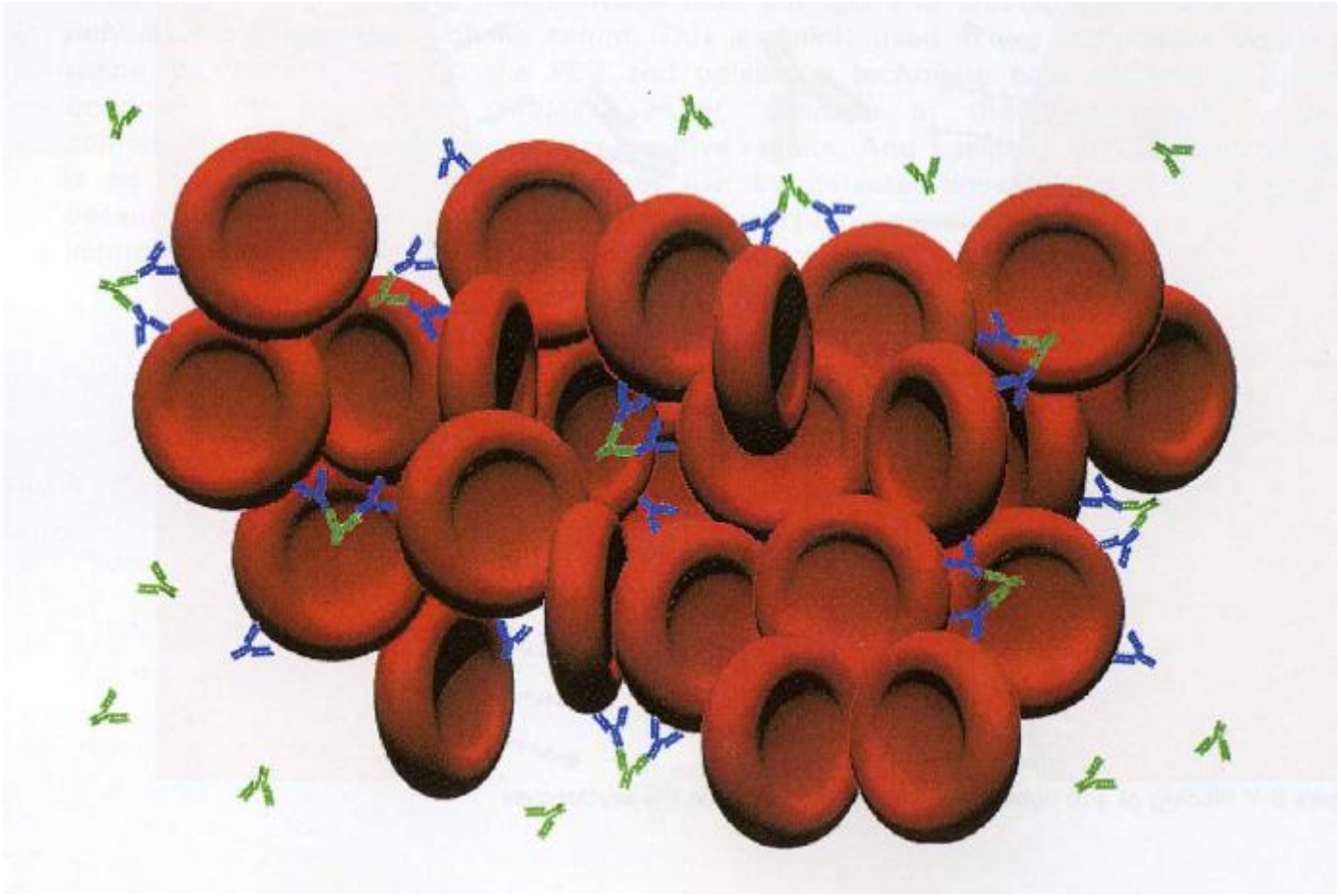
Platelets incompatibles transfusion could cause platelet refractoriness: platelets transfused are destroyed, so the transfusion is less efficient

•Seldom cause HDN , usually mild



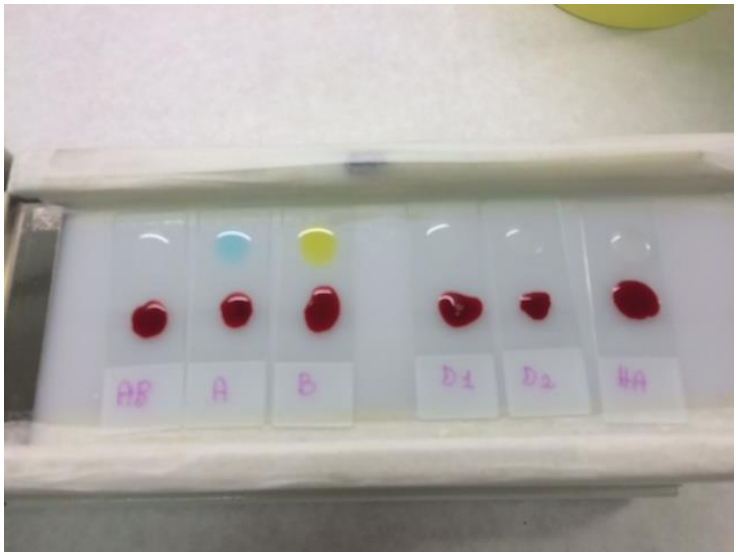
jaundice!

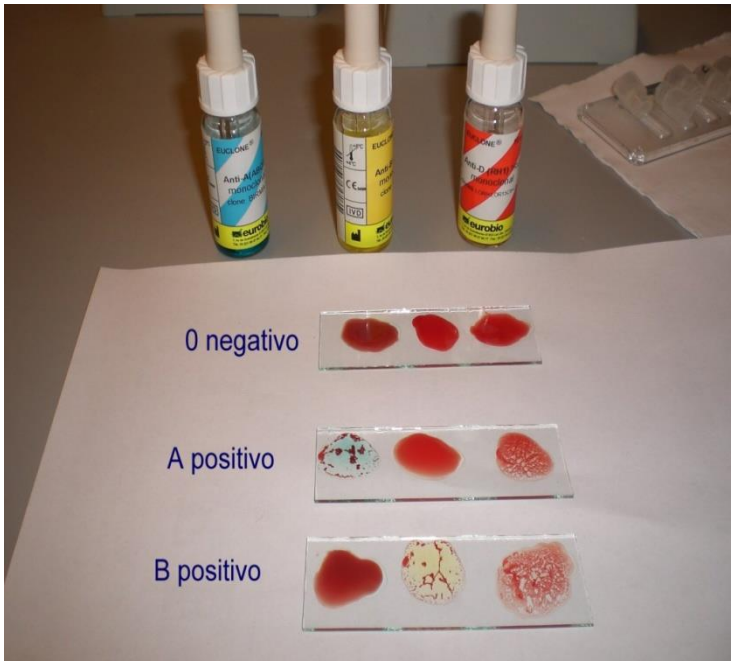
Lab. clinical practice: Agglutination of erytrocytes



ABO typing

The blood grouping and Rh(D) typing procedure is based on the principle of agglutination.
Normal red blood cells, possessing antigens, will agglutinate in the presence of antibodies directed toward those antigens. Commercial antisera are used to test patient and donor cells

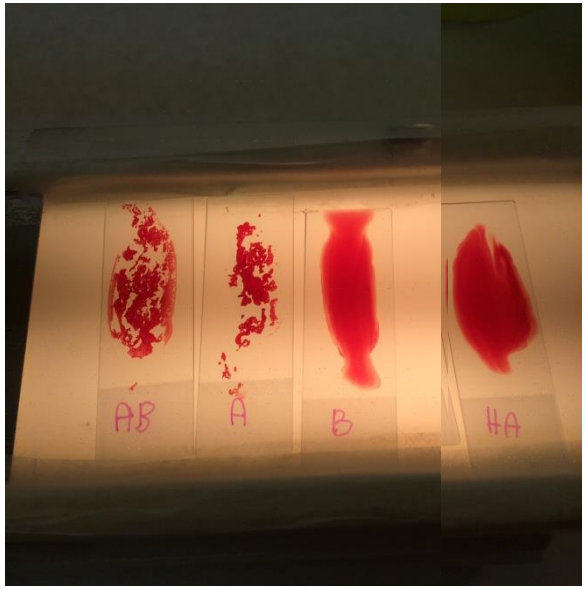




A positive

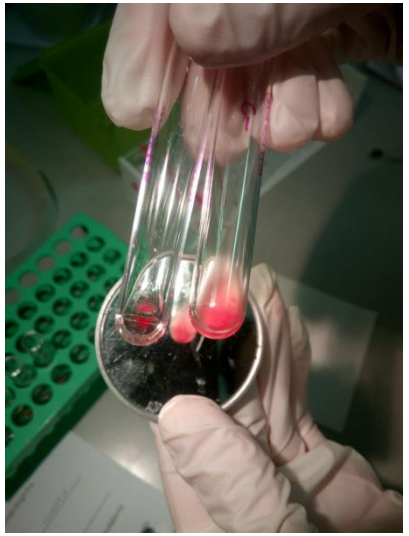
AB0 direct grouping
(we know the antibodies)

	Anti –A	Anti-B
GROUP A	pos	neg
GROUP B	neg	pos
GROUP AB	pos	pos
GROUP 0	neg	neg

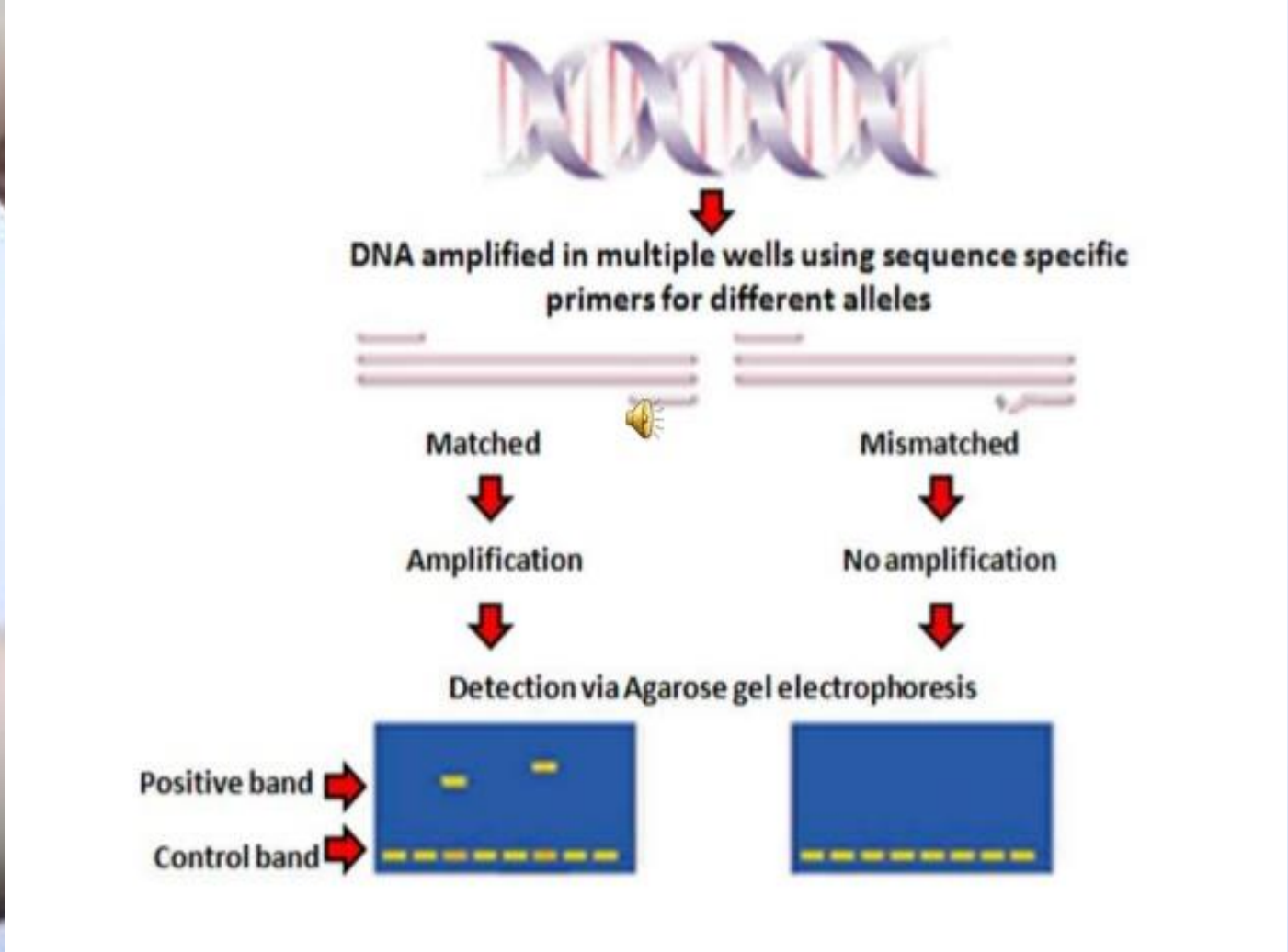


ABO reverse grouping
(we know the antigens)

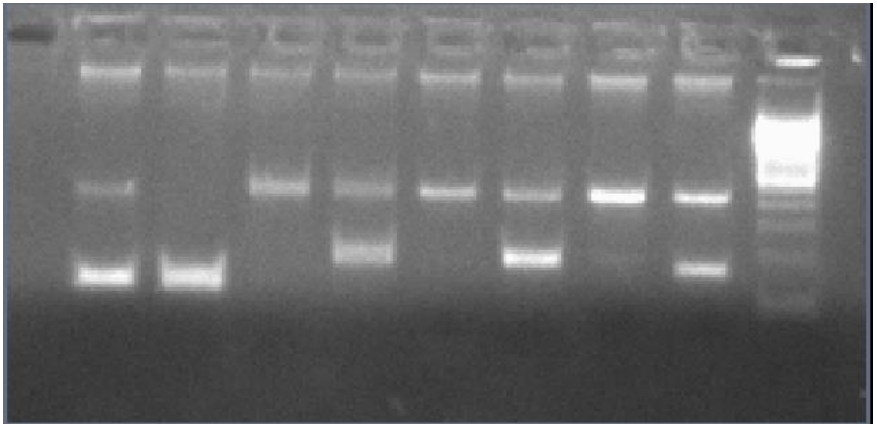
	A cells	B cells	0 cells
GROUP A	neg	pos	neg
GROUP B	pos	neg	neg
GROUP AB	neg	neg	neg
GROUP 0	pos	pos	neg



PCR-SSP


DNA amplified in multiple wells using sequence specific primers for different alleles
Matched
Mismatched
Amplification
No amplification
Detection via Agarose gel electrophoresis
Positive band
Control band

ABO typing



0A genotype, A phenotype

RBC-Ready Gene ABO IVD CE LOT S9AP102

Protocol for Documentation 2014-09

Date:

Reaction No.	1	2	3	4	5	6#	7	8		
PCR Product (Size in bp)	134	133	194	193	195	194	170	170		
Specificity	O^1	non O^1	O^2	non O^2	B	non B	A^2	non A^2		
Examples for results:									***Genotype**	***Phenotype**
Position 1–positive (O^1)	+	-	-	+	-	+	-	+	O^1O^1	O
	+	+	+	+	-	+	-	+	O^1O^2	O
	+	+	-	+	+	+	-	+	O^1B	B
	+	+	-	+	-	+	-	+	O^1A	A
	+	+	-	+	-	+	+	+	O^1A^2	A_2
	-	+	+	-	-	+	-	+	O^2O^2	O
Position 3-positive (O^2)	-	+	+	-	+	+	-	+	$^{**}O^2B$	B
	-	+	+	+	-	+	-	+	O^2A	A
	-	+	+	+	-	+	+	+	O^2A^2	A_2
Position 5-positive (B)	-	+	-	-	+	-	-	+	$^{**}BB$	B
	-	+	-	+	+	+	-	+	AB	AB
	-	+	-	+	+	+	+	+	A^2B	A_2B
Position 2/4/6-positive (non $O^1/O^2/B$)	-	+	-	+	-	+	-	+	AA	A
	-	+	-	+	-	+	+	+	AA^2	A
	-	+	-	+	-	+	+	-	A^2A^2	A_2
Results:										

* The specificity in table does not consider rare blood groups. You will find closer explanations about this in the product insert.

** An exception to the general reaction pattern is represented by the non o^2 reaction, in case of a o^2o^2, BB or BO^2 genotype the primer mix (No. 4) reacts negatively.

\# In rare cases the primers tend to show an unspecific band that it significantly larger than the internal control.

The positive sign (+) marks the appearance and the negative sign (-) marks the absence of a DNA fragment. You can enter the band pattern of the current samples in the last lanes. The size of the internal control in all reaction mixes is 434 bp. In the case of heterozygote samples all four "non" reactions (2,4,6,8) and two of the blood group specific reactions (1,3,5,7) must be positive. If you only find one DNA fragment in specific reactions (1,3,5,7) connected with positive amplifications in all four "non" reactions (2,4,6,8) the sample is heterozygote with A.

Gel picture	**Notes:**
	Result:

inno-train Diagnostik GmbH, Niederhöchstädter Straße 62, D-61476 Kronberg/Taunus, Germany
Tel. 0049-(0)6173 / 6079-30 Fax: 0049-(0)6173 / 6079-50, E-mail: info@inno-train.de, www.inno-train.de

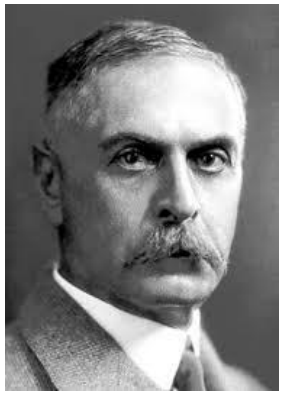
The Rh System

- The most important blood group system after AB0 in transfusion medicine
- 1937 Levine and Landsteiner
- A very important protein of blood groups
    - Highly immunogenic (90% possibility to became immunized)
    - Trasfusions
    - Pregnancies
    - Variability in populations
    - RhD+ 85% of caucasians, 94% Africans, 98% Asiatics
    - High genetic polymorphism

Discovered in 1937 by Levine and Landsteiner

- In **1939 by Levine and Stetson found an antibody directed at the D antigen in the serum of a woman whose fetus had fatal hemolytic disease of the newborn**.

- **The Rh system was identified by the work of Landsteiner and Wiener who found that human RBCs were agglutinated by an antibody, apparently common to all rhesus monkeys and 85% of humans. This factor was named the Rh factor.**



- **Landsteiner and Wiener immunized guinea pigs and rabbits with the RBCs of Rhesus monkeys, the antibody produced by these animals agglutinated 85% of human RBCs**. Later the antigens detected by the rhesus antibody and by the human antibody were established as dissimilar, but the system had already been named.

- **This contribution to medical science was the most significant event in blood group systems research since the discovery of the ABO system 40 years earlier.**

The Rh/D factor

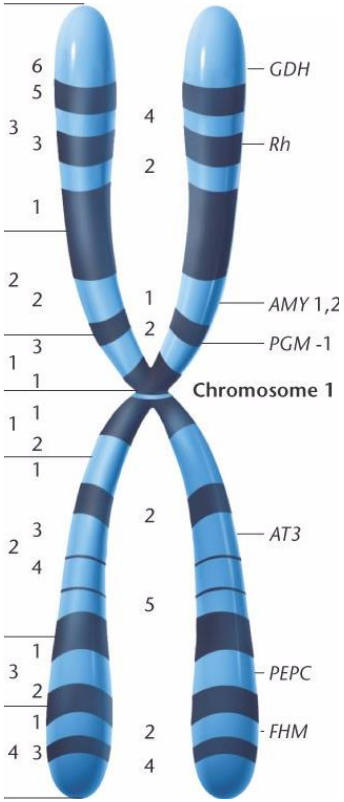
The **Rh/D factor is** not the single entity as originally thought but **a complex system of antigens**.

There are actually two genes, **RHD** and **RHCE**, accounting for five main antigens: **D, C, c, E, e**.

The D antigen is the strongest of the Rh system and most potent antigenically and therefore the most important in haemolytic disease and in transfusion reactions.

Rh positive is D positive !

The Rh/D factor



- ✓ Two genes (**RHD, RHCE**) in close proximity on **CHROMOSOME 1** encode the erythrocyte **Rh proteins, RhD and RhCE**; **one carries the D antigen**, and **the other carries CE antigens** in various combinations (ce, Ce, cE, or CE).
- ✓ The genes each have ten exons, are 97% identical.
- ✓ Individuals who lack RhD protein, “Rh or D negative”, most often have a complete deletion of the RHD gene

Rh System

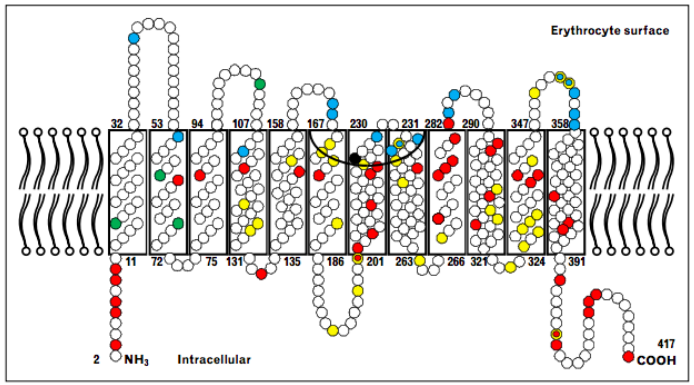
Rh+ Phenotypes

CcDee
CcDEe
CcDEE
ccDee
ccDEE
CCDee
CCDEe
CCDEE
ccDEe

Rh- Phenotypes

Ccdee
CcdEe
Ccdee
Ccdee
ccdEE
CcdEe

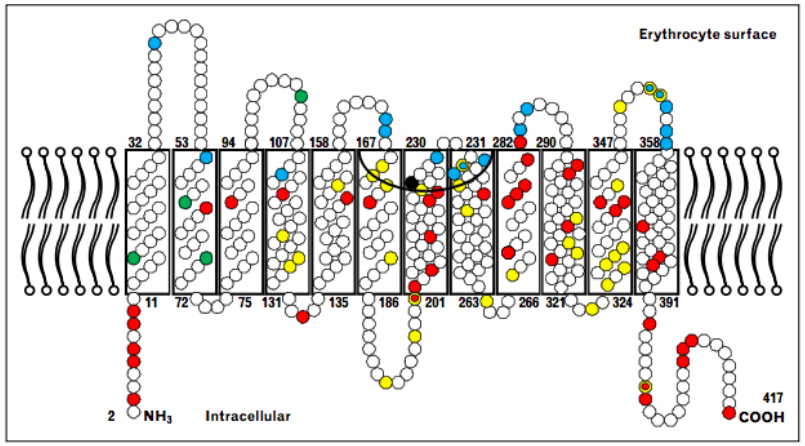
Figure 1 Model of Rhesus proteins in the red blood cell membrane



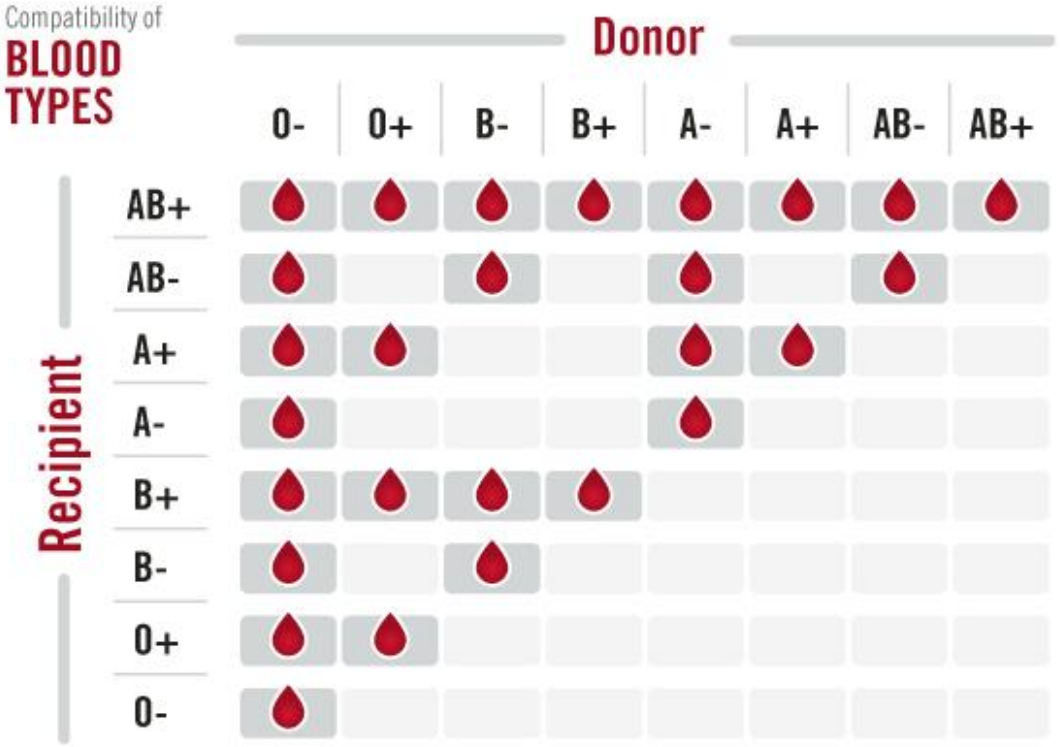
Rh system

In the Rh system there are no natural antibodies: it is necessary to have an immunization (through pregnancy or transfusion) to produce the specific antibody.

Figure 1 Model of Rhesus proteins in the red blood cell membrane

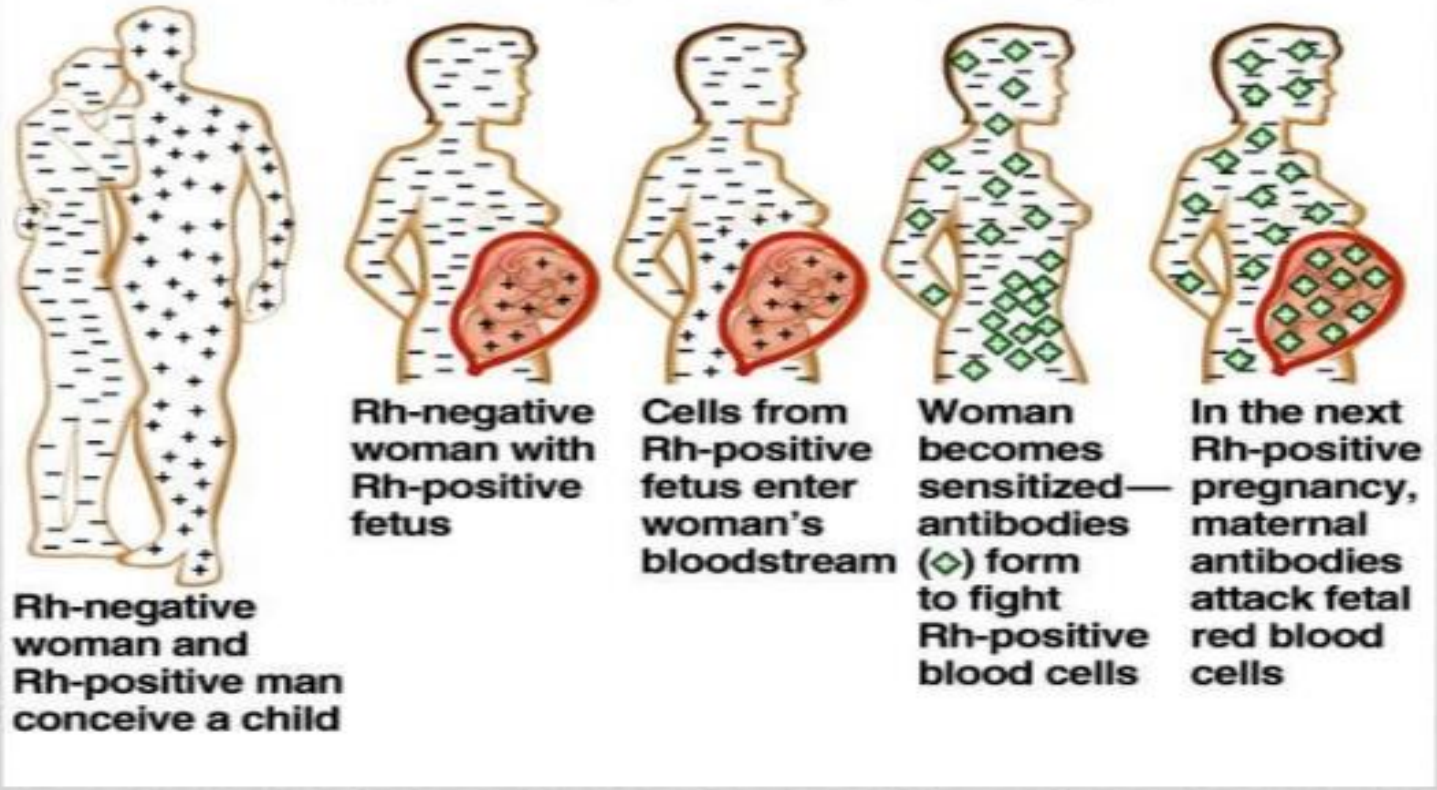


Blood donation



Now you are able to observe again this picture taking into account the RhD system

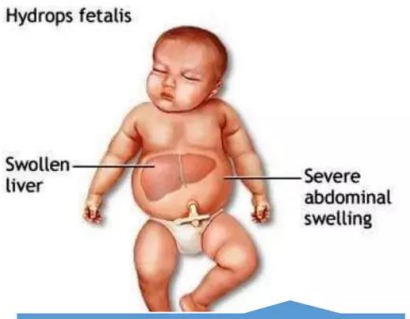
Remember that anti-D antibodies are not natural and it is necessary to have immunizing events such as pregnancies or previous blood transfusions



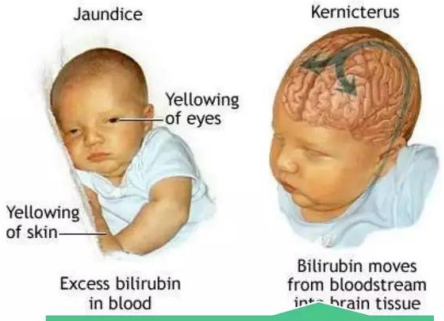
This figure shows pathophysiology of HDN

Retrieved from: http://tmedweb.tulane.edu/pharmwiki/lib/exe/fetch.php/rhod.png

Manifestations of the **Hemolytic Disease of the Fetus & Newborn (HDFN)**



Hydrops Fetalis

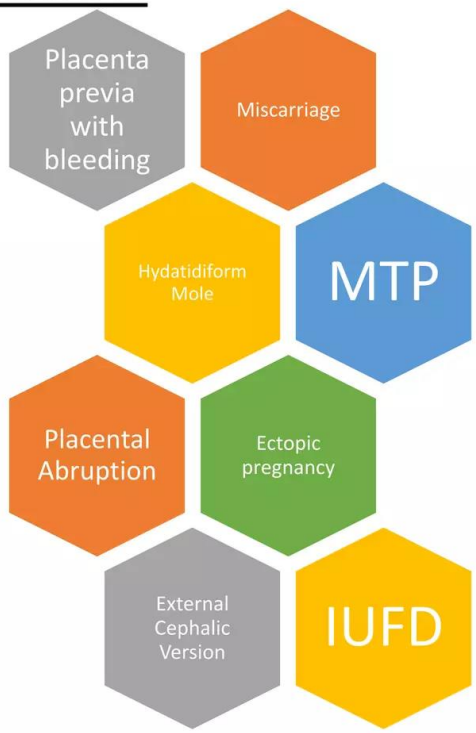


Icterus gravis neonatorum

Congenital anemia of the newborn

To prevent Active Immunization

- Rh anti-D immunoglobulin (IgG) is administered intramuscularly to the mother following childbirth.
- Other conditions:



When to administer?

- Within 72 hours or preferably earlier following delivery or abortion
- If >72 hours, may be given up to 14-28 days after delivery
- Baby born is Rh-positive & direct Coombs' test is negative
- When Rh factor of the fetus cannot be determined

During pregnancy, if woman is Rh negative & has no antibody, she hould have one dose of 300µg Rh IgG at 28 weeks & again after birth (within 72 hours)

Dose

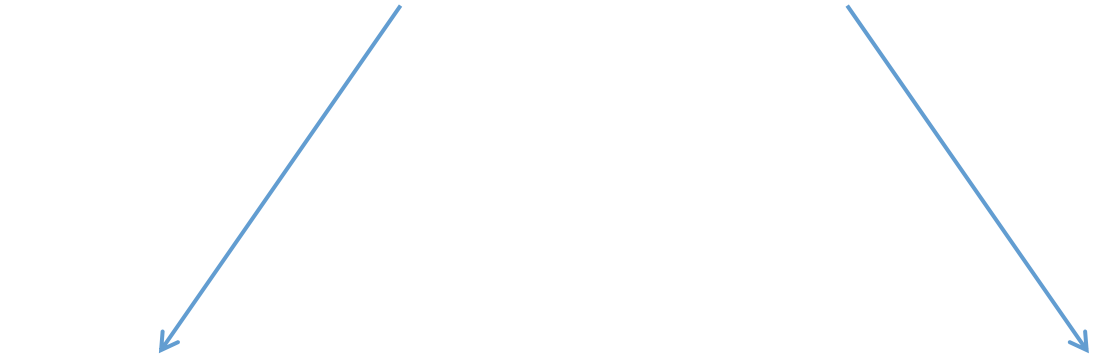
- Anti D-gammaglobulin 300 µg intramuscularly following delivery
- 50 µg of Rh-immune globulin IM within 72 hours (induced/spontaneous abortion, ectopic, molar pregnancy or CVS in 1st trimester)
- Women with pregnancy >12 weeks should have full dose 300 µg.

Generally 300µg dose will protect a woman from fetal haemorrhage of upto 30mL of fetal whole blood.

Calculation of dose

- By estimate volume of fetal blood entering into maternal circulation by "Kleihauer-Betke test"
- If 80 fetal erythrocytes in 50 low power fields in maternal peripheral blood films = transplacental hemorrhage to 4mL of fetal blood.
- More accurate test : immunofluorescence & flow cytometry.
- If fetomaternal hemorrhage > 30 mL whole blood, dose is 10µg for every 1mL of fetal whole blood.

D VARIANT



WEAK D

PARTIAL D

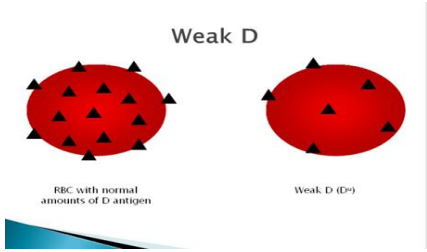
D weak

First described by Stratton on 1946 as Du,

Weak D expression results from single point mutations in RHD that encode amino acid changes predicted to be intracellular or in the transmembrane regions of RhD with reduced number of D antigen sites on the RBCs.

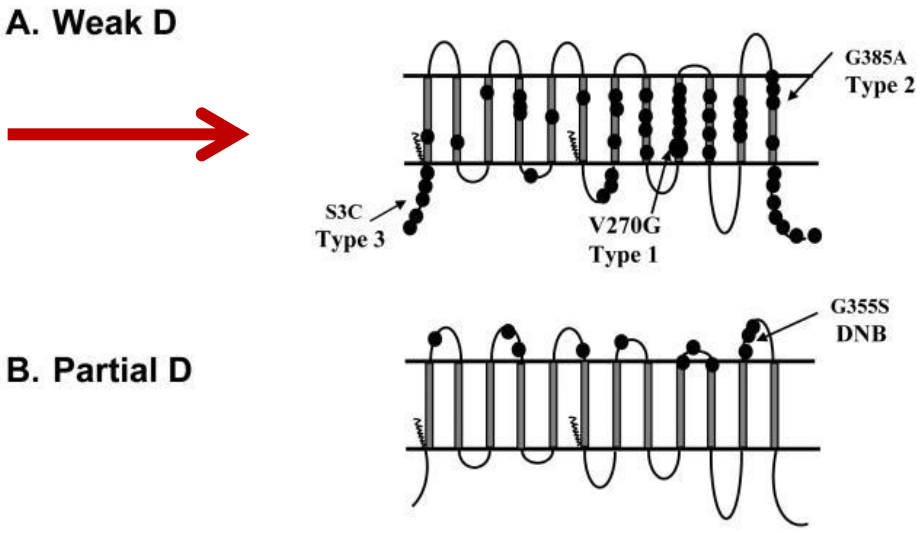
Over 99 different mutations, the most common being a Val270Gly designated Type 1, cause weak D expression . Mutations are catalogued on the Rhesus Base and blood group mutation websites and are updated regularly.

Occurs in an estimated **0.2%-1% of Caucasians**.



D weak

Mutations are catalogued on the RhesusBase and blood group mutation websites and are updated regularly



Some Weak D Types

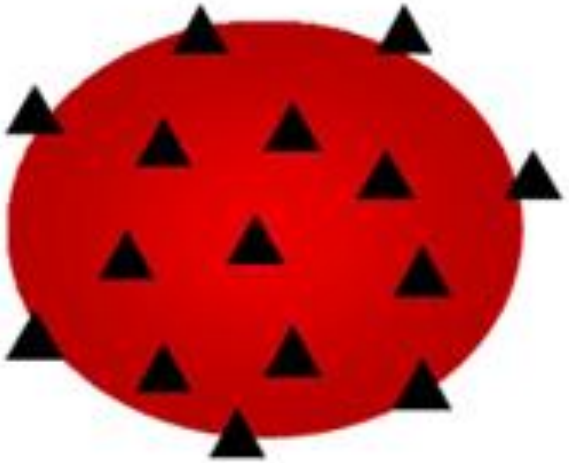
- Type 1
- Type 2
- Type 3

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

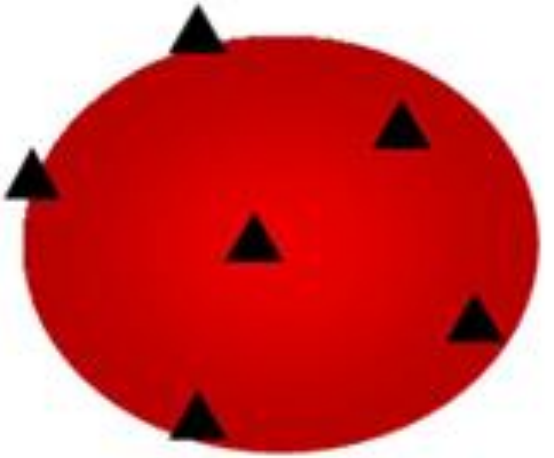
- Type 4.2
- Type 5
- Type 11
- Type 15
- Type 19
- Type 20

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

Weak D



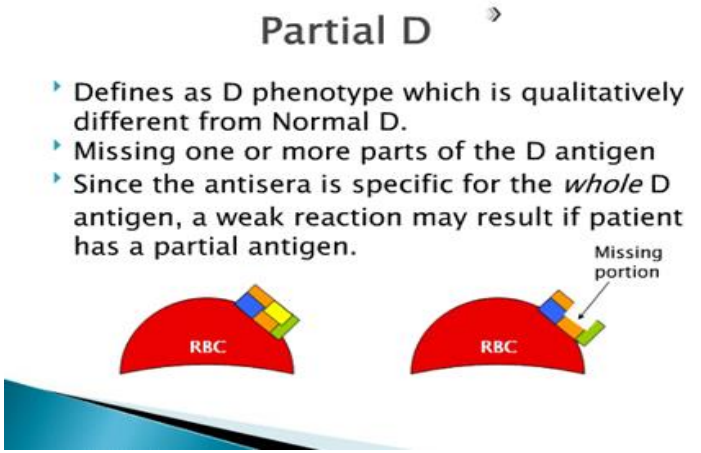
RBC with normal amounts of D antigen



Weak D (D^u)

D partial

RBCs with **partial D antigen type as D-positive**, but individuals often produce anti-D when stimulated by transfusion or pregnancy.
Some partial D, similar to weak D, result from point mutations in RHD that cause single amino acid changes. **These changes** are located on the **EXTRACELLULAR REGIONS** and **ALTER OR CREATE NEW EPITOPES.**
Many partial D result from hybrid genes that have regions of RHD replaced by the corresponding regions of RHCE. These replacements can involve short regions encompassing several codons, entire exons, or large regions of the gene, and the novel sequence of amino acids that result from RhD joined with RhCE can generate new Rh antigens

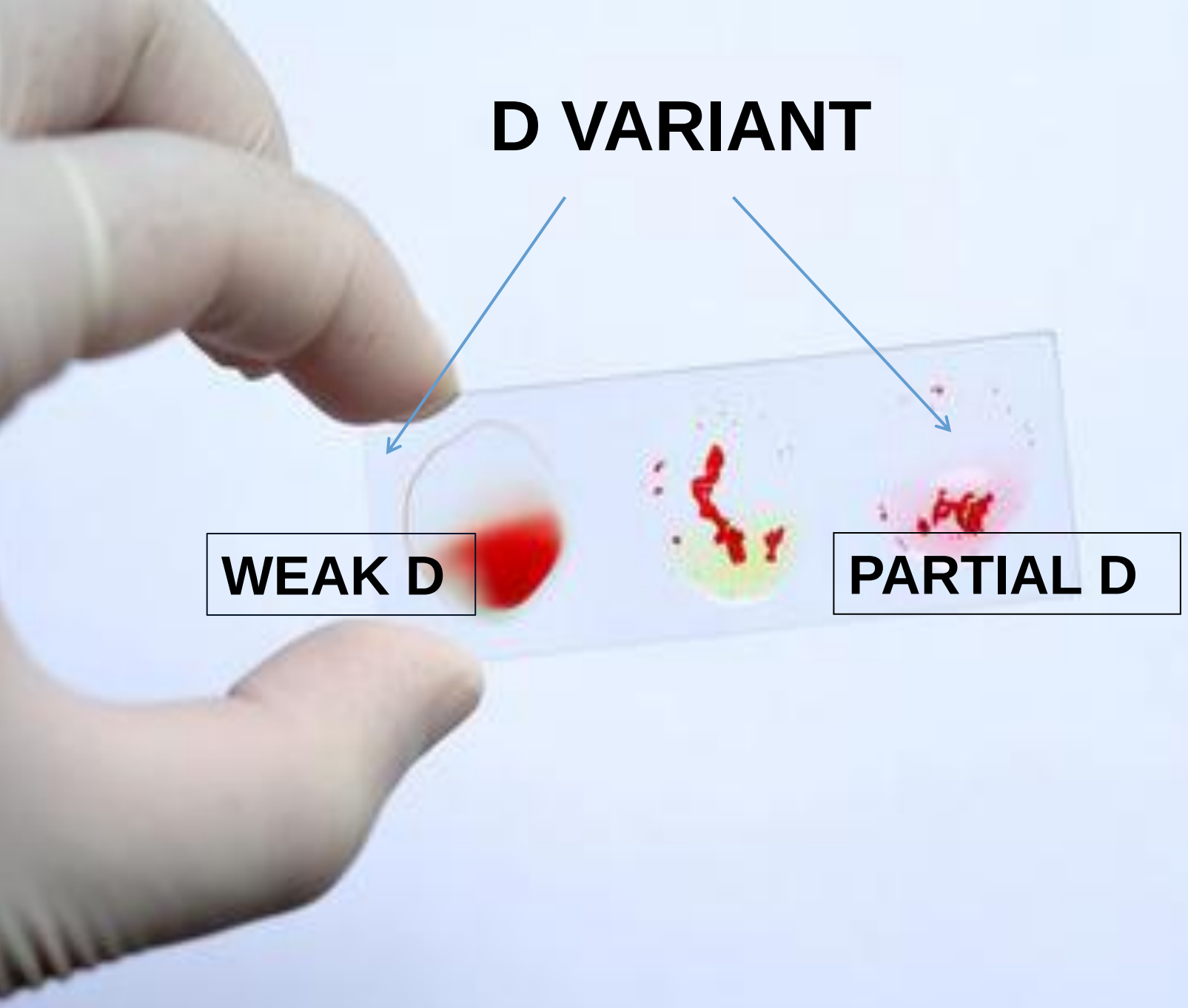


D partial

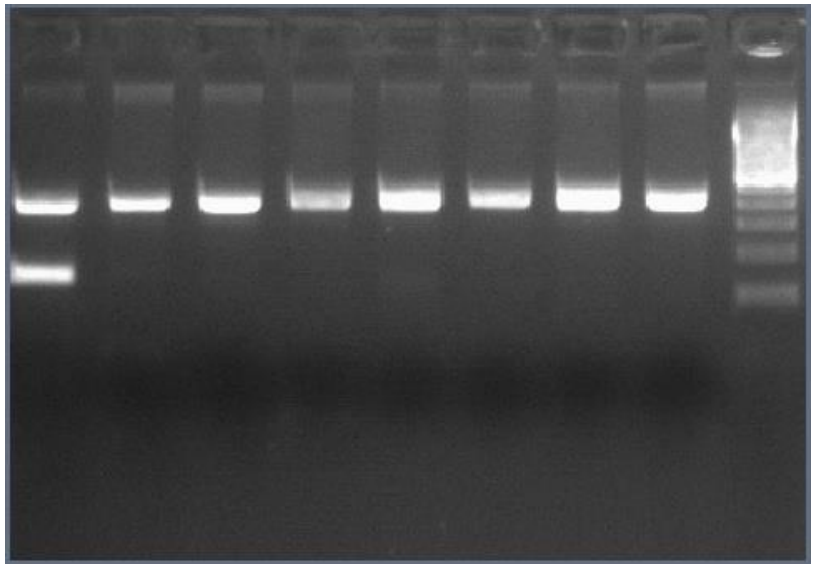
Partial D

- Defines as D phenotype which is qualitatively different from Normal D.
- Missing one or more parts of the D antigen
- Since the antisera is specific for the *whole* D antigen, a weak reaction may result if patient has a partial antigen.

D VARIANT



Weak D typing



Weak D type 1

BAG HEALTH CARE

BAGene

CE LOT 1102 WD

IVD 2012-08

REF 6647

Weak D-TYPE

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

Proben-ID / *Sample-ID:*

Name:

Geb.-Datum / *Birthdate:*

Ergebnis / *Result:*

Datum / *Date:*

Unterschrift / *Signature:*

Gelbild / *Gel Image*

Version 2.1 - 02/2011

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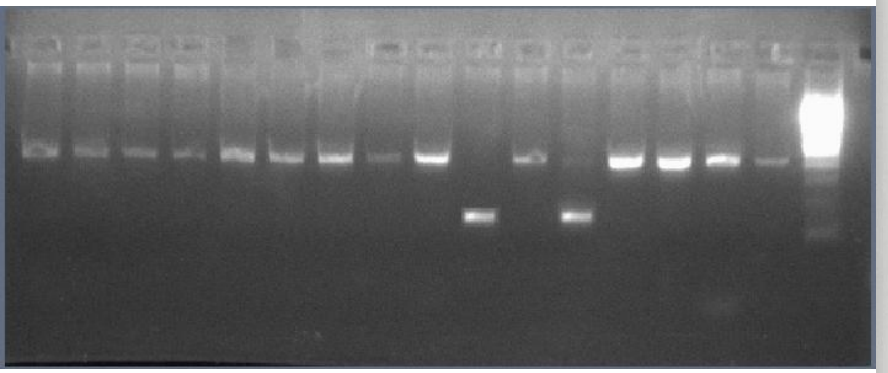
Fax: +49 (0) 6404/925-421
service@bag-healthcare.com



Rh negative ccdee

RBC-Ready Gene CDE IVD CE LOT S9CP032

Protocol for Documentation 2014-02

Reaction No.	1	2	3[B]	4[A]	5	6	7	8	9	10	11	12	13[#]	14[#]	15[#]	16[#]	
PCR-Product (Size in bp)	124	149 305	139	152 303	130	122	186	147	106 298	145	157	155	166 107	166 165	139 135	139 133	Typically associated haplotype
Specificity	D_1	$D_{5'}C$ D_3	D_4	D_5 psi	D_6	D_7	D_8	D_{10}	C „W"	c	E	e	D^{VII} DHMi	D^{VI} DAU	DNB 697A	DNB 697C	
	D Exon Block								CcEe Block				CATPA Block				
D, #[F]	+	149 305	+	152	+	+	+	+									
d	-	-	-	-	-	-	-	-									
C	-	149	-	-	-	-	-	-	106	-	-	-					
C^W									106 298	-	-	-					
c^W (rare)									298	+	-	-					
c									-	+	-	-					
E									-	-	+	-					
e									-	-	-	+					
D cat IIIa	+	149	-	152	+	+	+	+									cDe
D cat III type 5 + 6	+	149	-	152	+	+	+	+									open
D cat III type 7 **	+	-	-	152	+	+	+	+									open
D cat IIIb **	+	305	+	152	+	+	+	+									cDe
D cat IIIc	+	149	+	152	+	+	+	+									cDe
D cat III type 4	+	149	+	152	+	+	+	+									open
D cat IVa	+	149	+	152	+	-	+	+									cDe
D cat IVb D cat IV type 5	+	149 305	+	152	+	-	-	+									open
D cat IV type 3	+	149 305	+	152	-	-	-	+									cDe
D cat IV type 4	+	149 305	+	152	+	-	+	+									cDe
D cat VI typ 1[A]	+	149 305	-	-	+	+	+	+									cDE
D cat VI typ 2	+	149 305	-	-	-	+	+	-									cDe
D cat VI typ 3	+	149	-	-	-	+	+	-									cDe
D cat VI typ 4	+	149	-	-	+	+	+	+									cDe
D cat Va type 2, D cat Va, D cat V type 7, DCS	+	149 305	+	-	+	+	+	+									open
D cat V type 3, DBS-0, DBS-1, D cat Va type 6 (identical to DV type 3?)	+	149 305	+	-	+	+	+	+									cDE
D cat V type 1, D cat Va FK, D cat Va type 9, D cat Va TO	+	149 305	+	152	+	-	-	+					-	-	-	133	open
D cat V TT, D cat Va type 8	+	149 305	+	?	+	+	+	+					-	-	-	133	open
D cat Va (SM), D cat Va type 4	+	149 305	+	152	+	+	+	+					-	-	-	133	cDe
D cat Va (DHK,DYO), D cat Va type 5	+	149 305	+	152	+	+	+	+					-	-	135	-	cDe
D cat VII	+	149 305	-	152	+	+	+	+					166	166	-	-	
DNB	+	149 305	+	152	+	+	+	+					-	-	139	139	cDe
DAU-0,1,2,3	+	149 305	+	152	+	+	+	+					-	165	-	-	cDe
DAU-4	+	149 305	+	152	+	+	+	+					-	165	135	-	cDe
DHMi	+	149 305	+	152	+	+	+	+					107	-	-	-	cDE
DHMii	+	-	-	-	+	+	+	+									cDE
DBT type 1	+	149 305	+	-	-	-	+	+									Cde
DBT type 2	+	149 305	+	-	-	-	-	+									open
DAR (weak D type 4.2)[A] weak D type 4.0, 4.1, 14	+	149 305	-	152	+	+	+	+									cDe
weak D type 4.2.1, 4.2.2[F]	+	149 305	-	-	+	+	+	+					-	-	-	133	
DFR type 1	+	149 305	-	152	+	+	+	+									CDe
DFR type 2	+	149 305	-	152	+	+	+	+									open
DHAR (Rh33)	-	-	-	152	-	-	-	-									cDe
D psi	+	149 305	+	303	+	+	+	+									
RHD-CE(1-9)-D(10)	-	-	-	-	-	-	-	+									cdE
RHD-CE(2-9)-D	+	149	-	-	-	-	-	+									Cde
RHD-CE(3-7)-D d(C)ce[F]	+	149	-	-	-	-	+	+									Cde
RHD-CE(4-7)-D	+	149 305	-	-	-	-	+	+									cdE
RHD-CE(8-9)-D weak D type 45	+	149 305	+	152	+	+	-	+									CDELe
Result:																	
Reaction No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	

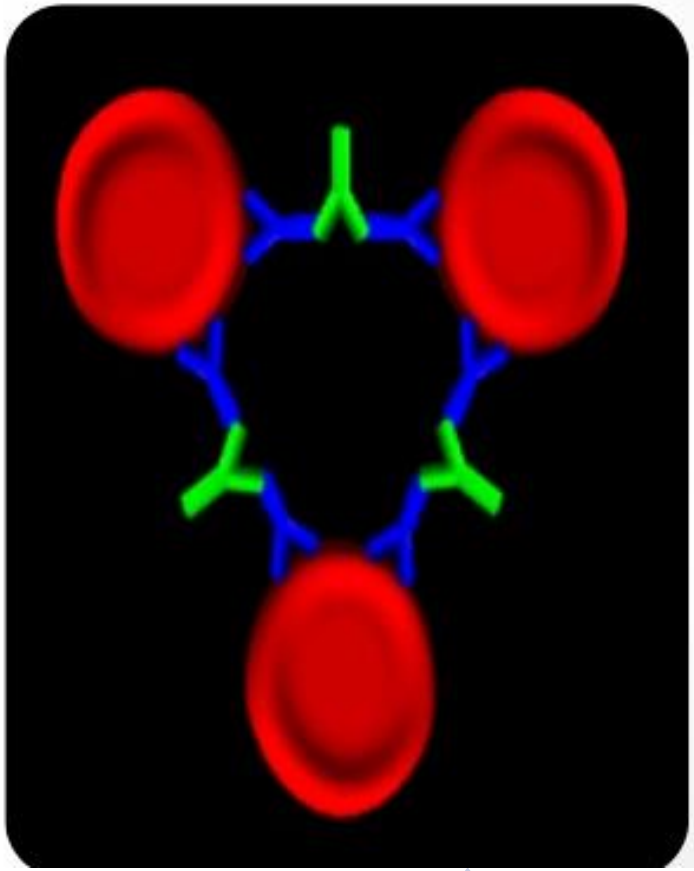
Legend for table see back

inno-train Diagnostik GmbH, Niederhöchstädter Straße 62, D-61476 Kronberg/Taunus, Germany
Tel. 0049-(0)6173 / 6079- 30 Fax: 0049-(0)6173 / 6079- 50, E-mail: info@inno-train.de, www.inno-train.de

Coombs Test
and Pretransfusion Testing

What is Coombs' Serum

- Serum from a rabbit or other animal previously immunized with purified human globulin to prepare antibodies directed against IgG and complement, used in the direct and indirect Coombs' tests. Also called *antihuman globulin*.



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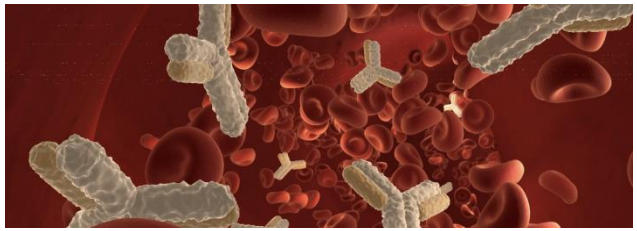

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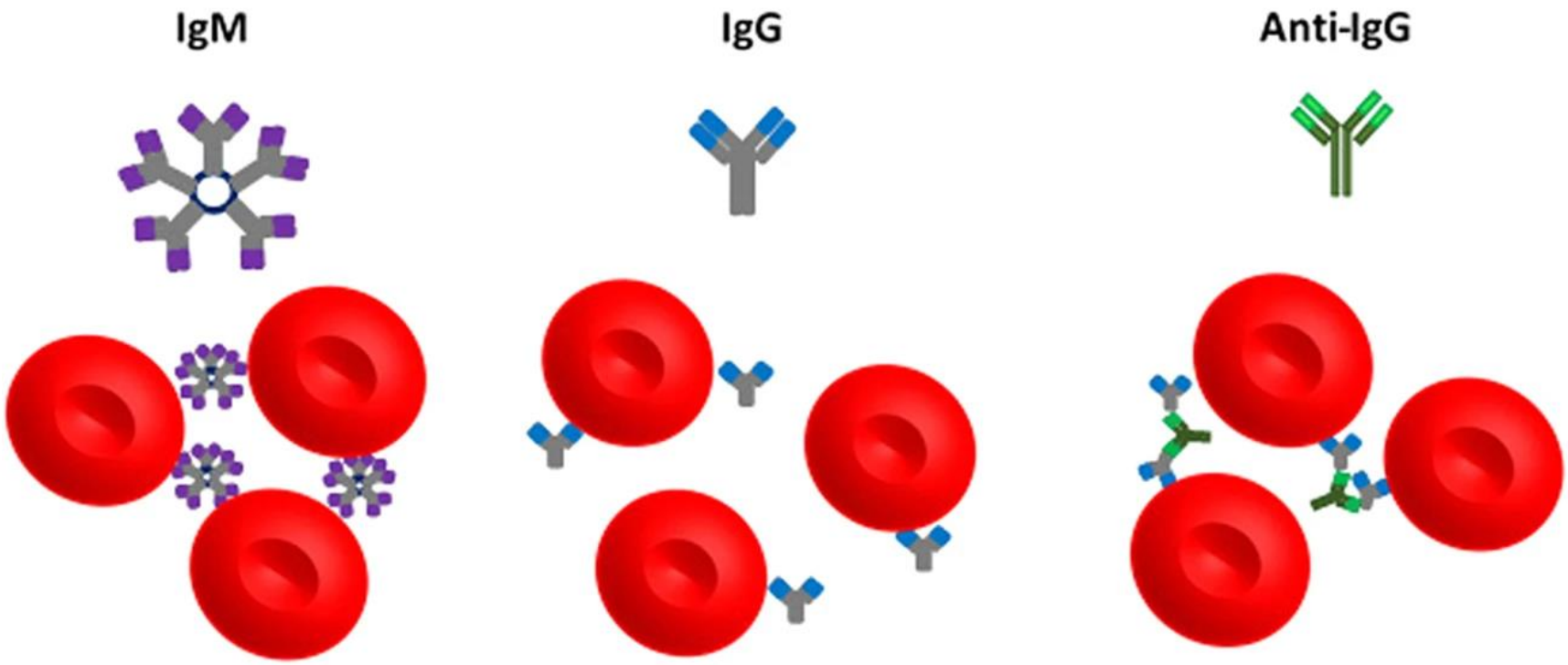
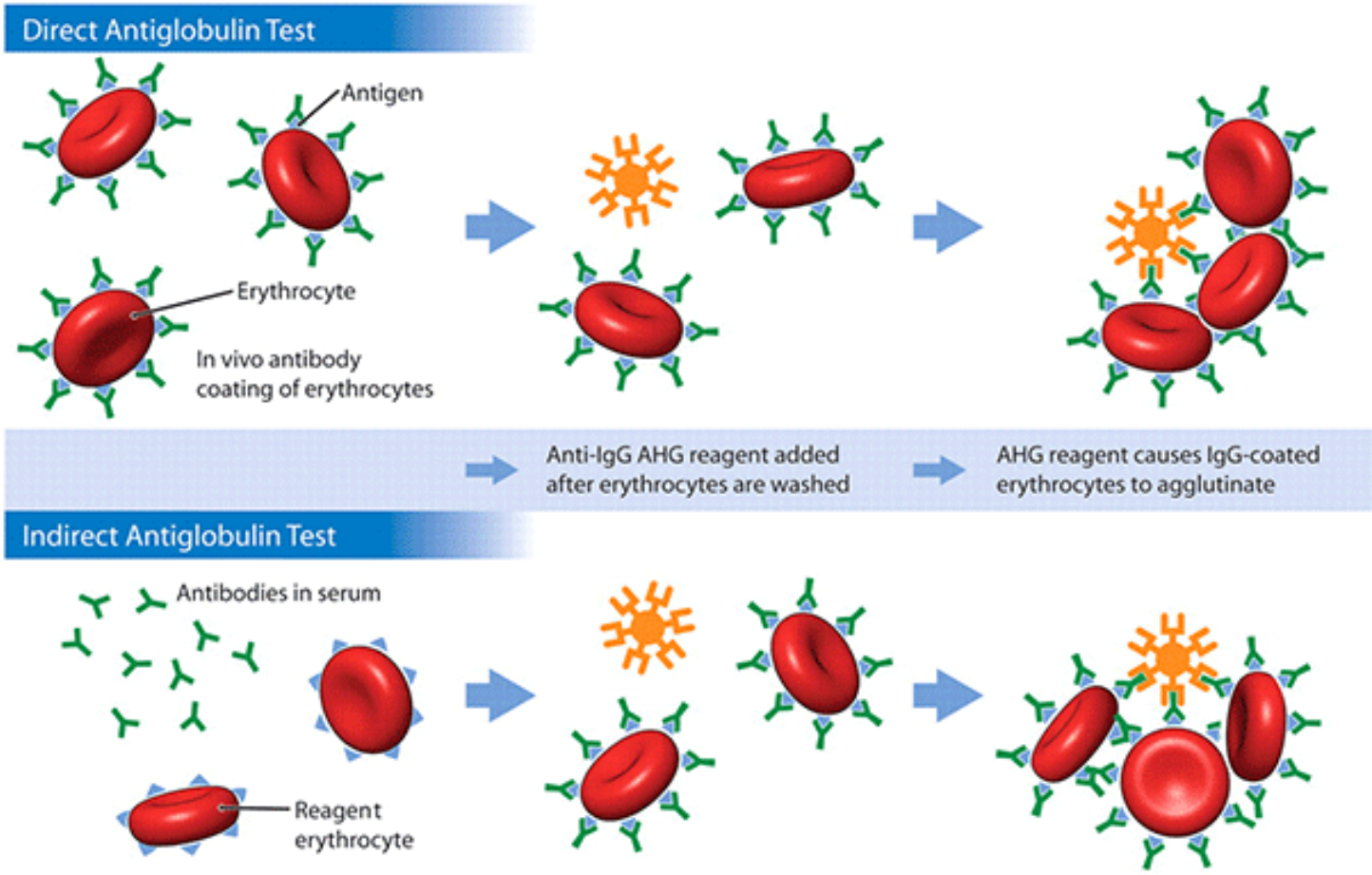


COOMBS TEST

Direct Coomb's Test

VS

Indirect Coomb's Test



Detection of irregular anti-erythrocyte antibodies using the indirect antiglobulin test

ANTI -

D C E c e K Fy^a Fy^b Jk^a Jk^b S s

Cross Matching

Cross Matching

Cross Matching

Donor red blood cells are mixed with patient plasma/serum

– To detect:

1. Most recipient antibodies directed against antigens on the donor red blood cells.

2. Major errors in ABO grouping, labeling, and identification of donors and recipient

A.E.Schmidt,
N.Blumberg
2016

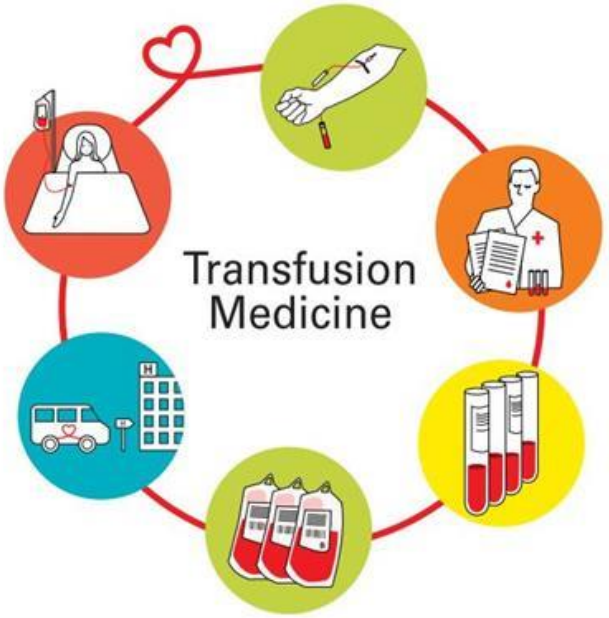
« BLOOD TRANSFUSION is the SECOND

most used MEDICAL PROCEDURES

in health care systems worldwide »


GIVE BLOOD GIVE LIFE


Thank You!



The primary responsibility of a blood transfusion service is to provide a safe, sufficient and timely supply of blood and blood products.

In fulling this responsibility, the BTS should ensure that the act of blood donation is safe and causes no harm to the donor.

It should build and maintain a pool of safe, voluntary non-remunerated blood donors and take all necessary steps to ensure that the products derived from donated blood are safe for the recipient, with a minimal risk of any infection that could be transmitted through transfusion.

All prospective blood donors should therefore be assessed for their suitability to donate blood, on each occasion of donation.

The purpose of blood donor selection is to:

- Protect donor health and safety by collecting blood only from healthy individuals
- Ensure patient safety by collecting blood only from donors whose donations, when transfused, will be safe for the recipients
- Identify any factors that might make an individual unsuitable as a donor, either temporarily or permanently
- Reduce the unnecessary deferral of safe and healthy donors
- Ensure the quality of blood products derived from whole blood and apheresis donations
- Minimize the wastage of resources resulting from the collection of unsuitable donations.

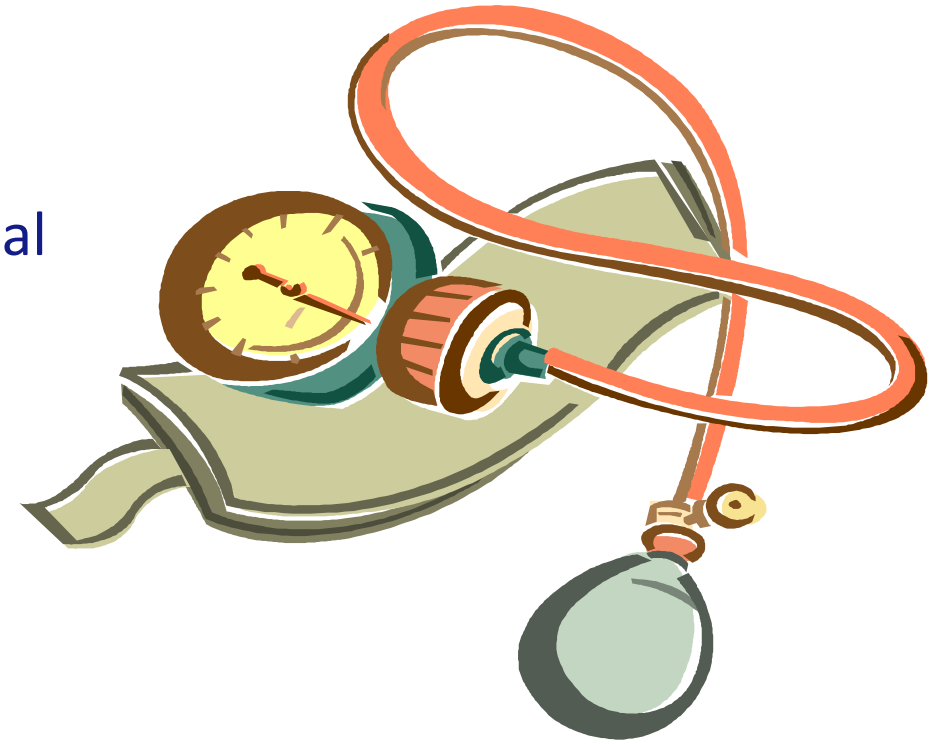
- 1 Donor registration
- 2 Pre-donation information
- 3 Completion of donor questionnaire
- 4 Donor interview and pre-donation counselling
- 5 Donor health and risk assessment
- 6 Informed consent.

Physical examination…

Age	18–65 years
Weight	≥ 50 Kg
Systolic Blood pressure	≤ 180 mmHg
diastolic blood pressure	≤ 100 mmHg
Pulse Rate	50 -100 b/min
Hb (females)	≥ 12,5 g/dL
(males)	≥ 13,5 g/dL

Physical examination…

With the aim to careful evaluate cardiovascular, respiratory and abdominal organs as well as the superficial lymphatic system



Donor questionnaire

A donor questionnaire is the key tool in donor selection for assessing donor health and safety and for reducing the risk of transmission of infection, in particular for infections for which no suitable screening tests are available.

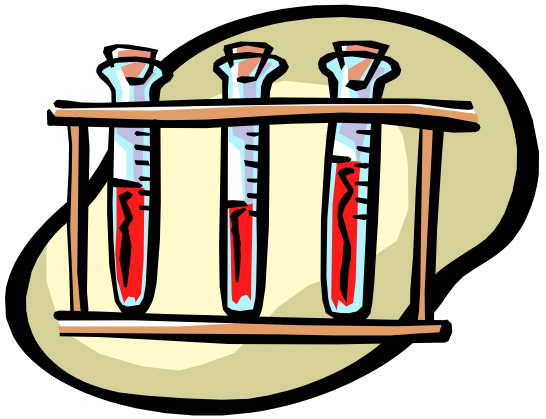
It is important to investigate any conditions of: habitual use of alcohol, use of drugs, use of steroids or hormones for the purpose of physical bodybuilding

Infectious Disease Markers in Blood Donors

- **Blood count**
- **HBsAg**
- **HIV antigen and Anti-HIV 1,2**
- **Anti-HCV**
- **HBV, HCV, HIV**
- **Anti-Treponema Pallidum**

Lab test to be performed in Blood Donors

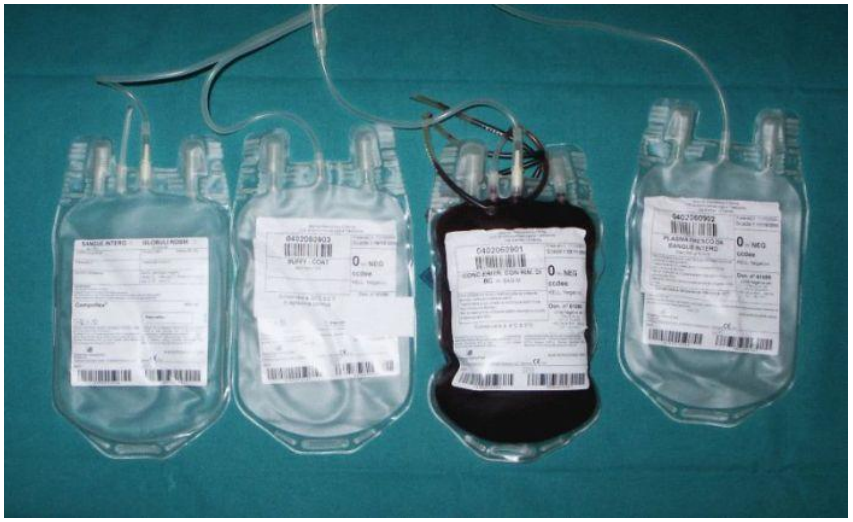
- Glycemia
- Creatinine
- ALT
- Cholesterolemia and HDL triglycerides
- Total proteinuria
- ferritin




HAEMONETICS
MCS 4
11

Collection of Blood

- Materials used are sterile and single use.
- Most important step is preparing the site to a state of almost surgical cleanliness.
- Bacteria on skin, if present, may grow well in stored donor blood and cause a fatal sepsis in recipient
- Use 16-17 gauge needle to collect blood from a single venipuncture within 15 minutes
- Collect 450 +/- 45 mLs of blood



The whole blood is a mixture of cells, colloids and crystalloids; it can be separated into **different blood components:**

- **packed red blood cell (PRBC) concentrate**
- **platelet concentrate**
- **fresh frozen plasma and cryoprecipitate.**

Each blood component is used for a different indication; thus the component separation has maximized the utility of one whole blood unit.

Apheresis is a procedure where required single or more than one component is collected, and the rest of blood components are returned back to the donor.

The working principle of apheresis equipment is either by **centrifugation (**different specific gravity) or by **filtration** (different size).

The most commonly used equipments use the centrifugation principle and also give leucodepleted products. In this method, fixed quantity of blood is collected in a bolus called as Extracorporeal volume (ECV) and the **required component (e.g. Platelets) is separated and collected in the collection bag and the other components (e.g. red blood cells, leucocytes and plasma) are returned back to the donor.**

The various components that can be collected are - double unit red cell collection (red cells), platelets, leucapheresis (harvesting granulocytes, peripheral blood haematopoietic stem cell), plasmapheresis (collecting normal plasma) and therapeutic plasma exchange (for exchanging with normal plasma after collecting and discarding patient's plasma).

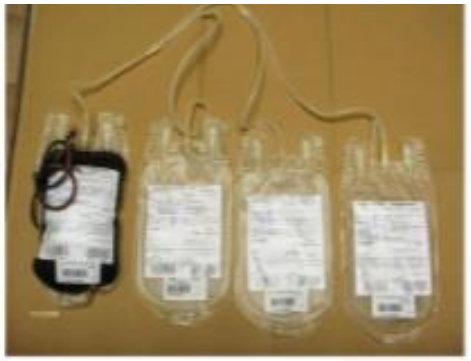
Permanent Deferrals

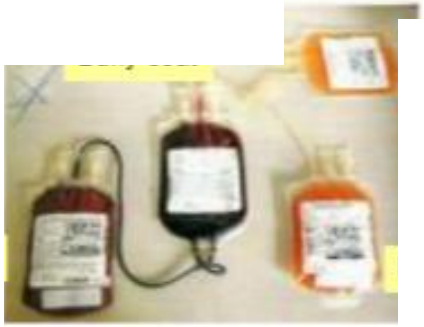
- HIV, HBV, or HCV positive
- Protozoan diseases such as Chagas disease or Babesiosis
- Received human pituitary growth hormone
- Lived in a country where Creutzfeld-Jacob disease is prevalent
- Most cancers except minor skin cancer and carcinoma in-situ of the cervix
- Severe heart disease, liver disease

Temporary Deferrals

- Certain immunizations
  - 2 weeks -MMR, yellow fever, oral polio, typhoid
  - 4 weeks -Rubella, Chicken Pox
  - 2 months – small pox
- Pregnancy
- Certain drugs and medications


Cryofuge 8500
Cryofuge 6000i





Storage

Storage

- RBCs

4°C (+/-2°C) (42 days in SAG-M)

- PLTs

20-24°C for 5 days, need to be stored with gentle agitation °C

- Fresh Frozen Plasma:

24 months T< -25°C

3 months - T between -18 °C e -25°C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A.E.Schmidt, N.Blumberg 2016


Thank You!