ORSZÁGOS VÉRELLÁTÓ SZOLGÁLAT

RhD typing

Bed side test

Antibody tests

Practice for IV year medical students

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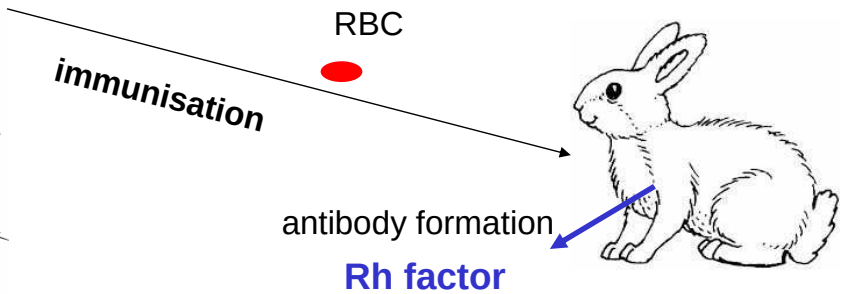
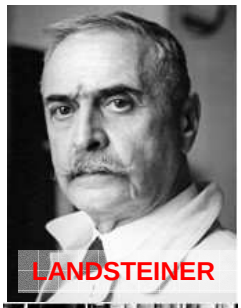
www.ovsz.hu

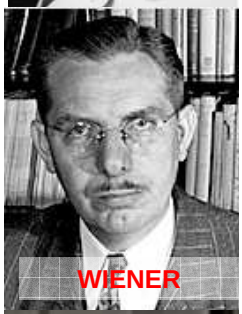
Rh Blood Group System

Discovery: 1940 K. Landsteiner and A. Wiener

Experience: Rabbits were injected with the red blood cells of the Rhesus monkey. The injection caused an antigenic reaction in the serum component of rabbit blood.




LANDSTEINER


WIENER


LEVINE

When blood from **humans was tested** with the rabbit serum, the red blood cells of 85% of the humans tested agglutinated such blood was typed Rh positive.

The blood of the remaining 15% lacked the factor and was typed Rh negative.

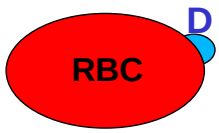
Agglutination 85% — **Rh positive RBCs**

No agglutination 15% — **Rh** negative **RBCs**

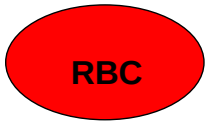
Rh Blood Group System

Antigens of Rh systems: D d E e C c

more than 50 antigens



D antigen is on the RBC: RhD positive



D antigen lack on the RBC: RhD negative

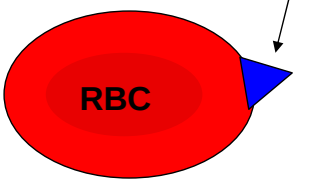
d is only a symbol of lack the D antigen

RhD negative means there is no D antigen on the cell membrane

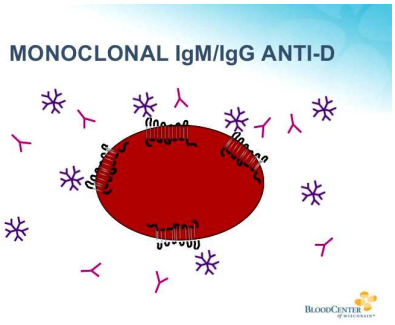
Basic of RhD grouping

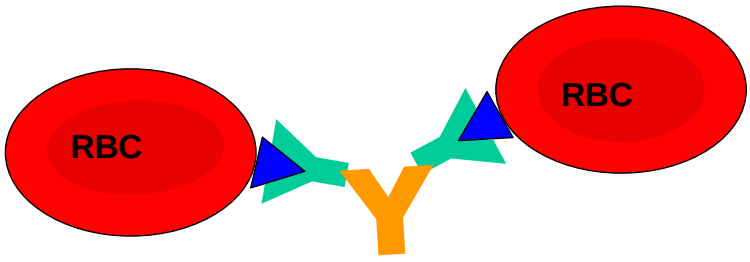
D antigen

Anti-D antibody



IgG antibody in vivo





Antigen-antibody reaction

Caracteristics of IgM and IgG antibodies

IgM	IgG
e.g. **ABO**	e.g. **Rh**
Pentamer (big)	Monomer (small)
10 binding sites	2 binding sites
complete	incomplete
naturally occuring	Immuno antibody
NOT accross the placenta	**ACCROSS** the placenta
Reaction temperature **+4 ºC to room temperature**	optimal reaction temperature **37 ºC**
Binds Complement	NOT binds Complement

Y → **HDN**

Monoclonal IgM anti-D is used to in vitro tests

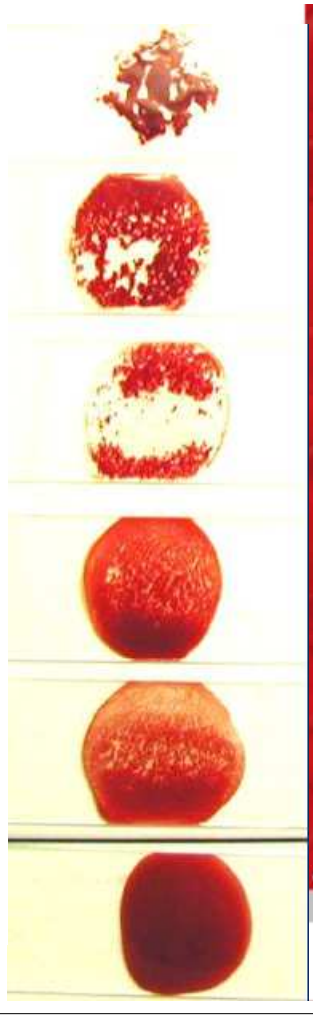
Reaction sheme of agglutination

1. ++++ strong — **positive**
   high, rough cloddy, hard frangible
2. +++
   large, separated
3. ++
   minor agglutination
4. +
   fine-grained
5. +/-
   very small

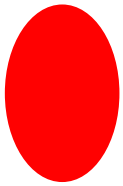
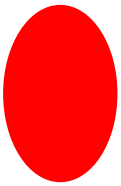
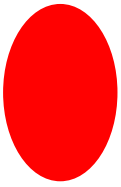
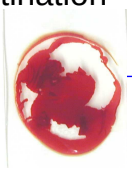
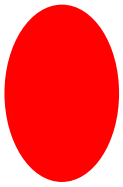
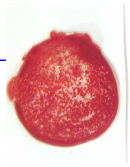
variant

6. No agglutination, homogene — **negative**

Anti-D + patient's RBC



INTERPRETATION OF RESULTS

anti-D	control	
		NO agglutination Rh D negative 
		STRONG (++++) agglutination Rh D positive 
		Weak agglutination (+ - +++) Rh D varians 

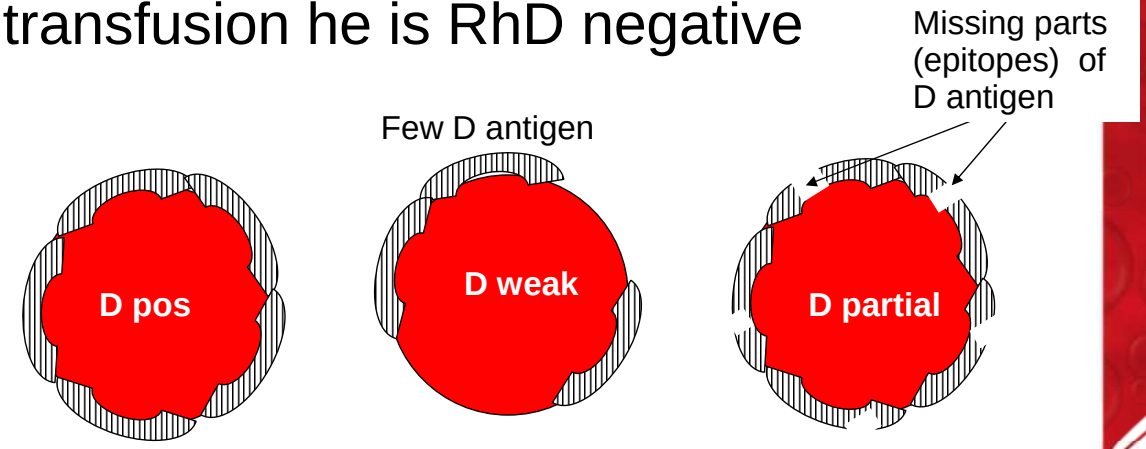
Negative control : anti-D control + patient's RBC

The control must be negative.

If any agglutination occurs the test is not valid.

What to do if the reaction is not ++++ positive?

1. Repeat the test
2. Send the sample to the Blood Bank
3. If the patient have to take transfusion he is RhD negative



Limitations of the Procedure:

Sources of errors

Antigen – antibody tests met a lot of requirements. Factors affecting the antigen-antibody reactions shoud be considered to establish the siutable reaction.
If the reaction conditions are not followed, false negative or false positive results can occur, which can lead to incorrect blood group determination.

- Medium of reaction (ionic strength) – **Serum**
- Antigen - antibody ratio (**50% suspension**)
- Reaction temperature (**+2O ºC – room temperature**)
- Reaction time **5 minutes**

Sympexis = rouleaux formation of RBCs

Physicochemical changes not real agglutination

The RBC's here have stacked together in long chains. This is known as "rouleaux formation" and it happens with increased serum proteins, particularly fibrinogen and globulins. Such long chains of RBC's sediment more readily. This is the mechanism for the sedimentation rate, which increases non-specifically with inflammation and increased "acute phase" serum proteins.

Causes: infections
multiple myeloma, chirrosis
(an increase in the ratio of immunoglobulins to albumin)
inflammatory and connective tissue disorders
cancer
diabetes mellitus
an increase in the ratio of RBCs to plasma volume
(anemia, hypovolemia)
macromolecules, contrast medium

Acute phase proteins, particularly fibrinogen, interact with sialic acid on the surface of RBCs to facilitate the formation of rouleaux. Rouleaux formation is retarded by albumin proteins, in vitro by physiological salin.

Main causes of false positive reactions

- Rouleaux formation
  marginal drying
- Little drops
- Late evaluation
- Contamination

Main causes of false negative reactions

- Early evaluation
- Inadequate
  antigen-antibody ratio
- Expired reagents and test cells

The sympexis may be differentiate from real agglutination with dropping of phys. saline.

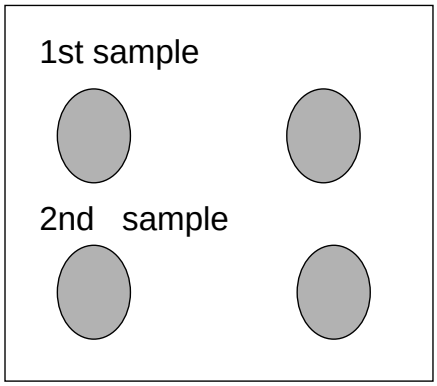
The sympexis dissolved but no agglutination.

TASKS

- RhD grouping with slide method – 2 tests

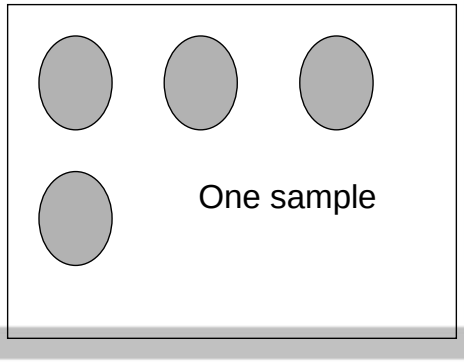
Two blood samples

1 empty tube respectively



- Bed side blood grouping

1 bed side card, one test

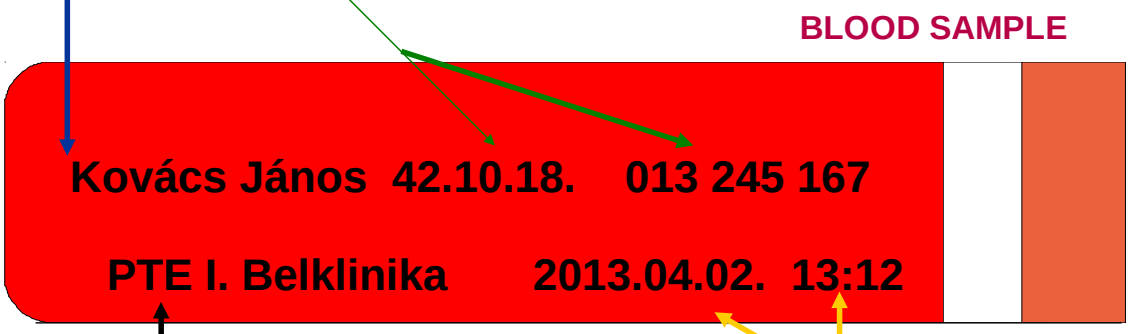


BLOOD SAMPLE LABELING

Details must include:

Patient's registered family name and given name (unabbreviated)

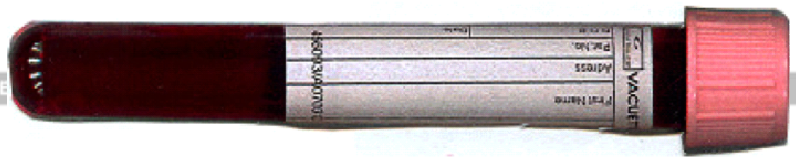
Date of birth or social security number (TAJ) (both preferred



Name and code of institute requiring

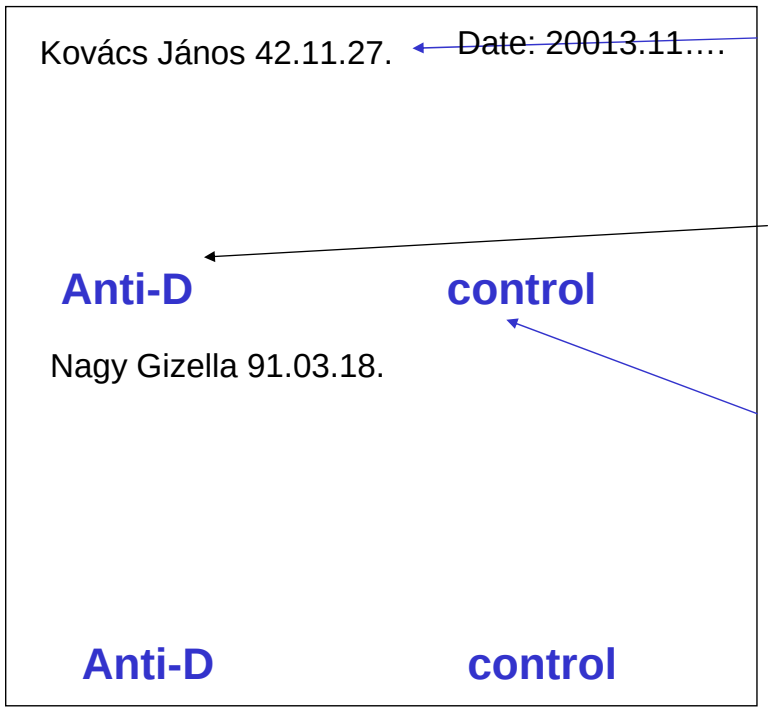
Date and time of collection

(Signature or initials of the collector)



RhD typing – slide method

Labeling of slide



Data:

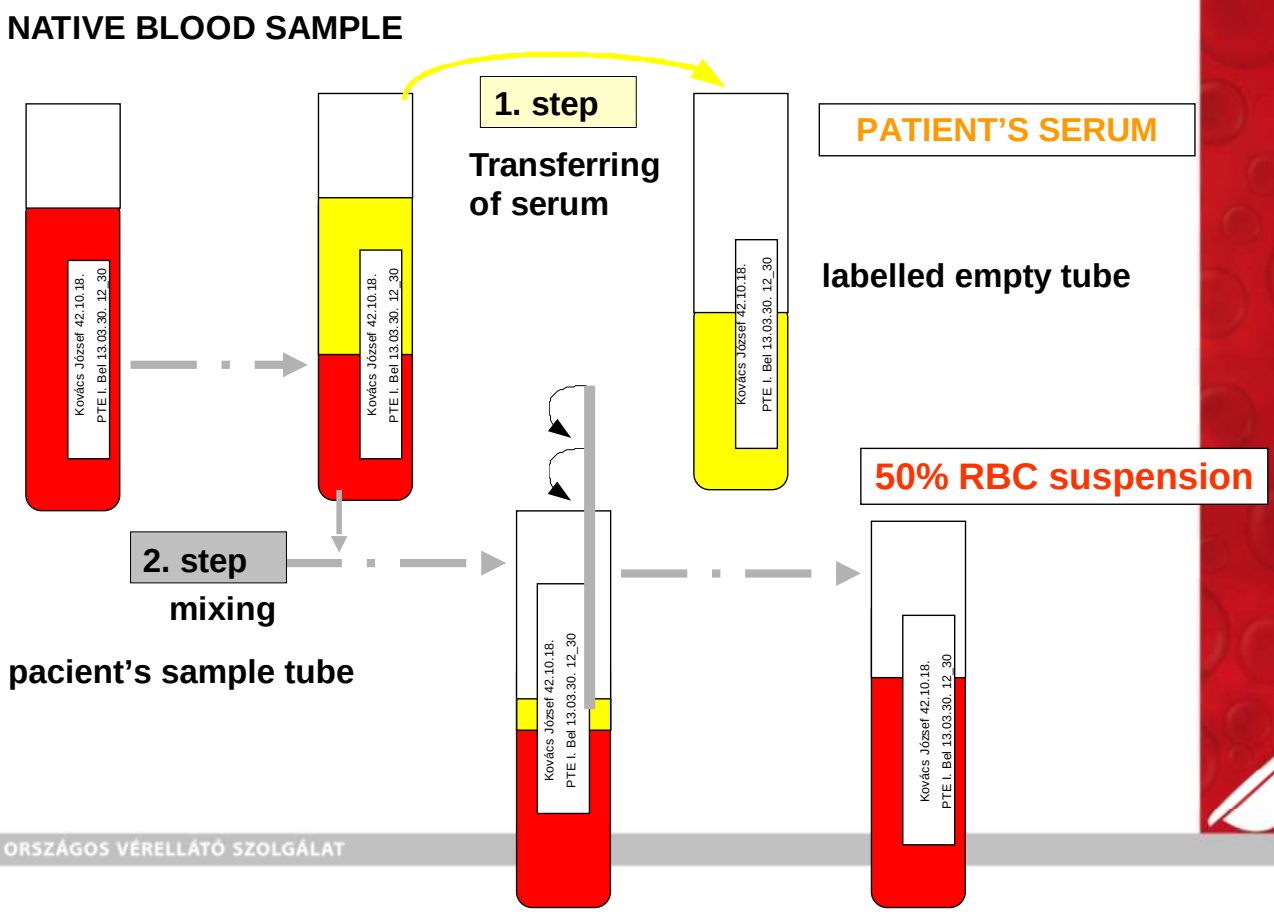
- Patient's name and code
- Reagent's name

  Anti-D
- Negative control

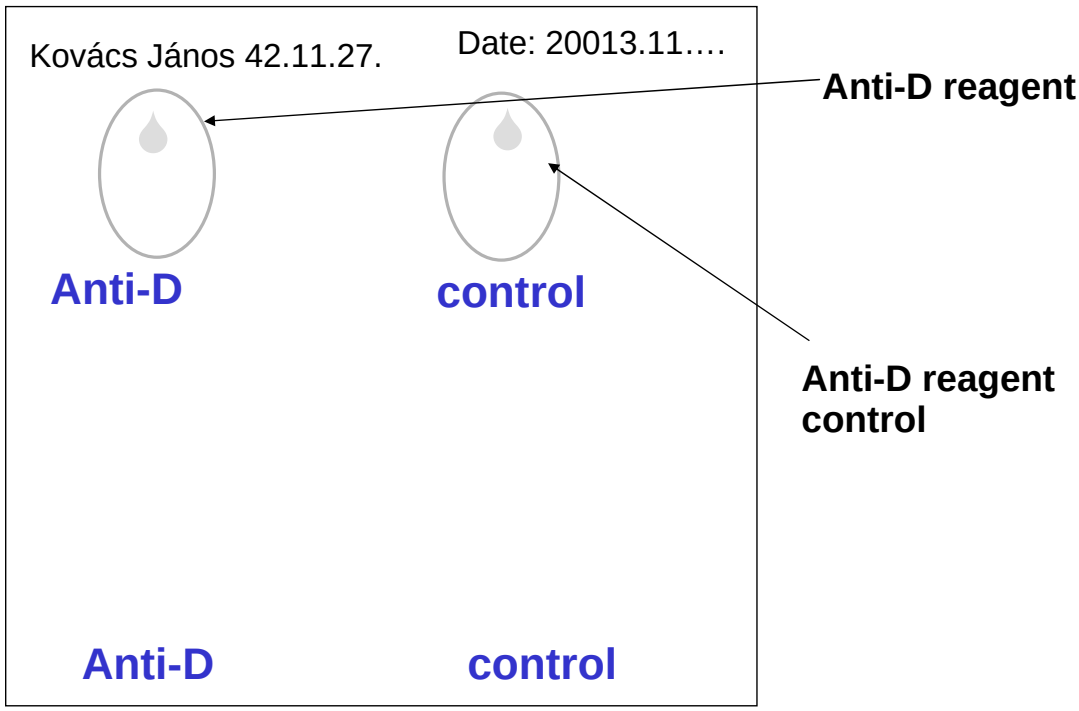
  Anti-D control

reagents + patient's RBC

PREPARATION OF 50% RBC SUSPENSION



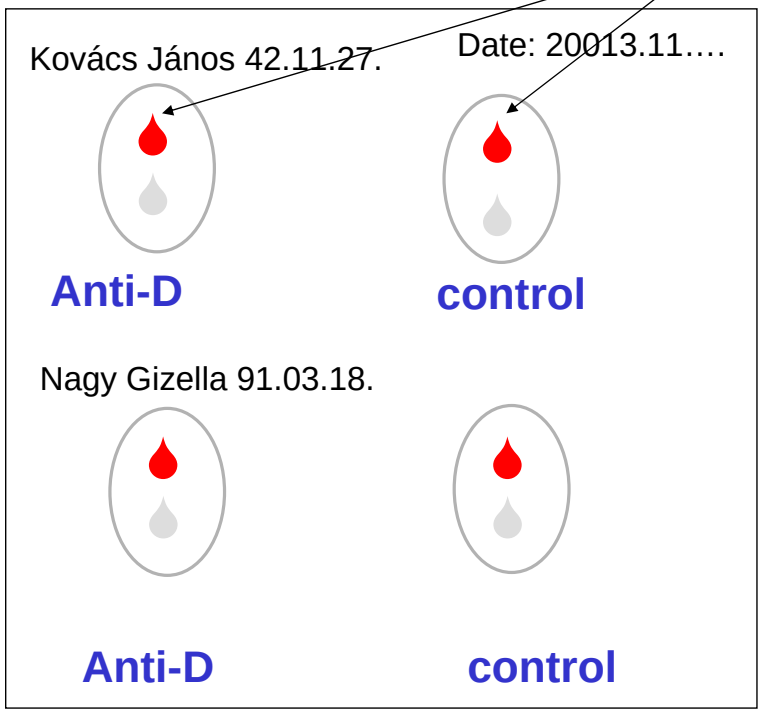
RhD typing



RhD typing

Dropping of RBC suspension

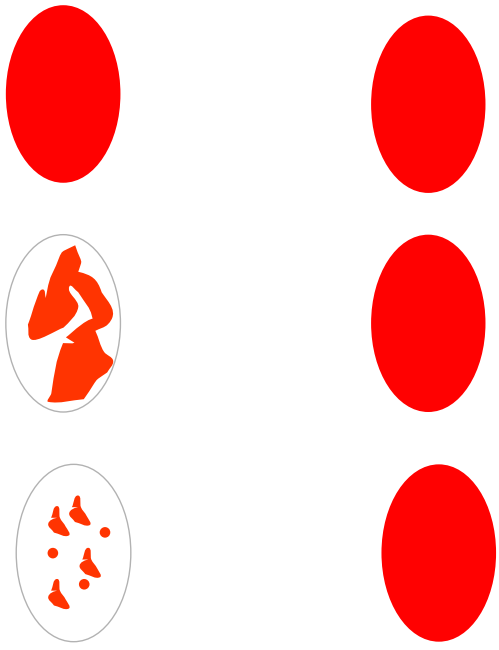
1 drpo of



- Patient's 50% RBC suspension to each reaction areas
- Mixing (wiping)
- Reaction time: 5 minutes
- Tilting
- Interpretation

INTERPRETATION OF RESULTS

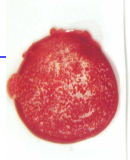
anti-D **control**



NO agglutination
Rh D negative

STRONG (++++) agglutination
Rh D positive

Weak agglutination (+ - +++)
Rh D varians

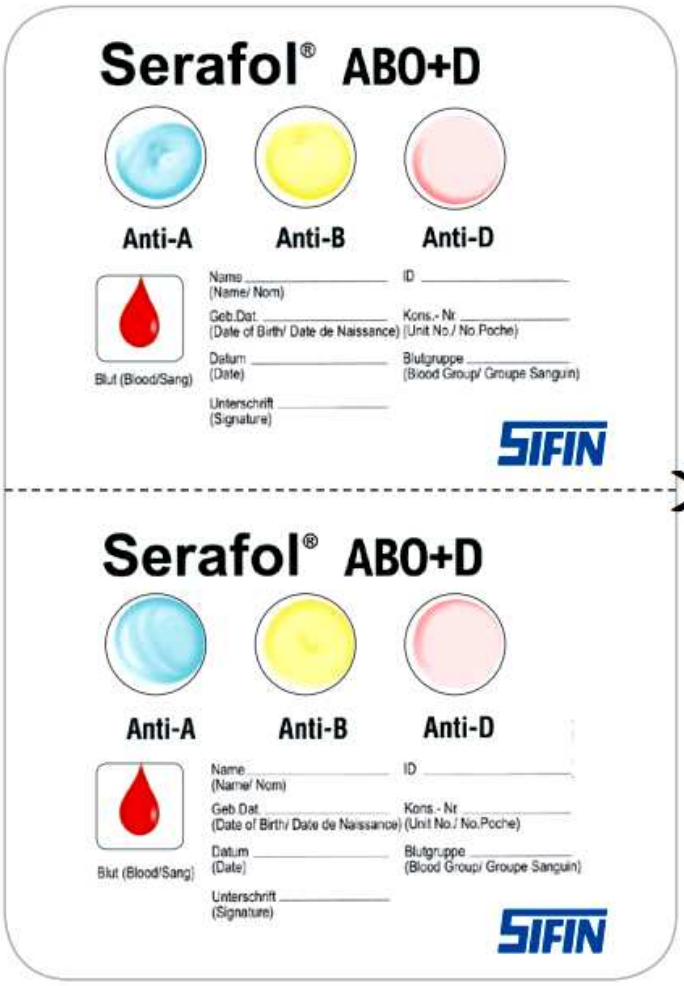


Negative control : anti-D control + patient's RBC

The control must be negative.

If any agglutination occurs the test is not valid.

Blood grouping on bedside card



ABO (forward) and RhD typing

Adhesive tapes

Stirring rods

Storage temperature: 2 – 25 °C

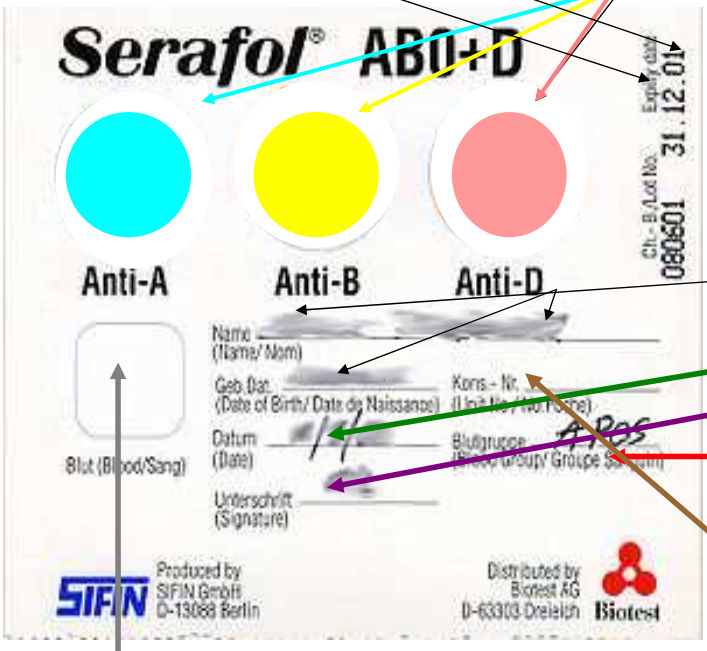
Lot number

Expiry date – important!

Expiry date

Lot number

Dried monoclonal specific antisera



Anti-A

Anti-B

Anti-D

Labelling of card

Name and codes

Date

Signature

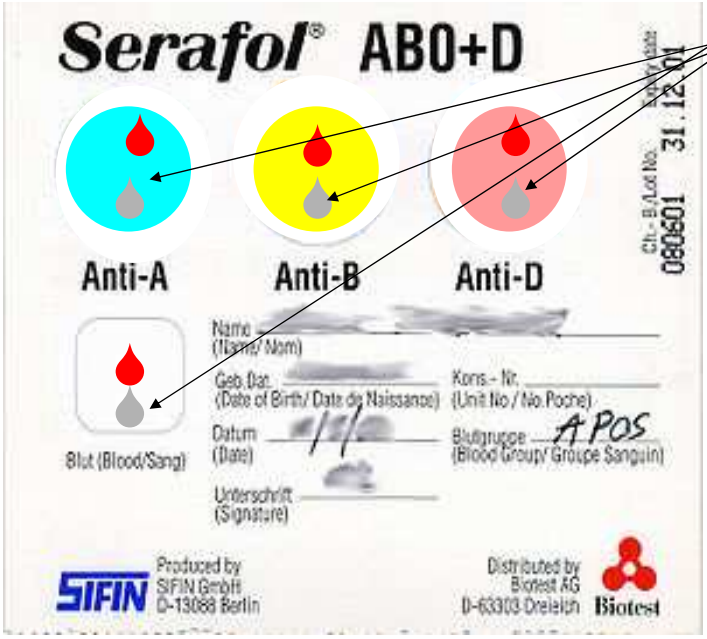
Blood group result

Unit code (RBC product grouping)

Autocontrol: physiological salin + patient's RBC

Test procedure

1 drop



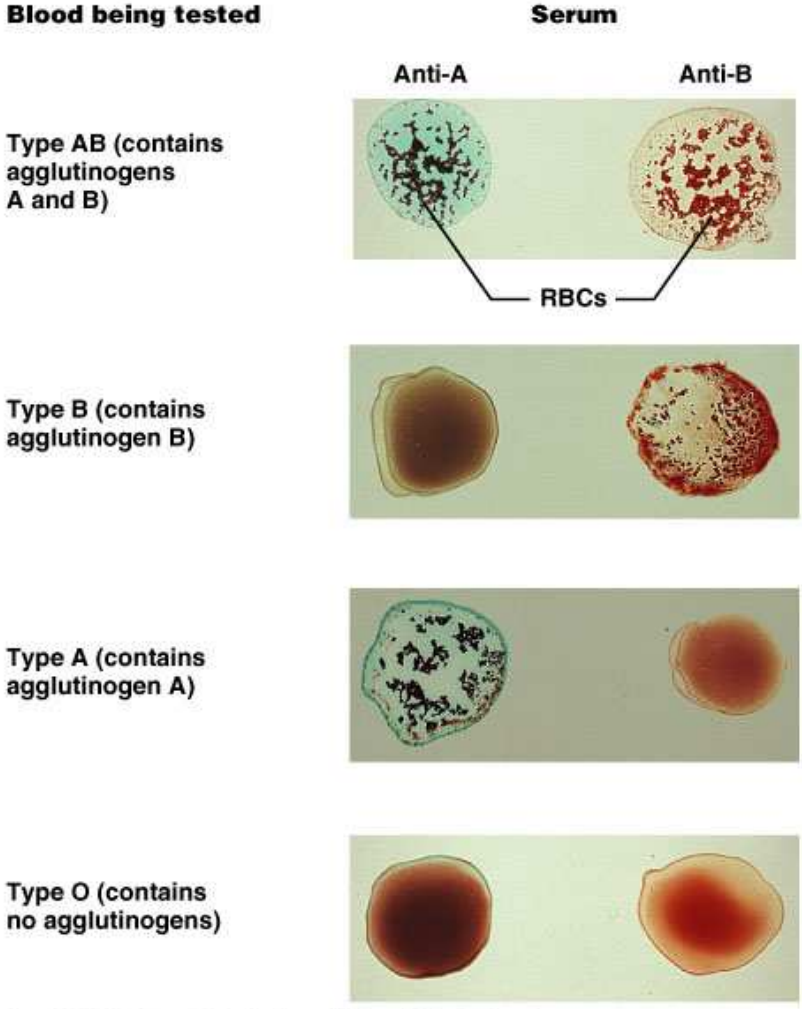
Physiological saline

to the 4 reaction fields respectively

Whole blood from patient or blood unit

The blood may be native, anticoagulated (except heparin) venous or capillary blood

Mixing until the reagent is completely dissolved
Spread material to be tested over the entire reaction field.
Repeat this procedure with a new or well cleaned mixing stick on the next reaction fields.



INTERPRETATION OF RESULTS

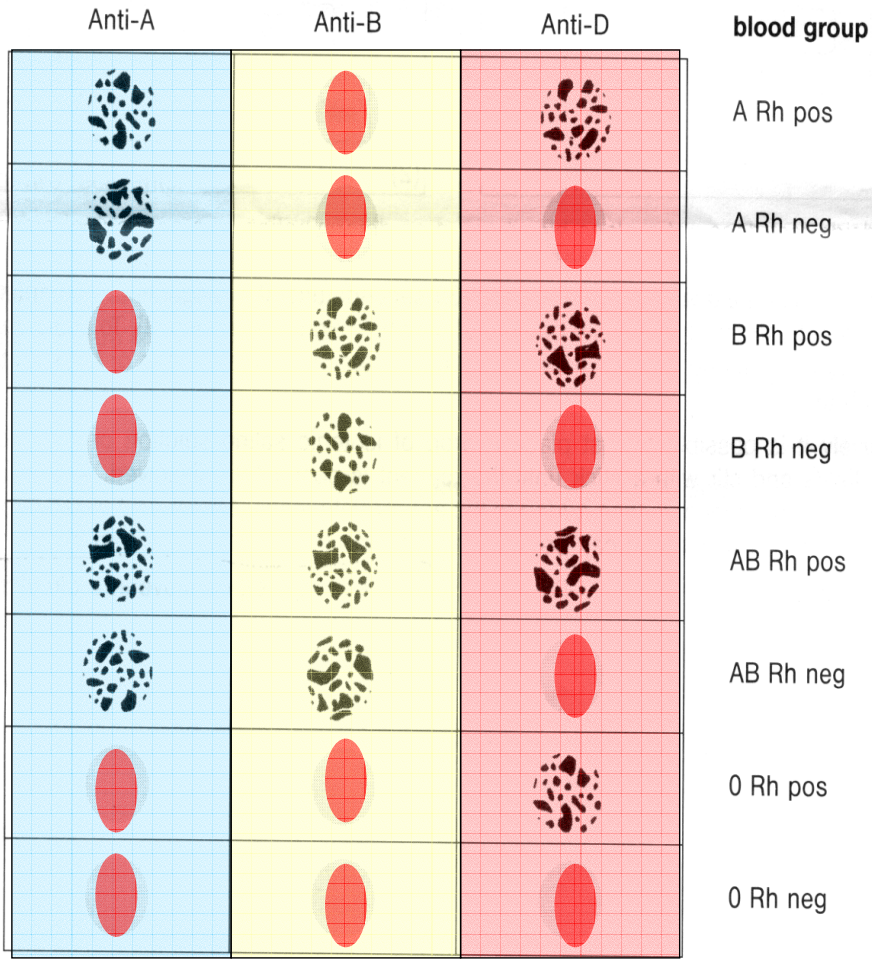
Anti-A	Anti-B	Anti-D	RESULT
positive	negative	negative	A RhD negative
positive	negative	positive	A RhD positive
negative	positive	positive	B RhD positive
negative	positive	negative	B RhD negative
positive	positive	positive	AB RhD positive
positive	positive	negative	AB RhD negative
negative	negative	negative	O RhD negative
negative	negative	positive	O RhD positive

Sources of errors:

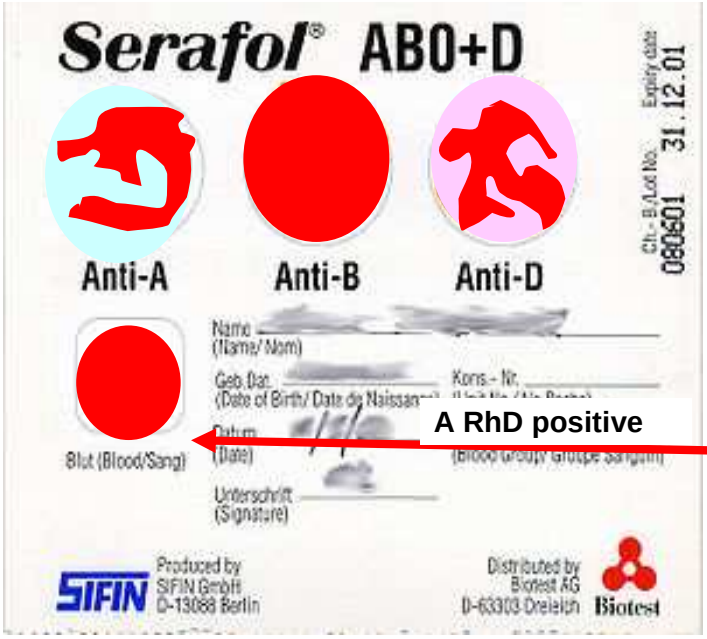
Fals positive: rouleaux formation, drying out, cold reactive antibody
Fals negative: Blood drop too small or too large, expiry date has passed or card was stored improperly, red cell suspension (10%), hematocrit under 15 %

Bed side test

Interpretation of results



INTERPRETATION OF RESULTS

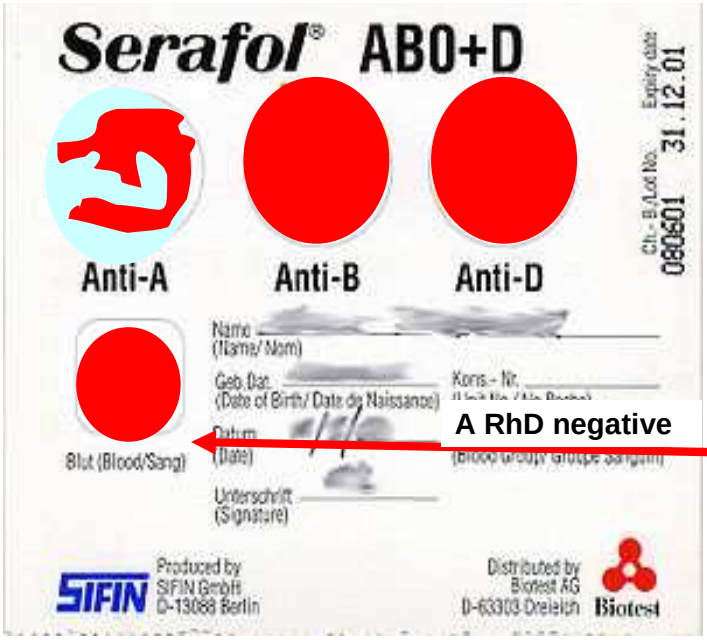


A RhD positive

Agglutination with anti-A and anti-D

Autocontrol shoud be negative

INTERPRETATION OF RESULTS

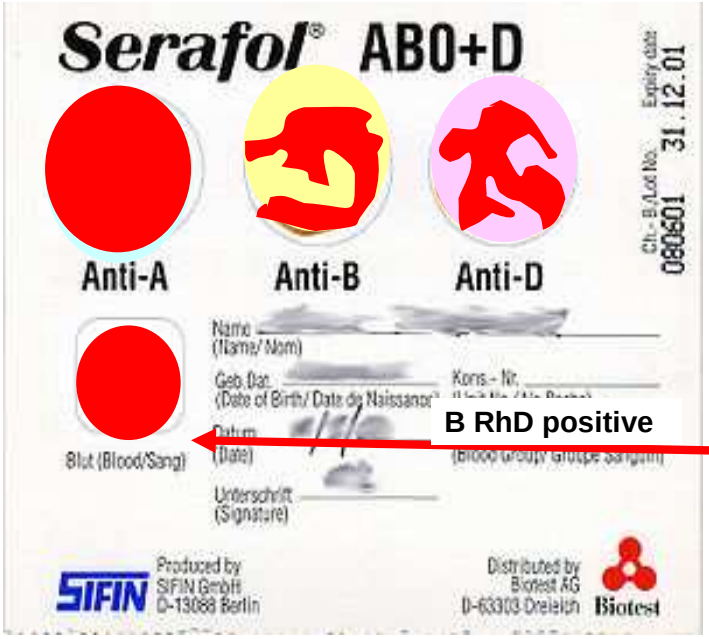


A RhD negative

Agglutination with anti-A only

Autocontrol shoud be negative

INTERPRETATION OF RESULTS

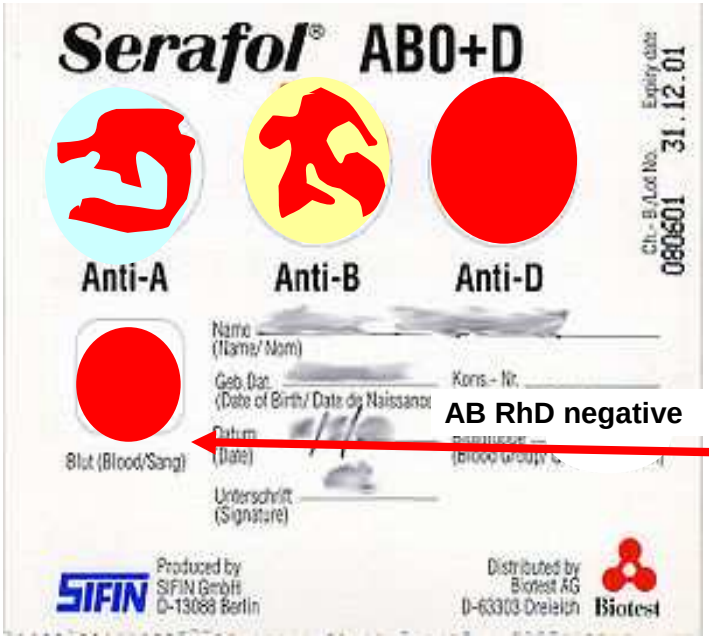


B RhD positive

Agglutination with anti-B and anti-D

Autocontrol shoud be negative

INTERPRETATION OF RESULTS

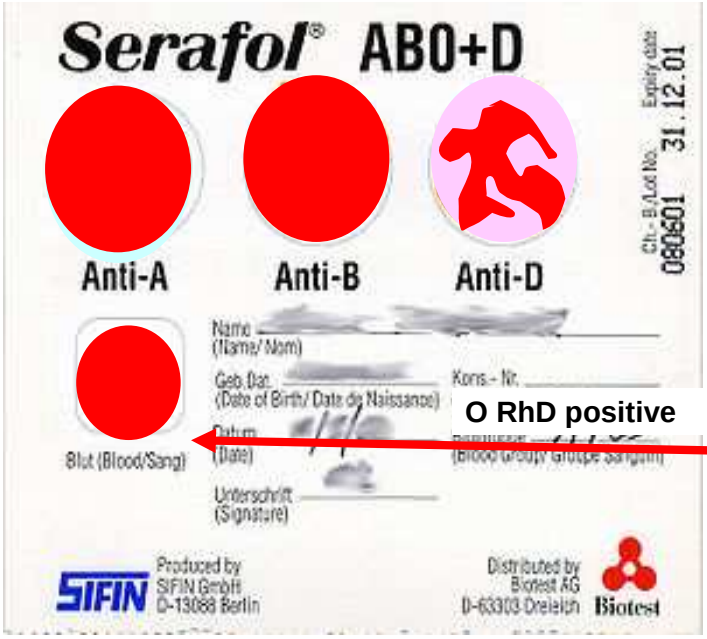


AB RhD negative

Agglutination with anti-A and anti-B

Autocontrol shoud be negative

INTERPRETATION OF RESULTS



O RhD positive

Agglutination with anti-D only

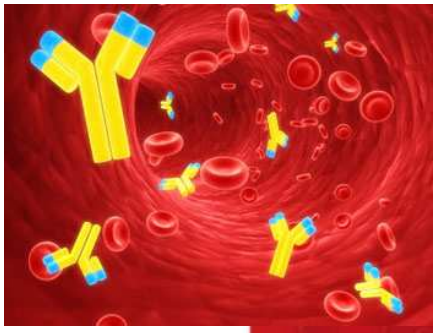
O RhD positive

Autocontrol shoud be negative

RECORD YOUR RESULT ON THE CARD

M101151321007 21 X

Antibody detection



Immune antibodies are IgG origin

Transfusions, pregnancy, transplantation

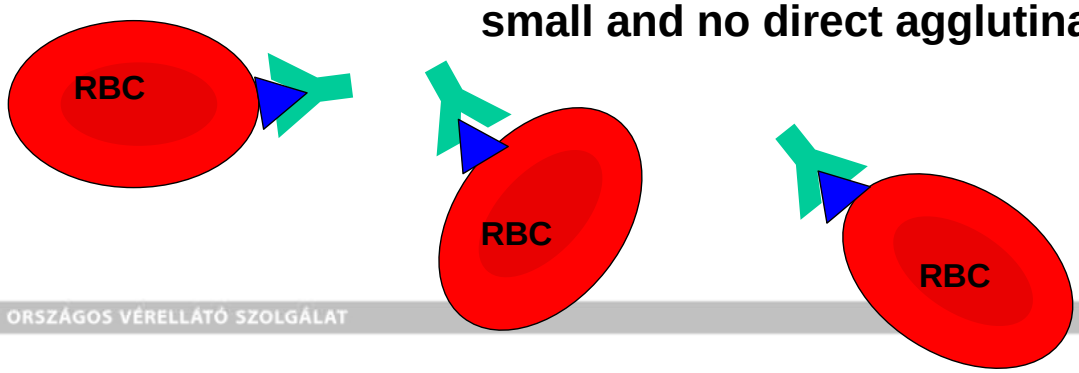
Clinical importance of immune antibodies:

May cose agglutination or hemolysis in vivo – maybe fatal

Hemolytic disease of the newborn

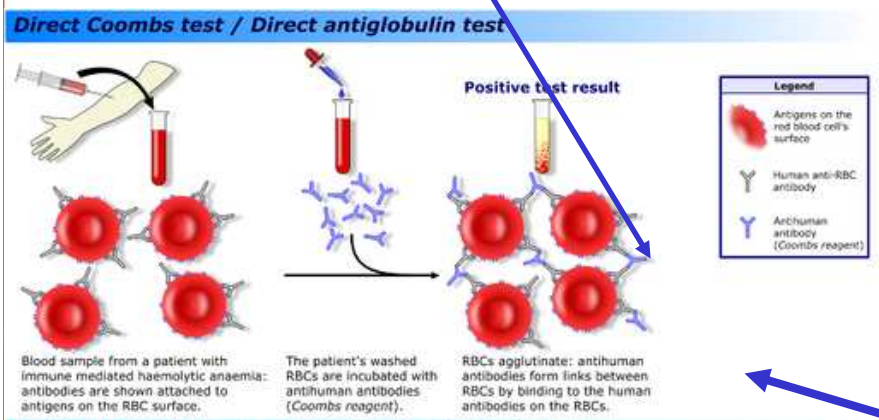
Type of antibodies **IgG** **incomplet**

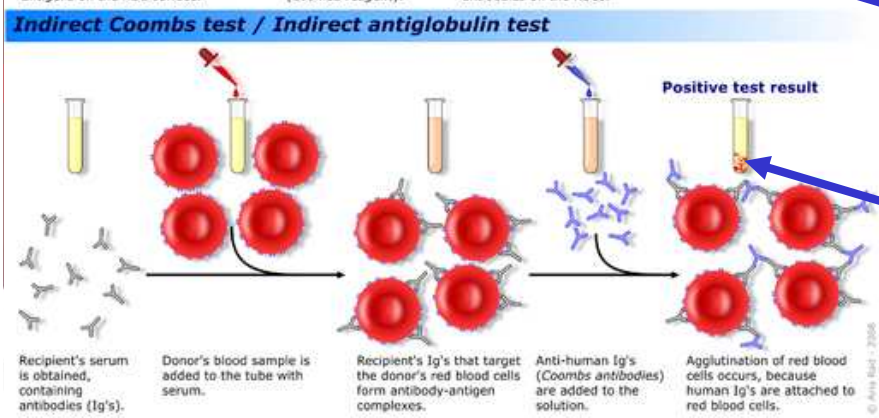
small and no direct agglutination



Coombs reaction

Anti-human globulin (Coombs) antibody are produced by immunizing non-human species with human serum.





Animal anti-human antibodies will also bind to human antibodies, commonly **IgG** or IgM that may be fixed onto antigens on the surface of red blood cells and in the appropriate test conditions this can lead to agglutination of RBCs.

The phenomenon of agglutination of RBCs is important here, because the resulting clumping of RBCs can be visualised; **when clumping is seen the test is positive and when clumping is not seen the test is negative.**

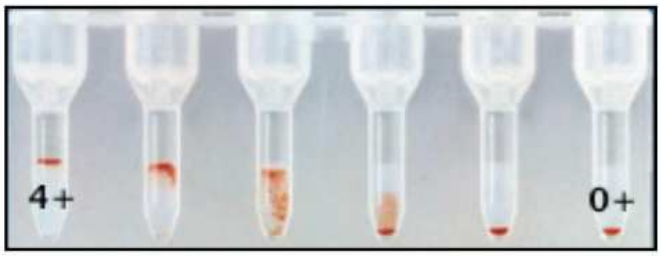
The DAT is used to detect IgG or C3 bound to the surface of the red cell.

The IAT is used to detect free red cell antibodies in patient serum.

Antibody tests

Test methods for antibody detection

Tube test, microplate test, **column agglutination**



3+ 2+ 1+ +/- negative

Gel separates agglutinates based on size and by binding to IgG-coated RBCs
a) Strong positive gives agglutinates at the TOP of the gel (left side of above)
b) Complete negative gives RBCs at the BOTTOM of the gel (right side)

Test procedure

Patent's serum (**unknown antibody**) + test cells (**known antigens**)

Reaction temperarure **+ 37 oC**

Importance of antibody identification

- HDN
- Transfusion reaction
- Compatibility

Patient's serum

+

Test cells

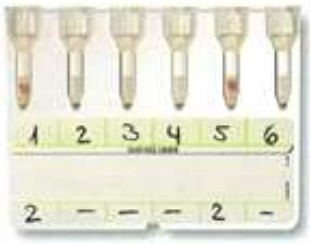
Types of the test

Antibody screening

ID-DiaCell I-II-III/Antigen Table (example)

	Rh-hr Donor	Rh						Kell						Duffy		Kidd		Lewis		P	MNS				Luth.		Xg	
		D	C	E	c	e	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	Xg^a	F/M
I	$C^wCD.ee$ $R_1{}^wR_1$ 754456	+	+	0	0	+	+	0	+	0	+	0	+	+	+	0	+	+	0	+	+	0	+	0	0	+	0	M
II	ccD.EE R_2R_2 047144	+	0	+	+	0	0	0	+	+	+	0	+	+	0	+	+	0	+	0	+	+	+	+	0	+	+	M
III	ccddee rr 101469	0	0	0	+	+	0	+	+	0	+	0	+	0	+	+	0	0	+	+	0	+	0	+	+	+	+	F

Antibody identification



	Rh-hr	Donor	Rh						Kell						Duffy		Kidd		Lewis		P	MNS				Luth.		Xg	
			D	C	E	c	e	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	Xg^a	F/M
1	$C^wCD.ee$	$R_1{}^wR_1$ 677783	+	+	0	0	+	+	0	+	0	+	0	+	+	0	+	0	0	+	+	+	0	+	0	0	+	+	
2	CCD.ee	R_1R_1 113683	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	+	0	0	+	0	+	0	+	0	+	0	
3	ccD.EE	R_2R_2 422278	+	0	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	0	0	+	+	
4	Ccddee	r'r 293832	0	+	0	+	+	0	0	+	0	+	0	+	+	0	0	+	+	0	+	+	+	+	+	0	+	+	
5	ccddEe	r"r 307849	0	0	+	+	+	0	0	+	0	+	0	+	+	+	+	+	0	+	+	+	+	0	+	+	+	0	
6	ccddee	rr 308478	0	0	0	+	+	0	+	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	nt	
7	ccddee	rr 439656	0	0	0	+	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	+	0	+	+	
8	ccD.ee	Ror 032656	+	0	0	+	+	0	0	+	0	+	0	+	0	0	+	0	0	0	+	0	+	0	+	0	+	0	
9	ccddee	rr 341656	0	0	0	+	+	0	0	+	0	+	0	+	+	0	0	+	0	+	+	0	0	+	0	+	0	+	0	
10	ccddee	rr 454253	0	0	0	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	0	+	+	0	0	+	+	
11	ccddee	rr 169276	0	0	0	+	+	0	0	+	+	+	0	+	+	0	+	+	0	+	+	+	+	0	0	+	0	+	+	

Examples: Anti-D

N°	GENOTYPE	Rhesus						Kell				Duffy		Kidd		Lewis		MNS				P	Lutheran		Colton *	
		D RH1	C RH2	E RH3	c RH4	e RH5	Cw RH8	K KEL1	k KEL2	Kp^a KEL3	Kp^b KEL4	Fy^a FY1	Fy^b FY2	Jk^a JK1	Jk^b JK2	Le^a LE1	Le^b LE2	M MNS1	N MNS2	S MNS3	s MNS4	P1 P1	Lu^a LU1	Lu^b LU2	Coa	Cob
IP	R1wR1 : RH(1,2,-3,-4,5,8)	+	+	0	0	+	+	0	+	0	+	0	+	0	+	0	+	+	0	0	+	0	0	+	nt	0
IIP	R2R2 : RH(1,-2,3,4,-5,-8)	+	0	+	+	0	0	0	+	0	+	+	+	+	+	+	0	+	+	0	+	+	+	0	nt	0
IIIP	rr : RH(-1,-2,-3,4,5,-8)	0	0	0	+	+	0	+	+	0	+	0	+	+	0	0	+	0	+	0	+	+	0	+	nt	0

Ol. verso : antigènes supplémentaires /ooc reverse : additional antigens

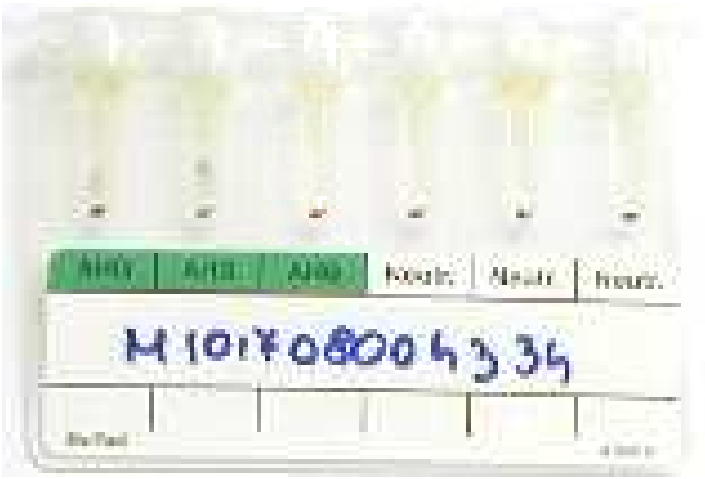
N°	GENOTYPE	*	Rhesus						Kell				Duffy		Kidd		Lewis		MNS				P	Lutheran	
			D RH1	C RH2	E RH3	c RH4	e RH5	Cw RH8	K KEL1	k KEL2	Kp^a KEL3	Kp^b KEL4	Fy^a FY1	Fy^b FY2	Jk^a JK1	Jk^b JK2	Le^a LE1	Le^b LE2	M MNS1	N MNS2	S MNS3	s MNS4	P1 P1	Lu^a LU1	Lu^b LU2
1/1P	R1wR1 : RH(1,2,-3,-4,5,8)		+	+	0	0	+	+	0	+	0	+	+	+	+	0	0	+	+	0	+	0	+	0	+
2/2P	R1wR1 : RH(1,2,-3,-4,5,8)		+	+	0	0	+	+	+	+	0	+	+	0	0	+	0	+	+	+	0	+	+	0	+
3/3P	R1R2 : RH(1,2,3,-4,5,-8)		+	+	+	0	+	0	0	+	0	+	+	+	+	0	0	+	+	+	0	+	+	0	+
4/4P	R2R2 : RH(1,-2,3,4,-5,-8)		+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	+	0	+	+	0	+
5/5P	R0r : RH(1,-2,-3,4,5,-8)		+	0	0	+	+	0	+	+	0	+	+	0	+	+	0	+	+	+	0	+	+	0	+
6/6P	r'r : RH(-1,2,-3,4,5,-8)		0	+	0	+	+	0	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+
7/7P	r"r : RH(-1,-2,3,4,5,-8)		0	0	+	+	+	0	0	+	0	+	0	+	+	0	0	+	+	+	+	+	+	+	+
8/8P	rr : RH(-1,-2,-3,4,5,-8)		0	0	0	+	+	0	+	+	0	+	+	+	+	0	+	0	+	0	+	0	+	0	+
9/9P	rr : RH(-1,-2,-3,4,5,-8)		0	0	0	+	+	0	0	+	+	+	0	+	+	0	0	+	+	+	+	+	0	0	+
10/10P	rr : RH(-1,-2,-3,4,5,-8)		0	0	0	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+

Examples:

Anti-D or

Anti-Jkb or ?

Anti-M



N°	Antigen table / GENOTYPE	Rhesus						Kell				Duffy		Kidd		Lewis		MNS				P	Lutheran		Colton *	
		D RH1	C RH2	E RH3	c RH4	e RH5	Cw RH8	K KEL1	k KEL2	Kp^a KEL3	Kp^b KEL4	Fy^a FY1	Fy^b FY2	Jk^a JK1	Jk^b JK2	Le^a LE1	Le^b LE2	M MNS1	N MNS2	S MNS3	s MNS4	P1 P1	Lu^a LU1	Lu^b LU2	Coa	Cob
IP	R1wR1 : RH(1,2,-3,-4,5,8)	+	+	0	0	+	+	0	+	0	+	0	+	0	+	0	+	+	0	0	+	0	0	+	nt	0
IIP	R2R2 : RH(1,-2,3,4,-5,-8)	+	0	+	+	0	0	0	+	0	+	+	+	+	+	+	0	+	+	0	+	+	+	0	nt	0
IIIP	rr : RH(-1,-2,-3,4,5,-8)	0	0	0	+	+	0	+	+	0	+	0	+	+	0	0	+	0	+	0	+	+	0	+	nt	0
	PATIENT																									

Examples:

Anti-M

N°	Panel table / GENOTYPE	*	Rhesus						Kell				Duffy		Kidd		Lewis		MNS				P	Lutheran	
			D RH1	C RH2	E RH3	c RH4	e RH5	Cw RH8	K KEL1	k KEL2	Kp^a KEL3	Kp^b KEL4	Fy^a FY1	Fy^b FY2	Jk^a JK1	Jk^b JK2	Le^a LE1	Le^b LE2	M MNS1	N MNS2	S MNS3	s MNS4	P1 P1	Lu^a LU1	Lu^b LU2
1/1P	R1wR1 : RH(1,2,-3,-4,5,8)		+	+	0	0	+	+	0	+	0	+	+	+	+	0	0	+	+	0	+	0	+	0	+
2/2P	R1wR1 : RH(1,2,-3,-4,5,8)		+	+	0	0	+	+	+	+	0	+	+	0	0	+	0	+	+	+	0	+	+	0	+
3/3P	R1RZ : RH(1,2,3,-4,5,-8)		+	+	+	0	+	0	0	+	0	+	+	+	+	0	0	+	+	+	0	+	+	0	+
4/4P	R2R2 : RH(1,-2,3,4,-5,-8)		+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	+	0	+	+	0	+
5/5P	R0r : RH(1,-2,-3,4,5,-8)		+	0	0	+	+	0	+	+	0	+	+	0	+	+	0	+	+	+	0	+	+	0	+
6/6P	r'r : RH(-1,2,-3,4,5,-8)		0	+	0	+	+	0	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+
7/7P	r"r : RH(-1,-2,3,4,5,-8)		0	0	+	+	+	0	0	+	0	+	0	+	+	0	0	0	+	+	+	+	+	+	+
8/8P	rr : RH(-1,-2,-3,4,5,-8)		0	0	0	+	+	0	+	+	0	+	+	+	+	0	+	0	+	0	+	0	+	0	+
9/9P	rr : RH(-1,-2,-3,4,5,-8)		0	0	0	+	+	0	0	+	+	+	0	+	+	0	0	+	+	+	+	+	0	0	+
10/10P	rr : RH(-1,-2,-3,4,5,-8)		0	0	0	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+