IMMUNOHEMATOLOGY

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ÚHKT

Immunohematology

- Specialized branch of medical science studying clinical and laboratory aspects of
  - antigens of blood cells (mainly erythrocytes /transfusion compatibility/, erythrocytes and thrombocytes /fetomaternal incompatibility/, leukocytes /TRALI, HLA system)

  and
  - immune system (mainly antibodies and complement /HTR, HON, AIHA/, event. efector cells /function tests/)

Immunohaematological Safety = Compatibility

Compatible Blood Transfusion = substitution with blood components selected to eliminate or minimise immunohaematologically mediated adverse effects

Compatibility Testing = all serologic tests and clerical checks involved in determining the compatibility between the donor and recipient

Adverse Immunohaematological Effects of Blood Transfusion

- Immediate post-transfusion haemolytic reaction

  ...intravascular haemolysis

  ... main cause: ABO incompatibility

- Delayed post-transfusion haemolytic reaction

  ... extravascular haemolysis

  ... main cause: alloantibodies to red cell antigens

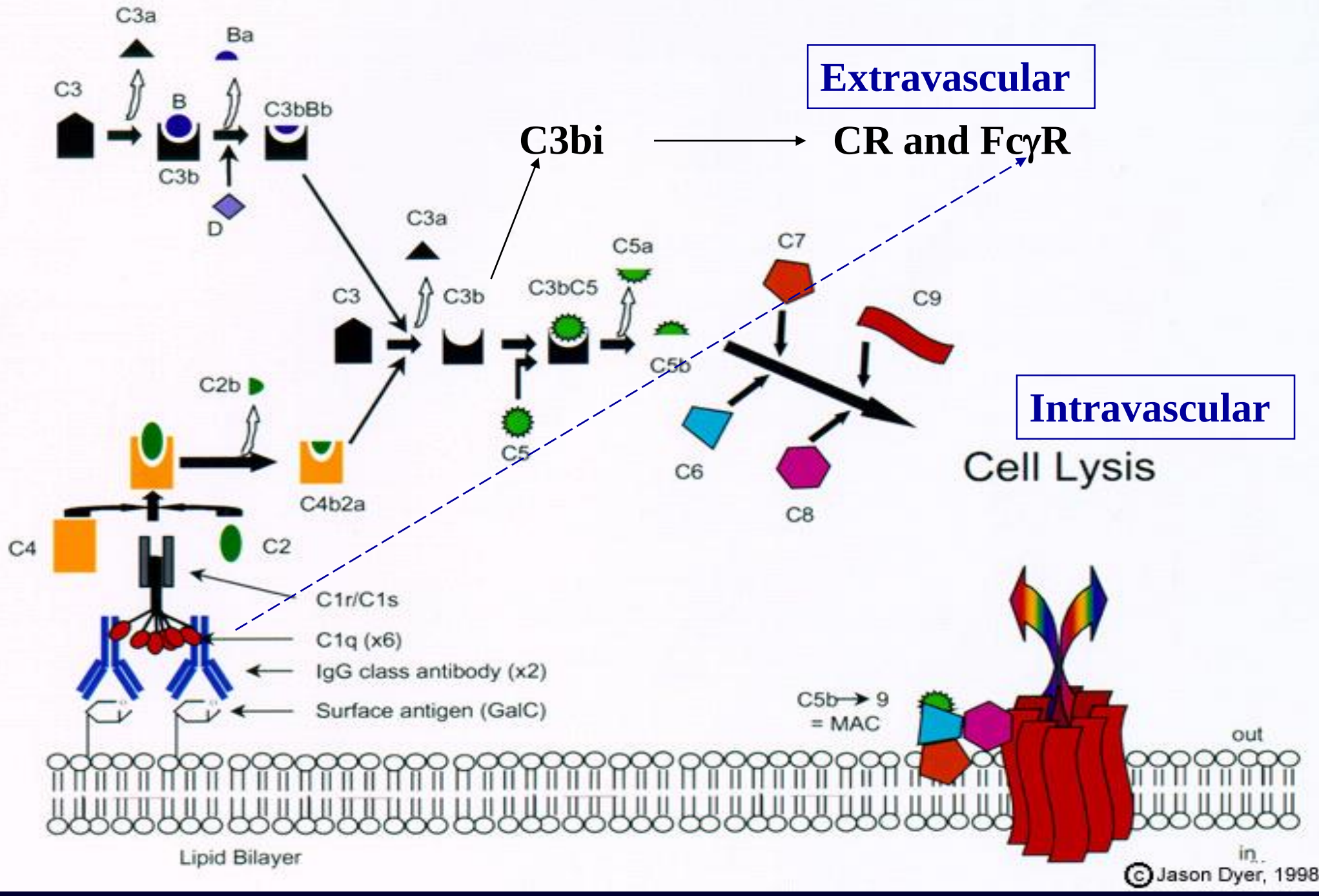
- Alloimmunization to blood group antigens

… danger for next transfusions and pregnancies

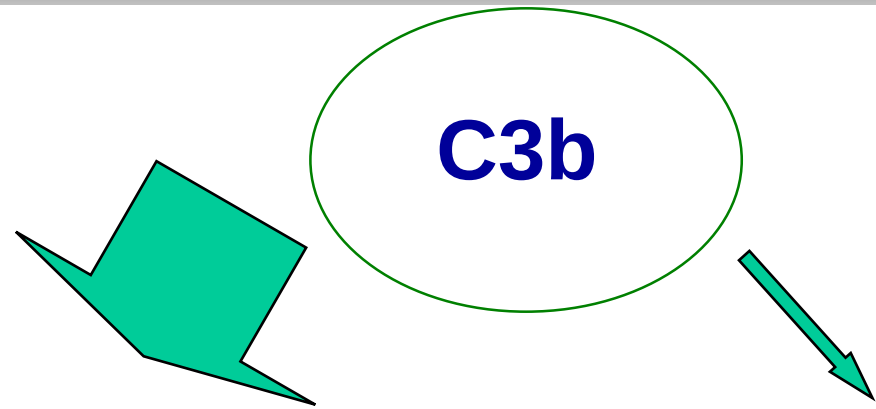
Intravascular Haemolytic Transfusion Reactions

Immediate transfusion reactions
Usually due to antibodies in the recipient reacting with transfused ABO incompatible red cells

- A and B antigens present at high density
- IgM, IgG1 or IgG3 anti-A, -B or -A,B avidly fix complement
- lysis due to C1>>>MAC cascade
- acute symptoms due to complement activation >>> C3a and C5a >>> serotonin and histamine release from mast cells (vasoactive) >>> hypotension and shock
- antigen/antibody/complement complexes >>> activate factor XII >>>coagulation cascade >>> DIC >>> fibrin deposition in kidney and haemorrhage


C3a
Ba
C3
B
C3bBb
C3b
D
Extravascular
C3bi
CR and FcγR
C3a
C5a
C7
C3
C3b
C3bC5
C9
C5b
C2b
C5
C6
C8
Cell Lysis
Intravascular
C4b2a
C4
C2
C1r/C1s
C1q (x6)
IgG class antibody (x2)
Surface antigen (GalC)
C5b→ 9
= MAC
out
in
Lipid Bilayer
© Jason Dyer, 1998

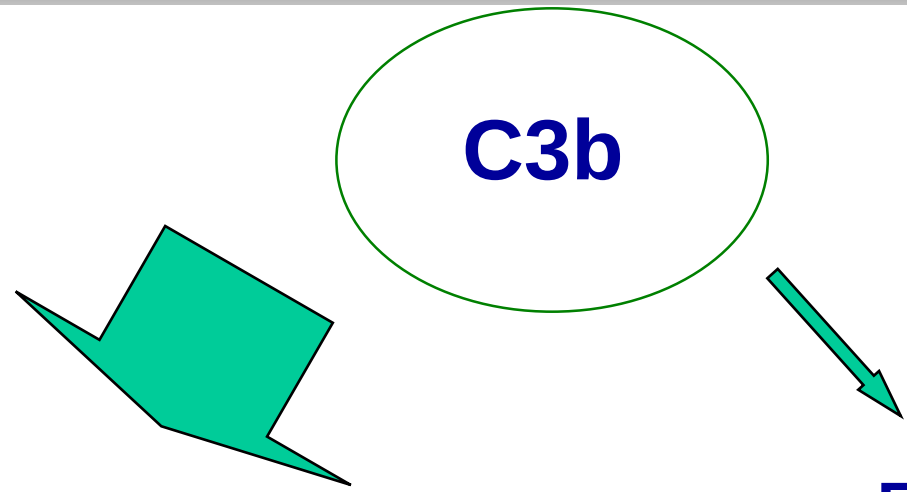
Complement and Red Cell Haemolysis



Intravascular
C-activation by large dose of red cells
>>> overwhelms regulatory and phagocytic systems
>>> MAC + vasoactive peptides

Extravascular
C-activation by small dose
>>> CR on MPS

Complement and Red Cell Haemolysis



<u>Intravascular</u>
"last resort"
lysis
complement cleavage products are vasoactive & chemotactic
>>> systemic inflammation
>>> severe systemic reactions

Extravascular

phagocytosis

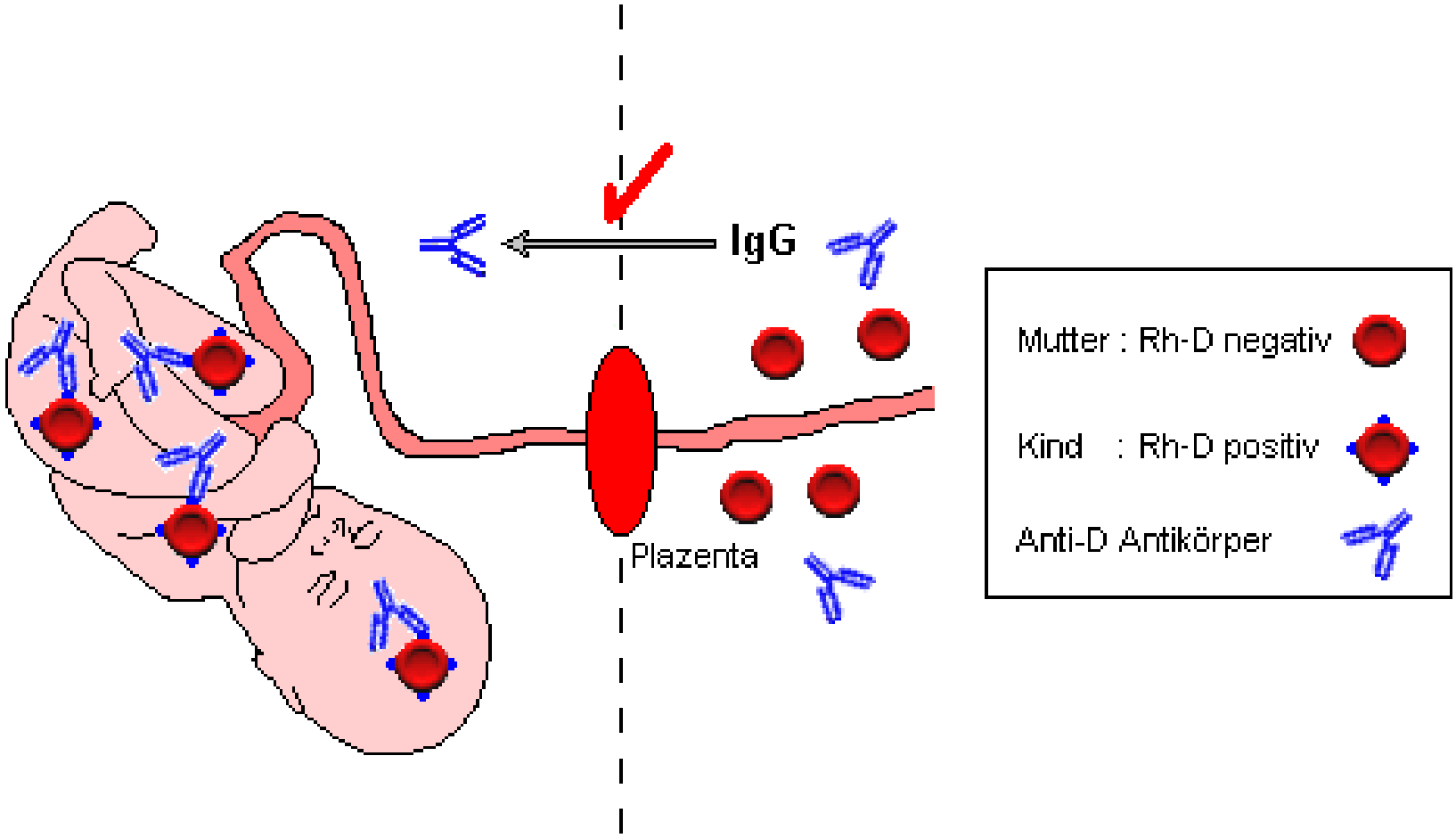
Extravascular Lysis

- **DHTR (Delayed Hemolytic Transfusion Reaction)**
- **HDN (Hemolytic Disease of Newborn)**
- **warm AIHA (Autoimmune Hemolytic Anemia)**

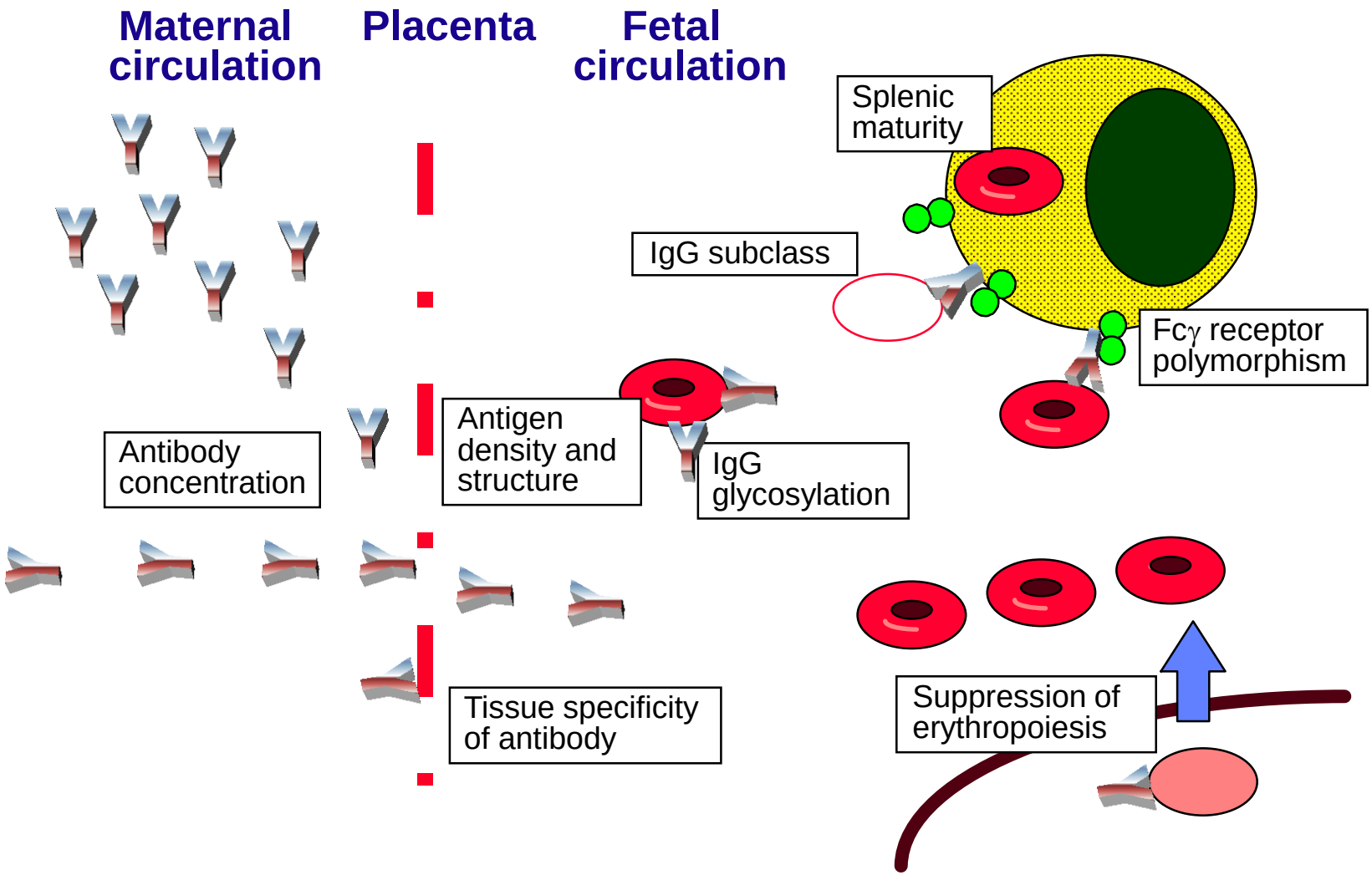
E-IgG (with or without C3bi) are almost always destroyed in the spleen.

E-IgM + C3 may be transiently captured by the liver.

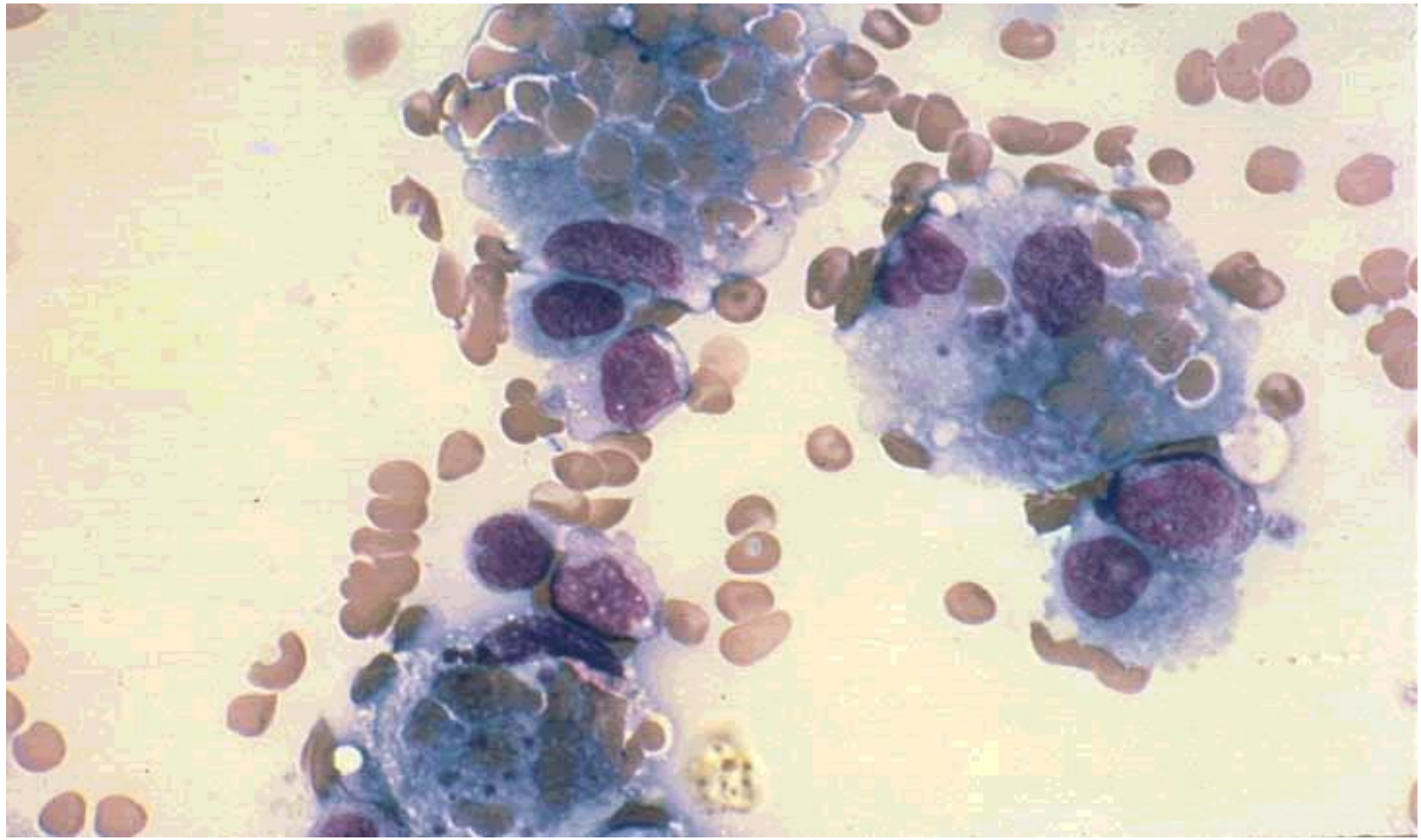
Morbus haemolyticus Neonatorum (MhN)



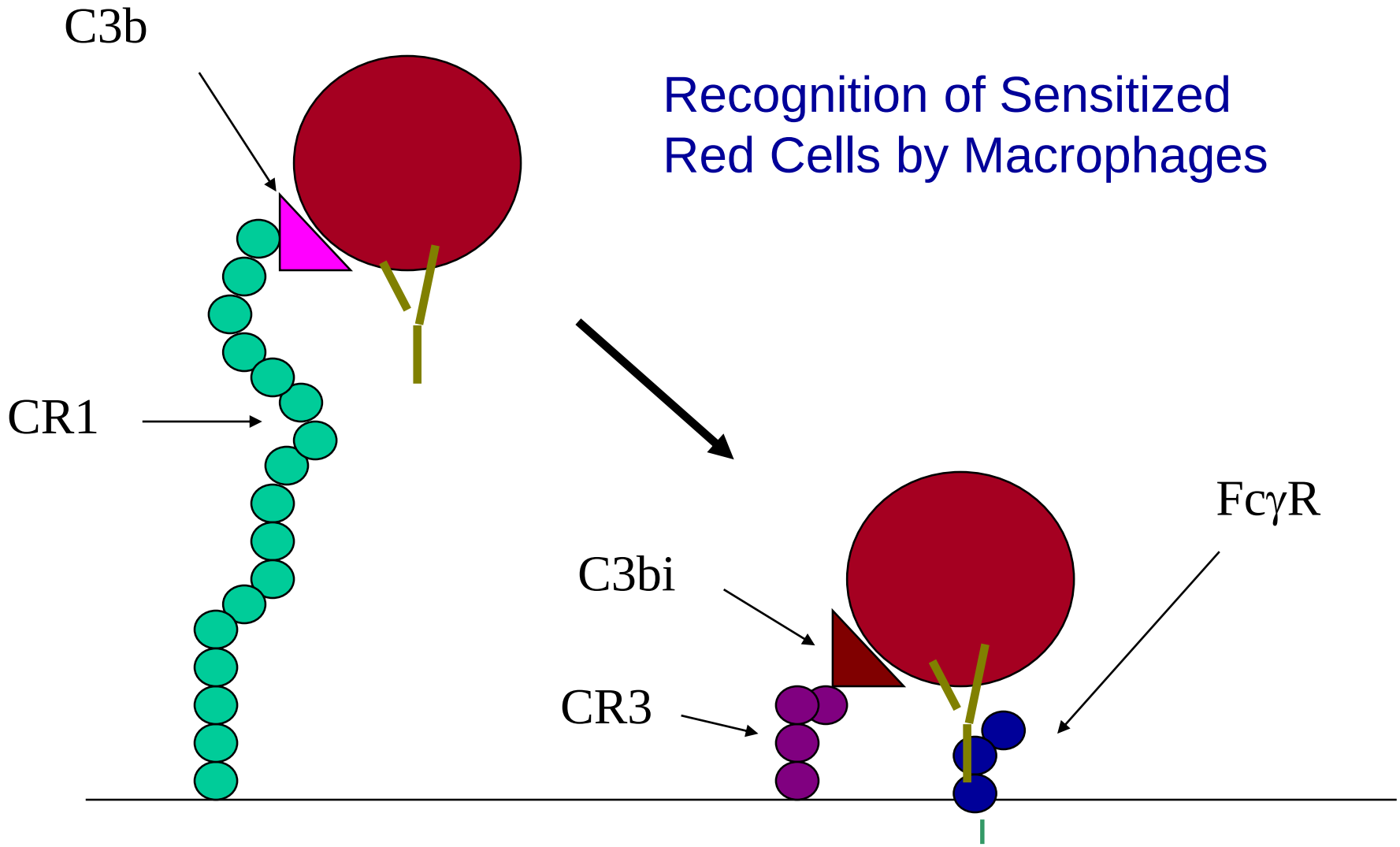
HDN is a Multifactorial Disorder



Erythrophagocytosis by Macrophages



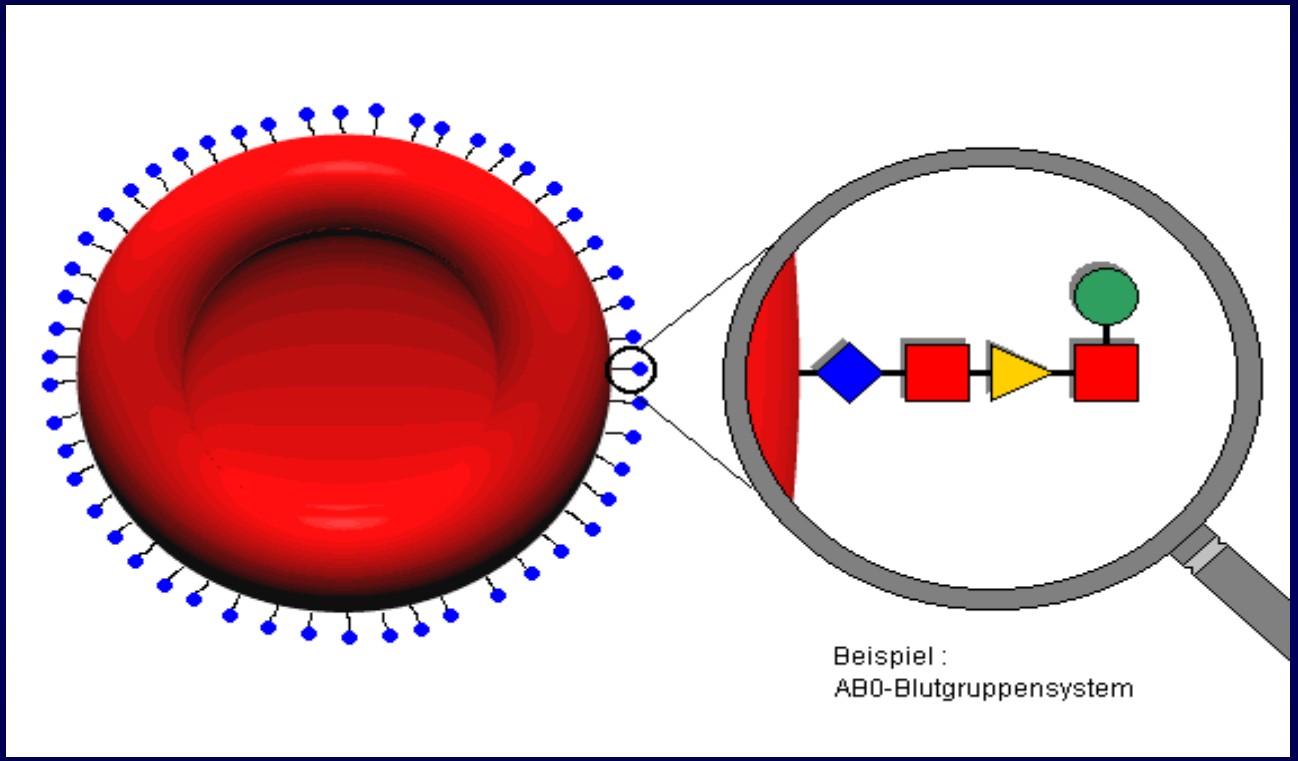
Recognition of Sensitized Red Cells by Macrophages



Antigens

- Membrane structures which are able to induce immune response:
  - allo-reaction - response to „foreign“ antigens
    - „different“ antigens in recipient/donor or in mother/fetus
  - auto-reaction - pathologic aggression of immune system to „own“ antigens /AIHA/

Antigen



2018 – 360 antigens, 322 in 36 BG systems, 14 in 5 BG collections, 17 in LFA and 7 in HFA series

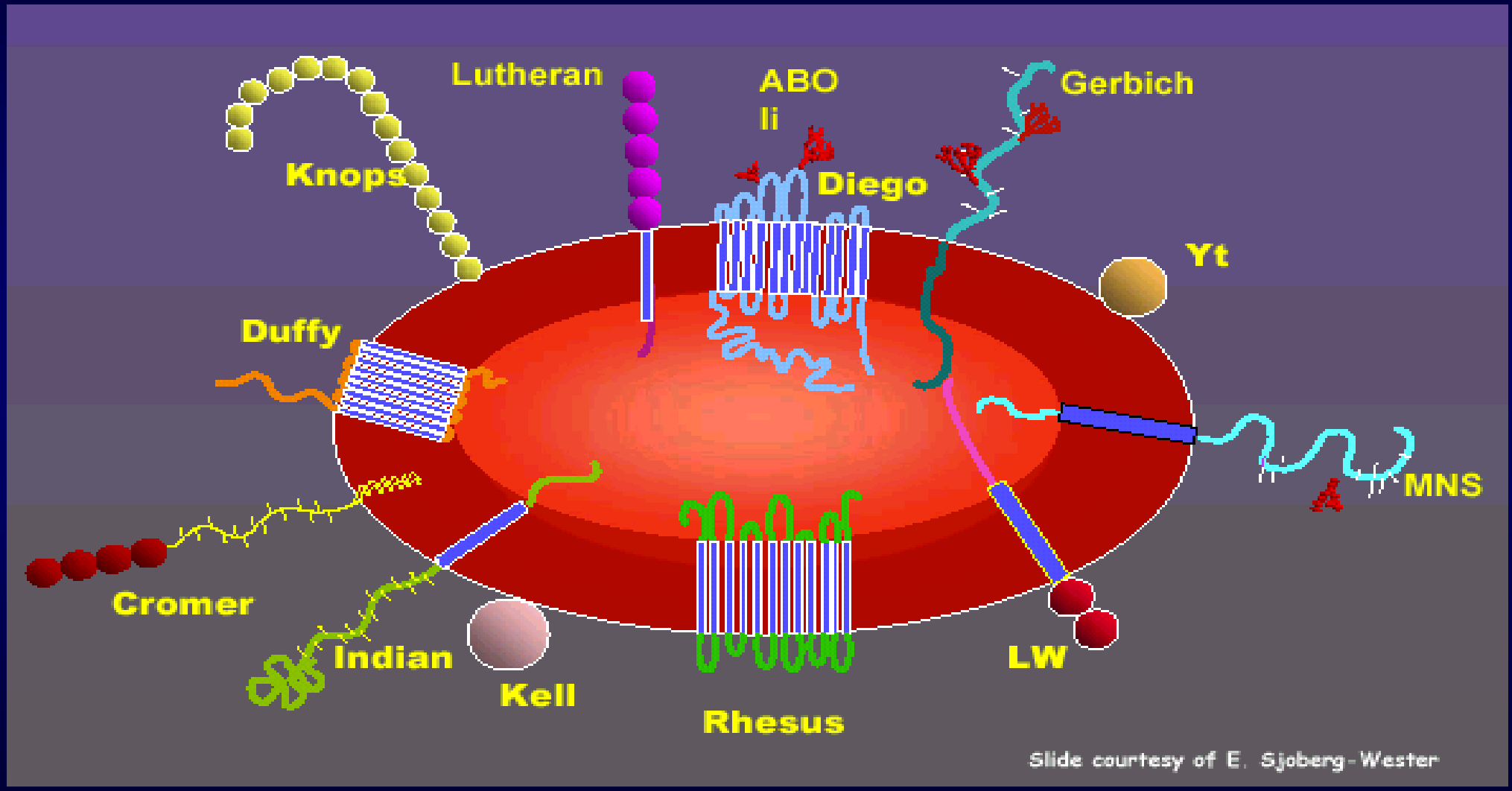
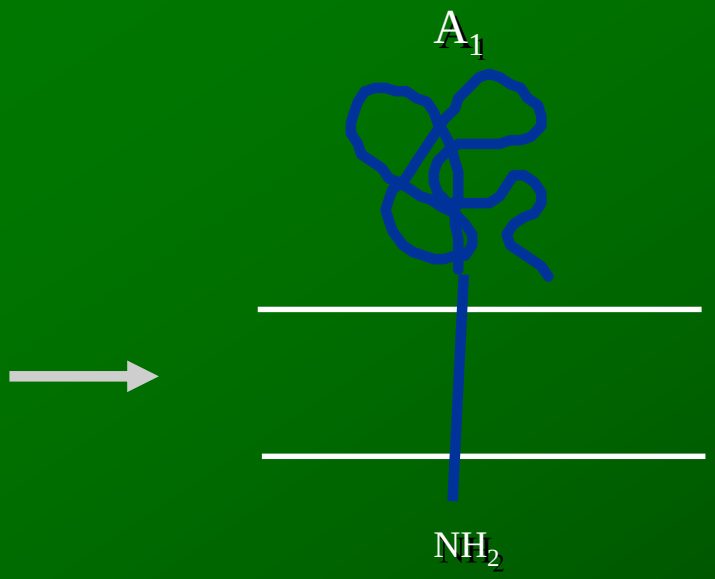


Table I. Functional classification of human blood groups.

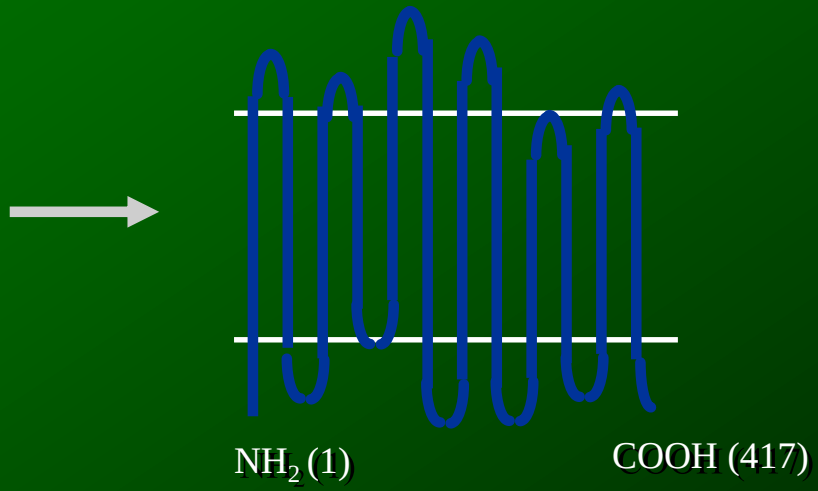
Molecular class	*Gene symbol*	*Symbol ISBT*	*Chromosome*	*Protein or lipid*[§]	*Size (kDa)*[°]	*Copies per RBC*	*Biological function*
Transporter or channel	DI	010	17q21	Band 3 (CD233)	90	10^6	Anion exchanger [AE1]
	CO	015	7p14	AQP-1/CHIP28	28**	$2x10^5$	Water channel
	JK	009	18q11-q12	Kidd	50	$15x10^3$	Urea Transporter [hUT-B1]
	RH	004	1p34-p36	Rh (CD240)	30-32	$2x10^5$	RhAG(CD241)* - Amonium Transporter
	XK	019	Xp21.1	Kx	37		Transporter?
Receptor	FY	008	1q22-q23	DARC (CD234)	35-45	$15x10^3$	Receptor *P. vivax* / chemokines / (HIV-1 ?)
	KN	022	1q32	CR1 (CD35)	170-280	10^3	Receptor *P. falciparum* / C3b, C4b
	MNS	002	4q28-q31	GPA/B (CD235A/CD235B)	36/20	$10^6/3x10^5$	Receptor *P. falciparum (EBA-175)*/ bacteria / viruses
	CROM	021	1q32	DAF (CD55)	70	$6\text{-}15x10^3$	Receptor *E. coli* / Enterovirus
	P	003	22q11-ter	Globoside		$10x10^6$	Receptor Parvovirus B19
Adhesion	IN	023	11p13	CD44	80	$5\text{-}10x10^3$	Ligands= Hyaluronate, Collagens I and VI, fibronectin, Serglycin, ETA-1
	LW	016	19p13	ICAM-4 (CD242)	42	$3\text{-}5x10^3$	Ligands= integrins $\alpha4\beta2$ (and $\alpha4\beta1$, $\alpha v\beta3$?)
	LU	005	19q12-q13	Lu/B-CAM (CD239)	78-85	$1.5\text{-}4x10^3$	Ligand= Laminin (chain $\alpha5$)
	XG	012	Xp22-p32	XG1(Xg^a)/XG2(CD99)	22-29	150/960	? ligand ?
	OK	024	19p13.2	EMMPRIN (CD147)/Ok^a	54-65		Leukocyte adhésion molecule M6 (ligand = ?)
	JMH	026	15q23-q24	JMH/SEMA7A (CDw108)	75-80		Semaphorin 7A (Cell attachment through RGD sequence ?)
Enzyme	ABO	001	9q34-q34.2	GlycosylTransferase	40-42		3-α-D-GalNAc/Gal-transferases (A/B)
	H	018	19q13	GlycosylTransferase			2-α-L-fucosyltransferase (H= FUT1 / SE= FUT2)
	LE	007	19p13	GlycosylTransferase			3/4-α-L-fucosyltransferase (FUT3)
	YT	011	17q22.1-22.3	Cartwright	160	$3x10^3$	Acetylcholinesterase
	KEL	006	7q32-q36	Kell (CD238)	93	$3\text{-}6x10^3$	Zn-Metalloproteinase
	DO	014	12p13.1-13.2	Dombrock	54-57		ADP-ribosyltransferase ?
Structure+	GE	020	2q14-q21	GPC/D (CD236C/CD36D)	32/23	$2x10^5$	Mechanical/elastic properties of red cell membrane and receptor *P. falciparum (BAEBL)*
Others	CH/RG	017	6p21.3	C4A/CAB fragments			*Complement fractions adsorbed on RBCs*
	SC	013	1p32-p34	Scianna	*60*		*unknown*
	RAPH	025	11p15	MER2	*40#*	*70-500#*	*unknown*

Principle of Detection (ABO, Rh)

ABO
9q34



Rhesus
1p34-36



Carbohydrates

(Glyco) Proteins

Receptory/adhez.molekuly

MNS
Sclanna
Dombrock
Gerbich
Raph

???

Lutheran
Duffy
Xg
LW
Indian
Oka
JMH

Rh
Kidd
Diego
Colton
Kx
GIL

membránový transport

Kell
Yt

enzymy

Ch/Rg
Cromer
Knops

regulace komplementu

Slide courtesy of Jill Storry

- Gen (DNA)
- RNA
- Glykosyltransferase
- Antigen

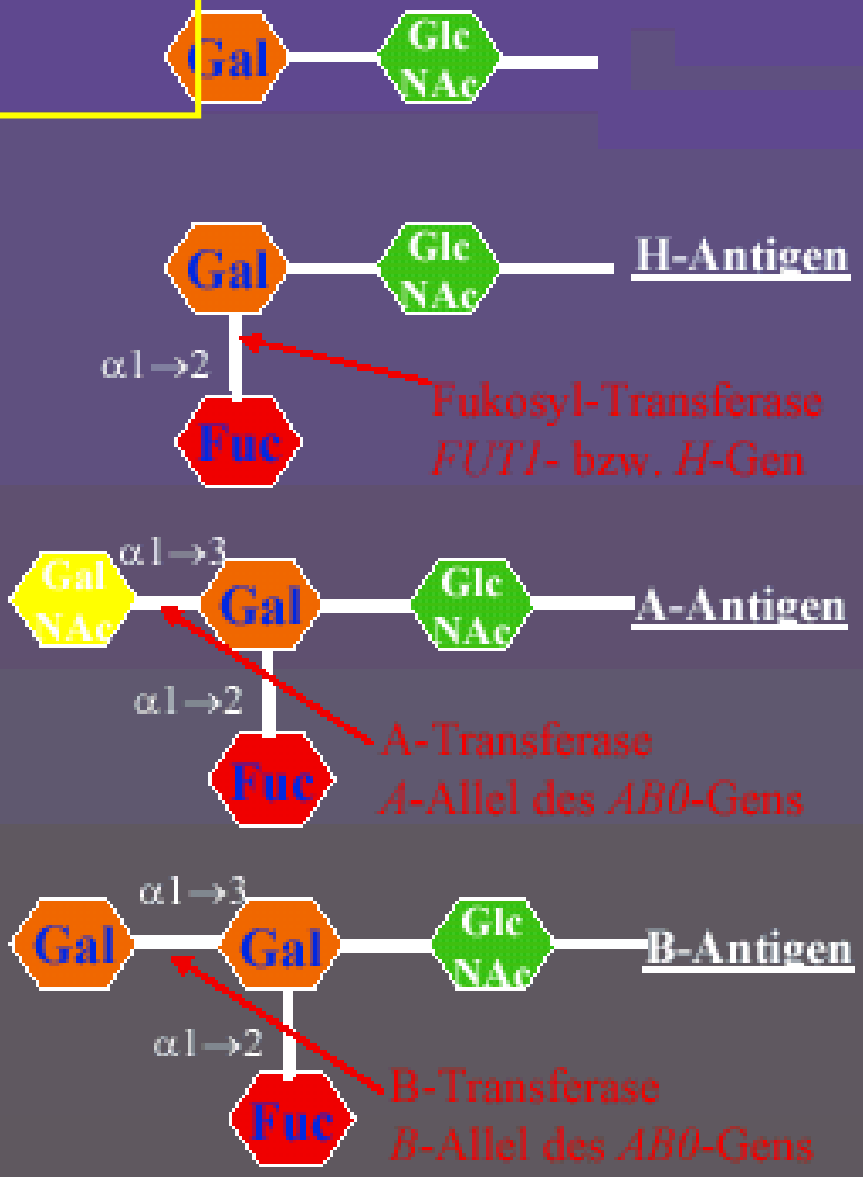


Fig. courtesy of ND Avent 2002

AB0 system

- Antigens:
  - terminal sugars of glycoproteins (65-75%) and glycolipids (25-35%) of rbc membrane
  - „histo-blood-group antigens“ … present on almost all epithelial tissues (exc.: not in CNS)
  - Subgroups with quantitative and qualitative differences (A1, A2 and several weak subgroups: A3, Am, Ax, Ael etc.)
- Antibodies:
  - „naturally occuring“ … produced in postnatal perion (detected in 3.-6.month, increasing to 5-6 year, then stable, decreasing in elderly and in immunodefficiencies)
  - Induced by exposition to foreign substances in the environment (bacteria, pollen, dust etc.) with similar biochemical configuration on basis of response to NON-SELF antigens
  - IgM (high potential of complement activation) and IgG (crossing placenta, mild forms of HDN)
  - anti-A, anti-B, (anti-A,B /in gr.0/), anti-A1 /in A2 v 2%, in A2B in 25%, frequent in weak subgroups)
  - anti-H (cold antibody, not clin.signif.except in 0h/Bombay/

AB0 system

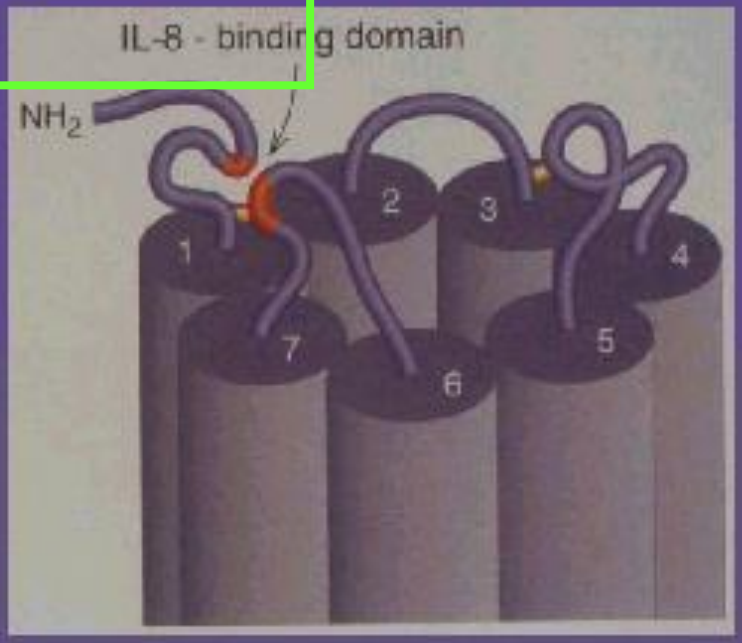
- Antigen (epitopes) number:
  - A1: 1×10^6
  - A2: $0{,}2\text{-}0{,}3 \times 10^6$
  - newborn A: $0{,}2\text{-}0{,}3 \times 10^6$
  - A3: 5×10^3

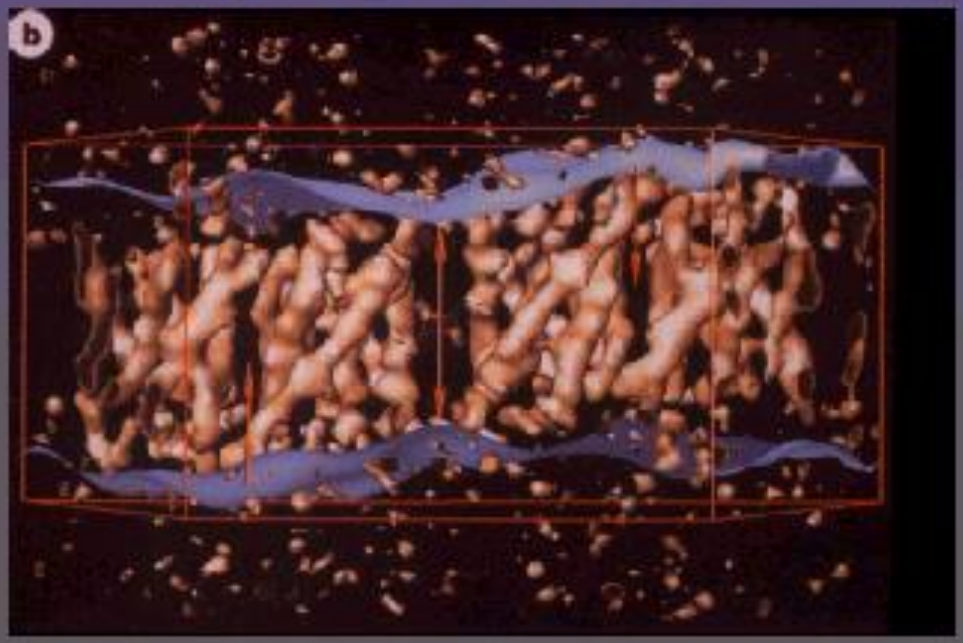
- Frequency:

	ÚHKT	Germany	USA(c)	USA(b)	USA(a)
A	40	48	41	27	27
B	18	9	9	19	25
AB	8,5	4	4	4	8
0	33,5	39	46	50	40

Duffy - Colton

- Gen (DNA)
- RNA
- Protein = Antigen

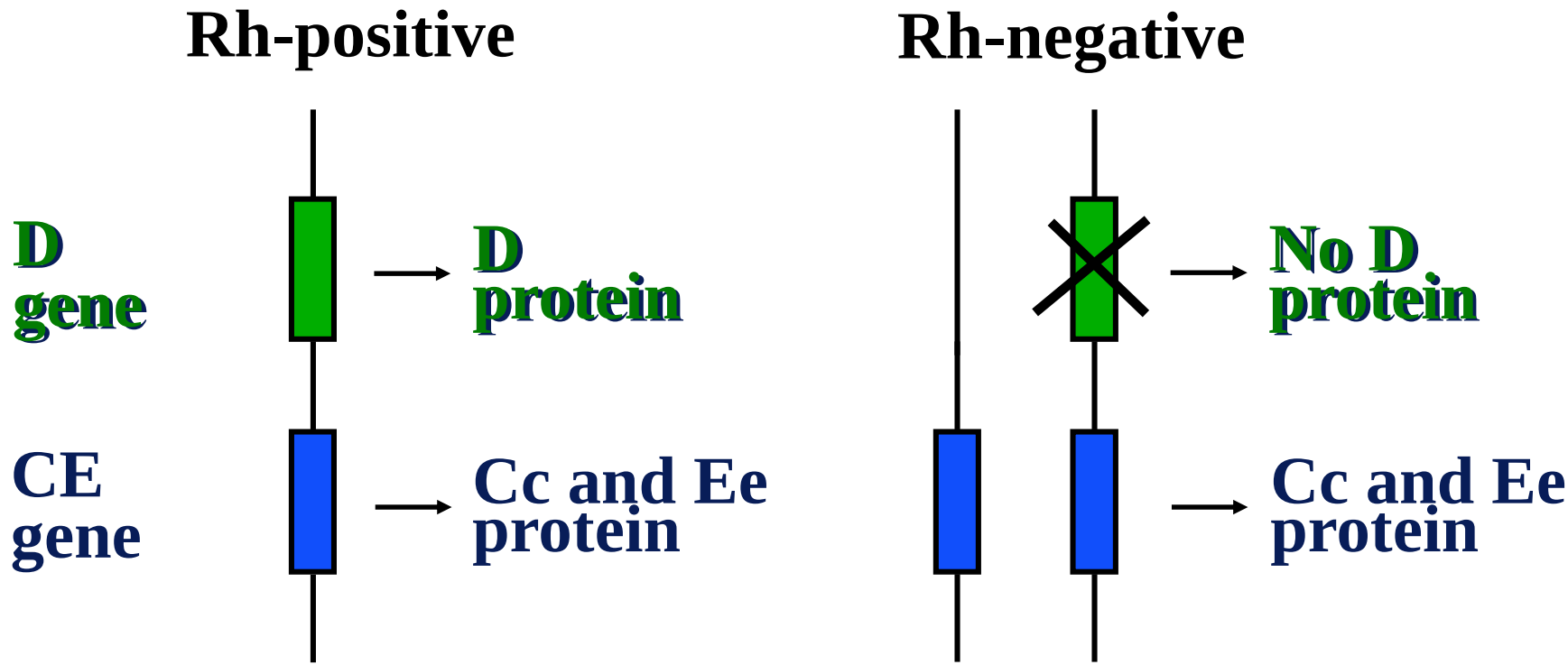




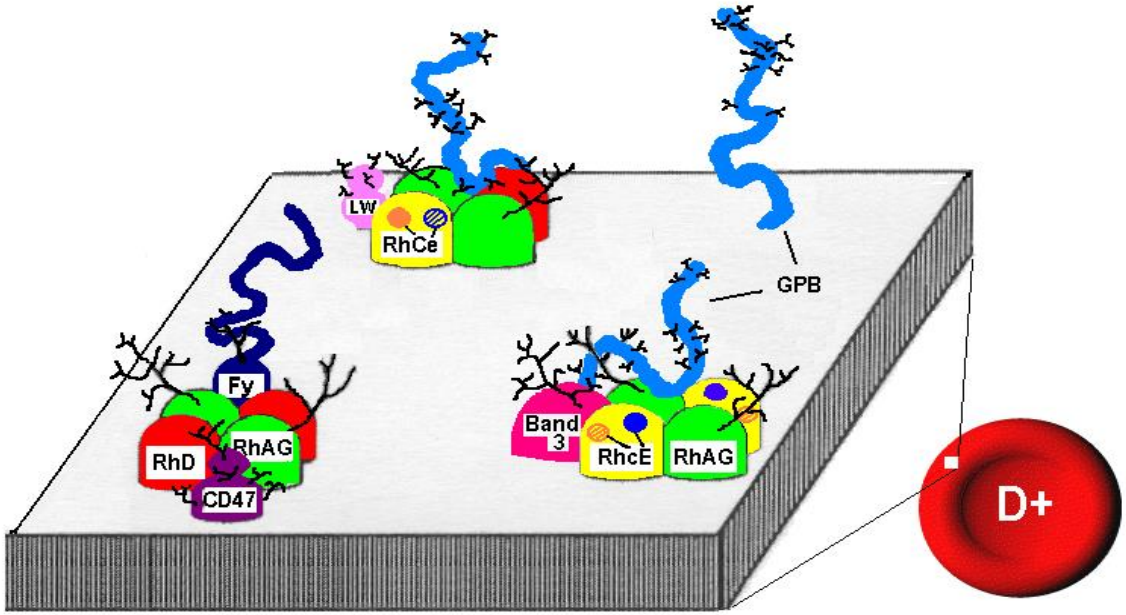
Rh system

- Antigens:
  - Second most important system
    - Most frequent cause of HDN
    - Most frequent alloantibodies
    - Frequent target of autoantibodies
  - RhD antigen – the most immunogenic rbc antigen – no allelic counterpart – D- person are lacking whole RhD protein
  - Very polymorfic system – more than 50 antigens, molecularly defined more than 100 allels
  - main Ags: D – C – c – E – e – Cw … a lot of ohters (mostly HFA or LFA)
  - Function: membrane transport of ammonium (susp.)
- Antibodies:
  - Immune origin (after contact with foreign rbcs), causing HDN and HTR by exravascular hemolysis, not activating complement

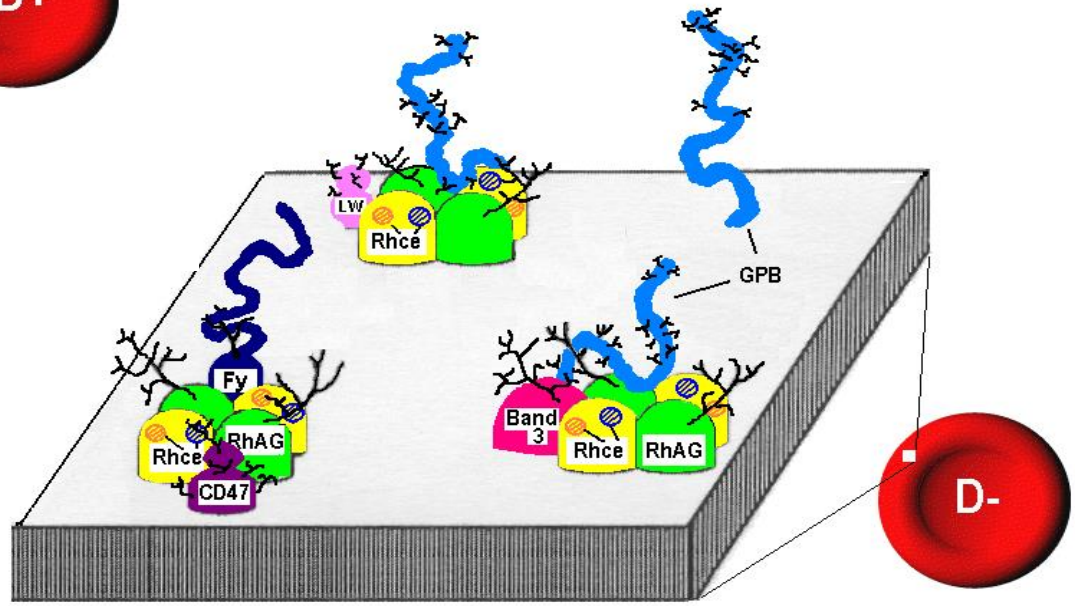
Defining the Rh Blood Group Antigens*



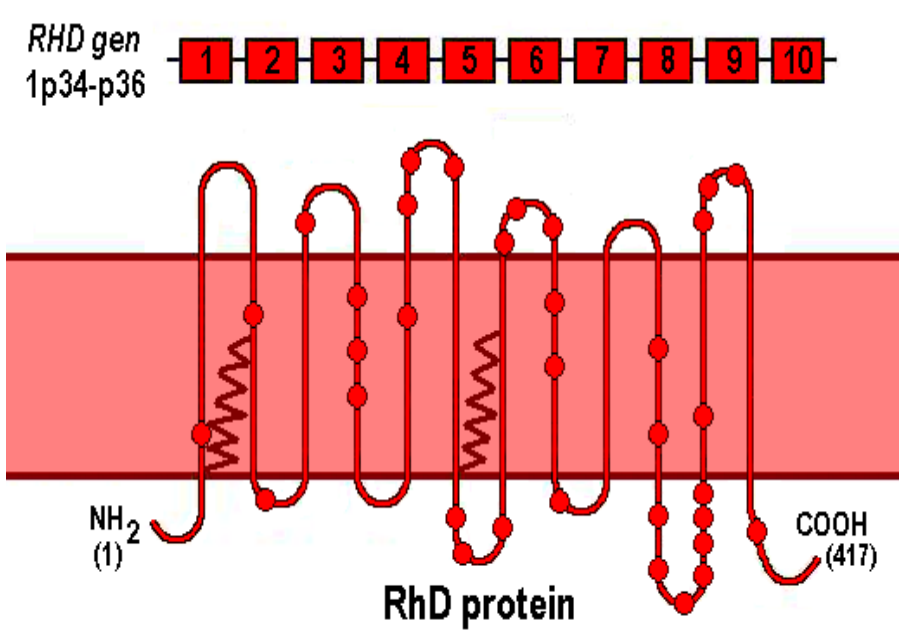
*J. P. Cartron in Blood Reviews (1994) 8, 199-212

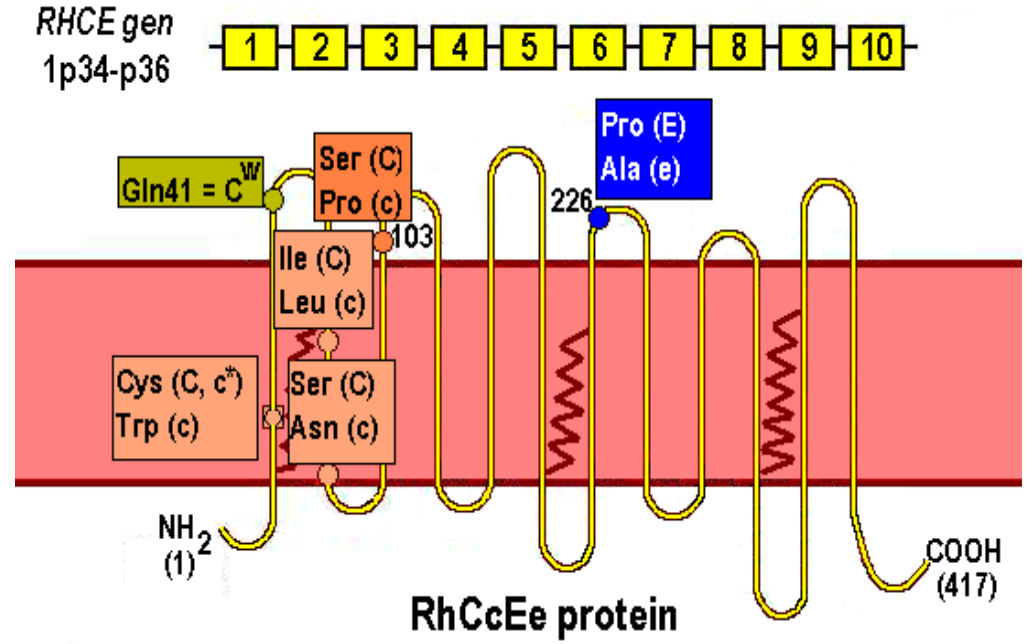


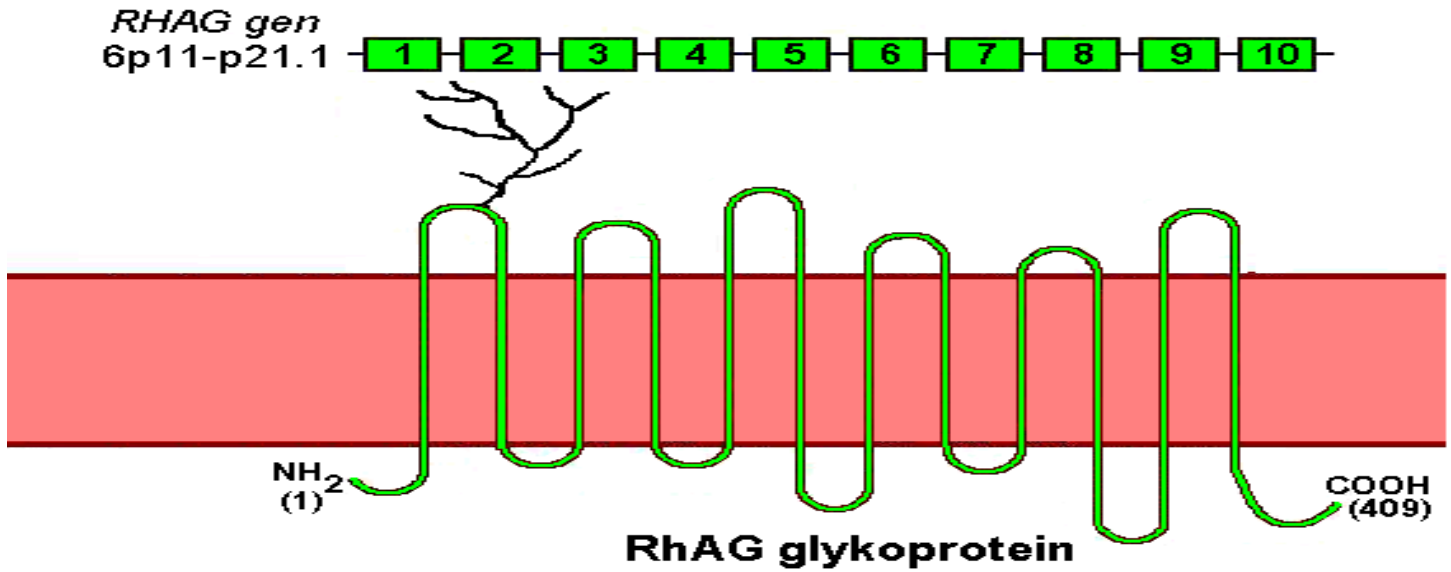
RhD+ (D+ C+ c+ E+ e+) *RHD-RHCe/RHD-RHcE*



RhD- (D- C- c+ E- e+) *RHce/RHce*


RHD gen
1p34-p36
1 2 3 4 5 6 7 8 9 10
NH2
(1)
COOH
(417)
RhD protein


RHCE gen
1p34-p36
1 2 3 4 5 6 7 8 9 10
Gln41 = CW
Ser (C)
Pro (c)
Pro (E)
Ala (e)
226
103
Ile (C)
Leu (c)
Cys (C, c*)
Trp (c)
Ser (C)
Asn (c)
NH2
(1)
COOH
(417)
RhCcEe protein

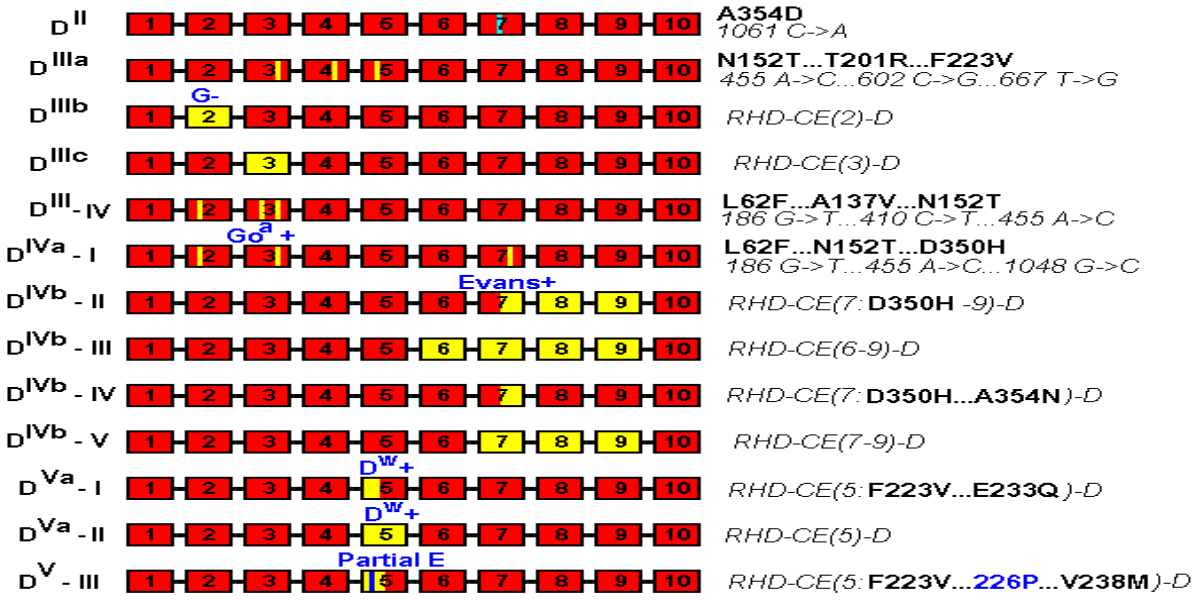

RHAG gen
6p11-p21.1
1 2 3 4 5 6 7 8 9 10
NH2
(1)
COOH
(409)
RhAG glykoprotein

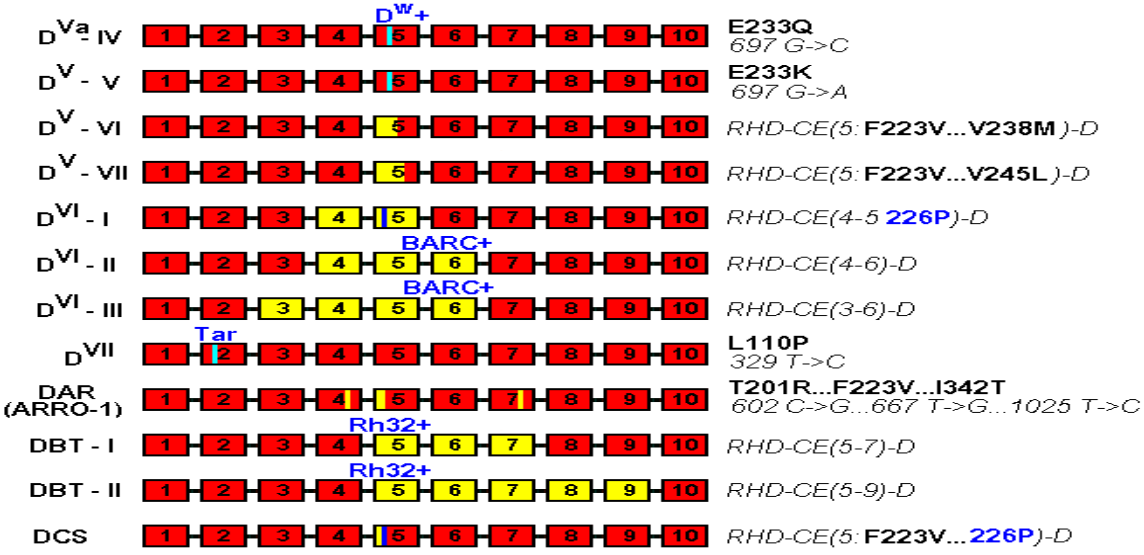
Rh system

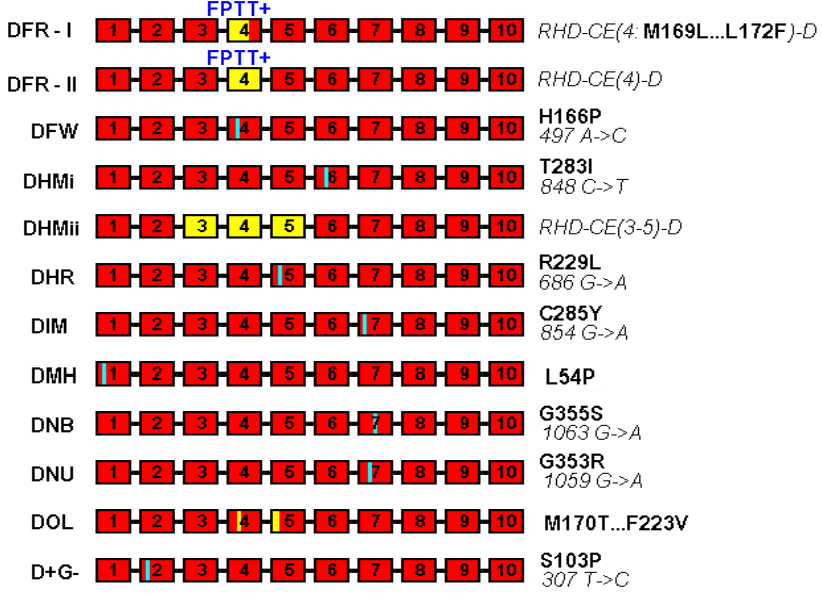
Frequency of Rh haplotypes:

			caucasians	africans	asians
DCe	R1	RH1,2,-3,-4,5	42	6	73
dce	r	RH-1,-2,-3,4,5	39	20	2
DcE	R2	RH1,-2,3,-4,-5	14	12	19
Dce	R0	RH1,-2,-3,4,5	2	59	3
dcE	r''	RH-1,-2,3,4,-5	1	<1	<1
dCe	r'	RH-1,2,-3,-4,5	1	3	2
DCE	RZ	RH1,2,3,-4,-5	<1	<1	<1
dCE	rY	RH1,2,-3,-4,5	<1	<1	<1

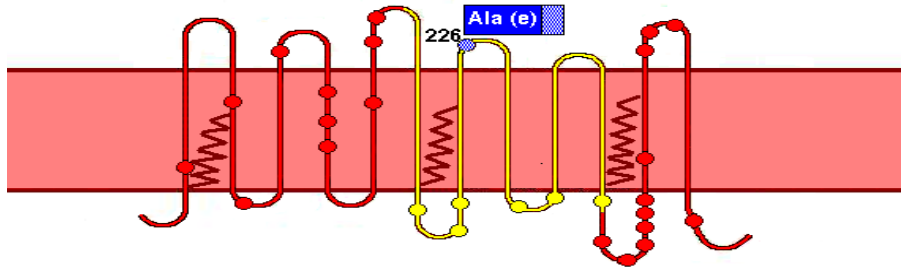
Rh varianty se změnami v *RHD*



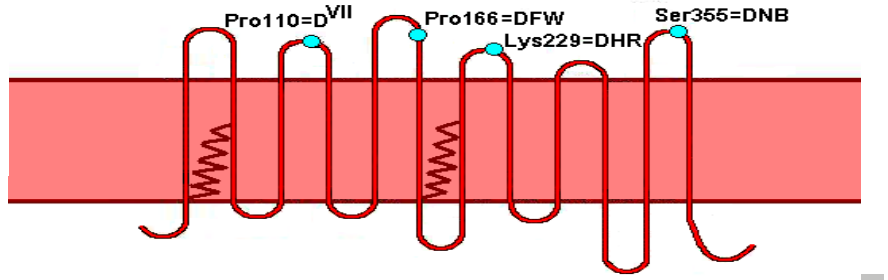




Varianta D VI -II (hybridní protein RhD-C*I*ce-D)



Příklady variant s bodovými mutacemi

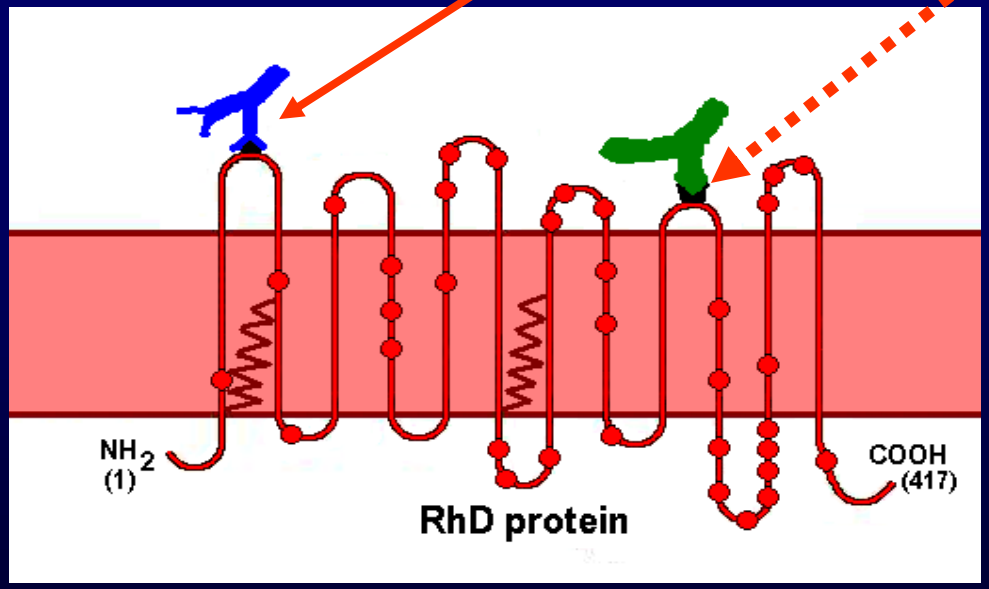


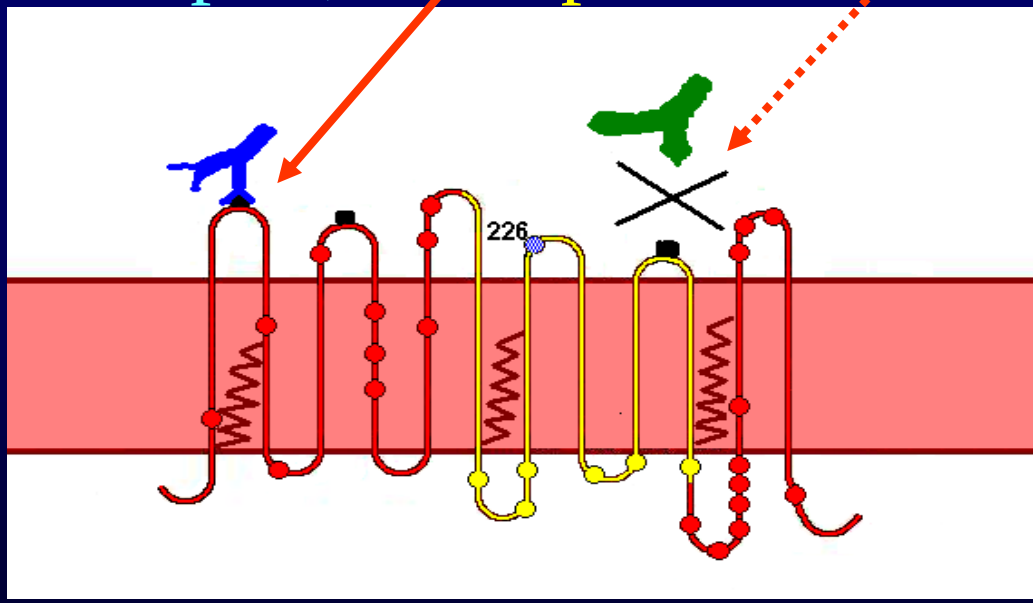
Serologická detekce variant:

• monoklonální protilátky reagují s malou přesně definovanou oblastí RhD proteinu = D epitopem

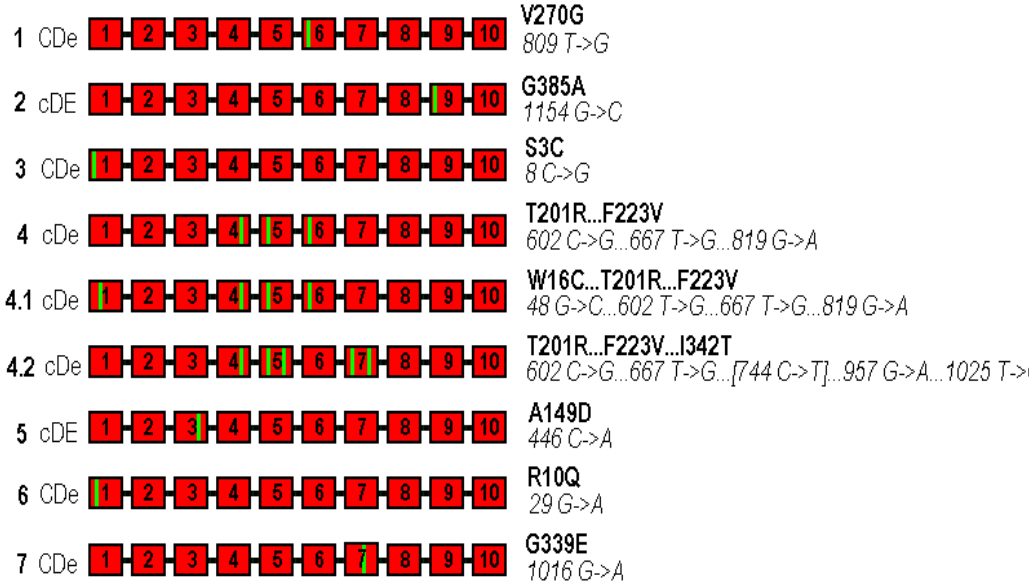
normální: anti-ep1+, anti-ep2 +

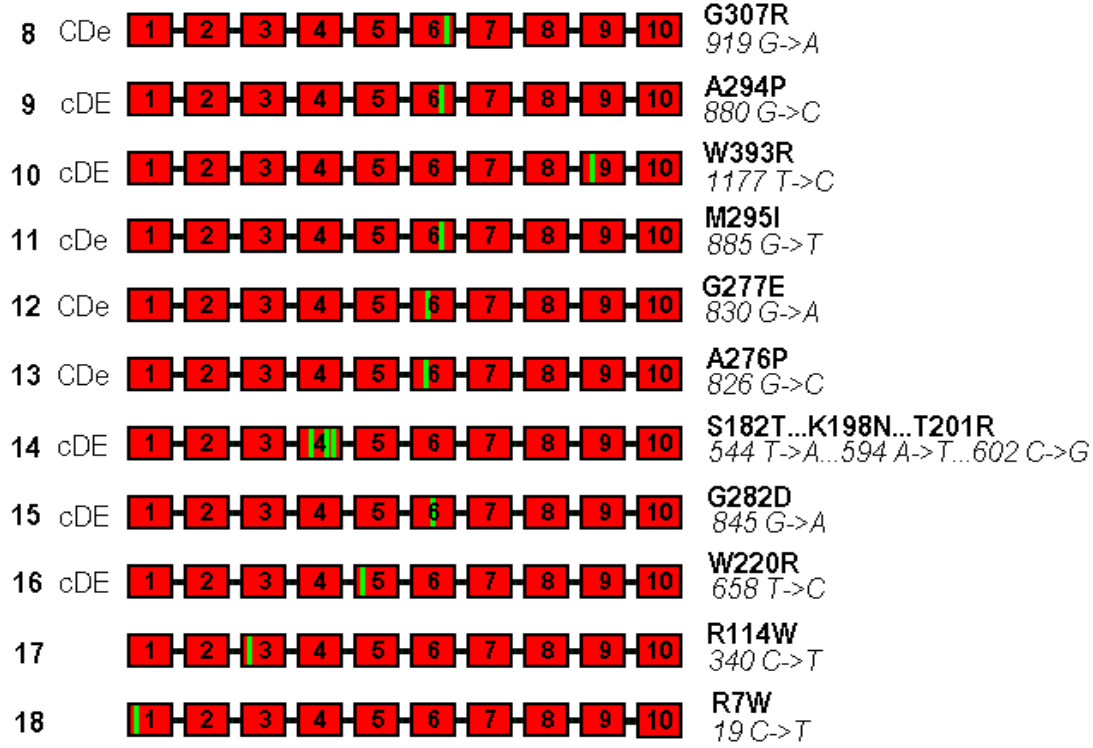
variantní: anti-ep1+, anti-ep2 -





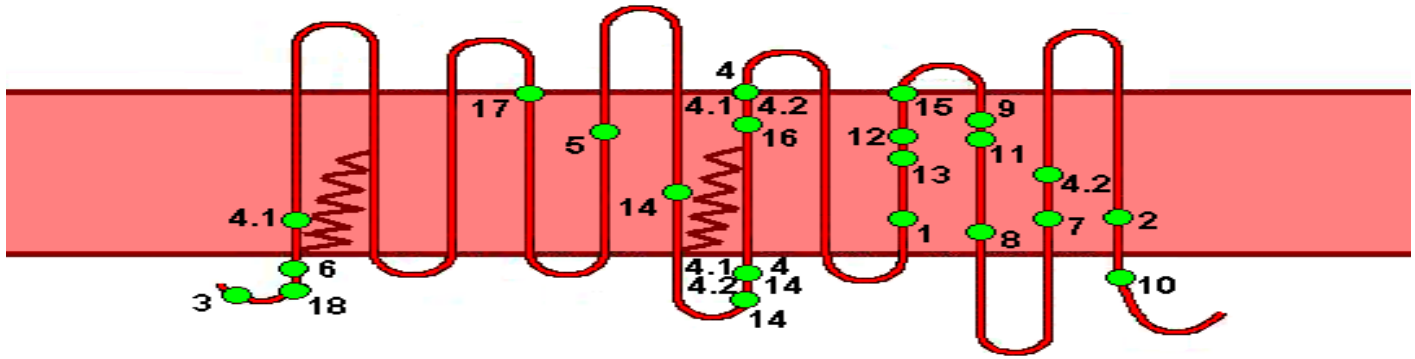
Mutace *RHD* u slabých D antigenů





Rh protein u slabých D antigenů

(mutace v transmembranozní a cytoplasmatické lokalizaci)

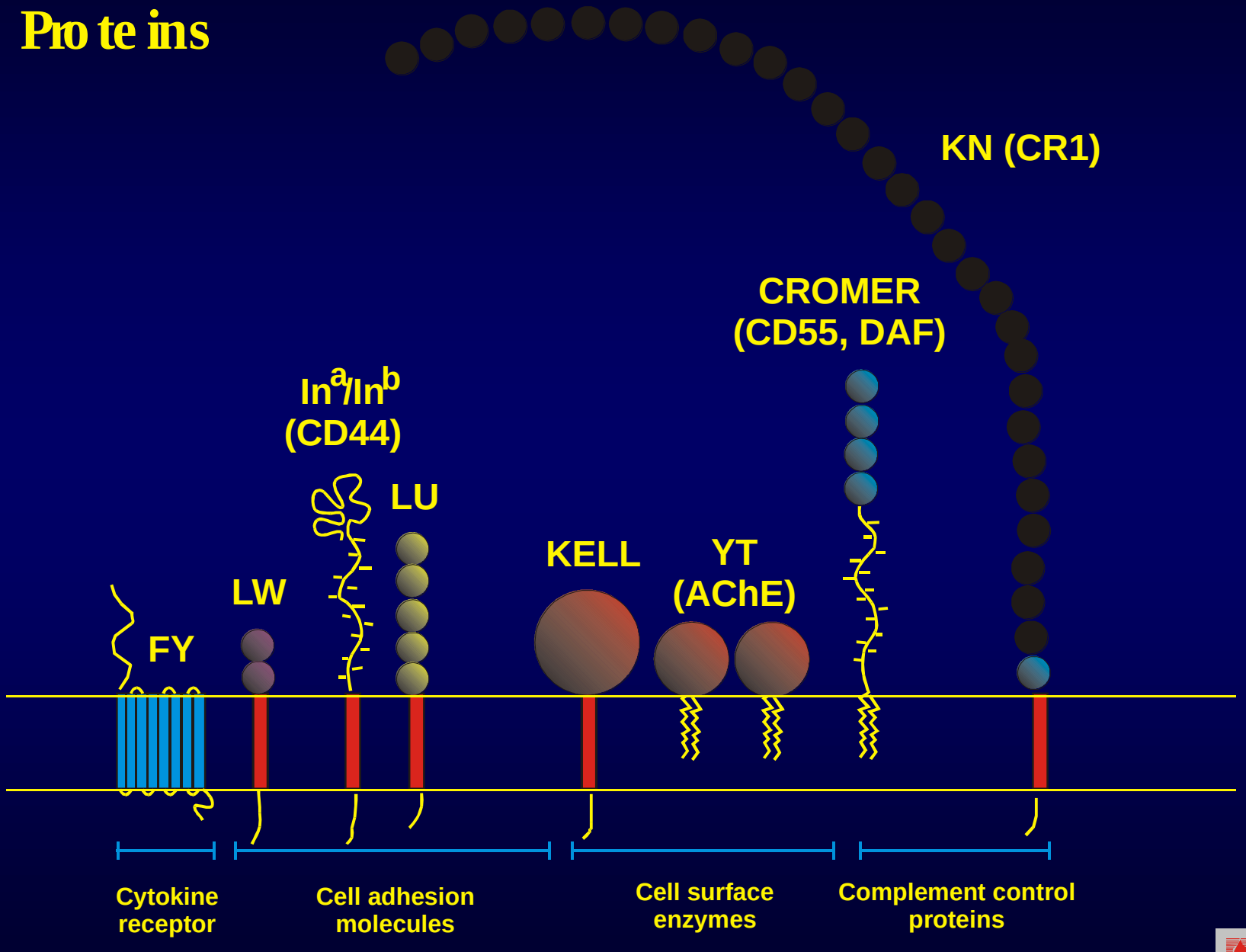


ÚHKT

Erytrocytární antigenní systémy

ISBT No.	System	ISBT-Symbol	Zahl der Antigene	Wichtigste Antigene	Chromosom Locus
001	ABO	ABO	4	A, B, AB1, A1	9 q - 34.1 - q 34.2
002	MNSs	MNS	38	M, N, S, s,U , Ena	4 q 28 - q 31
003	P	P	1	P1	22 q 11.2 - qter
004	Rhesus	Rh	45	D, C, E, c, e	1 p 36.2 - p 34
005	Lutheran	LU	18	Lua, Lub, Luab, Lu4	19 q 12 - q 13
006	Kell	KEL	21	K, k, Kpa, Kpb, Jsa	7 q 33
007	Lewis	LE	3	Lea, Leb, Leab	19 p 13.3
008	Duffy	FY	6	Fya, Fyb, Fy3, Fy4	1 q 22 - q 23
009	Kidd	JK	3	Jka, Jkb, Jkab	18 q 11.1 - q 11.2
010	Diego	DI	4	Dia, Dib, Wra, Wrb	17 q 12 - q 21
011	Cartwright	YT	2	Yta, Ytb	7 q 22
012	Xg	XG	1	Xga	Xp 22.32
013	Scianna	SC	3	Sm, Bu3, Sc3	1 p 36.2 - p 22.1
014	Dombrock	DO	5	Doa, Dob, Gya, Hy, Joa	DO Glykoprotein
015	Colton	CO	3	Coa, Cob, Coab	7 p 14
016	Land. /Wiener	LW	3	Lwa, LWb, Lwab	19 p 13.2 - cen
017	Chido / Rogers	CH/RG	9	Ch 1, Ch 2, Ch 3, Rg 1, Rg 2, WH	35875.75
018	Hh	H	1	H	19 q 13
019	Kx	XK	1	Kx	Xp 21.1
020	Gerbich	GE	7	Ge 2, Ge 3, Ge 4, Wb	2 q 14 - q 21
021	Cromer	CROM	10	Cra, Tca, Tcb, Tcc, Dra	1 q 32
022	Knops	KNOPS	5	Kna , Knb, McCa, Sla, Yka	1 q 32
023	Indian	IN	2	Ina, Inb	11 p 13

Models of the Structure of Minor Blood Group Active Proteins



ÚHKT

Clinical Significance of Antibodies Reactive at 37C

Usually	Sometimes	Never ?
ABO	Cartwright (Yt^a)	Bg
Rh	Lutheran (Lu^b)	Ch/Rg
Kell	Gerbich	Lewis (Le^b)
Duffy	Lan	Kn/Mc/Yk
S,s,U	Dombrock	JMH
P	At^a	Xg^a
Kidd	In^b	
	Vel	
	Cs^a	

From Garratty, 1998

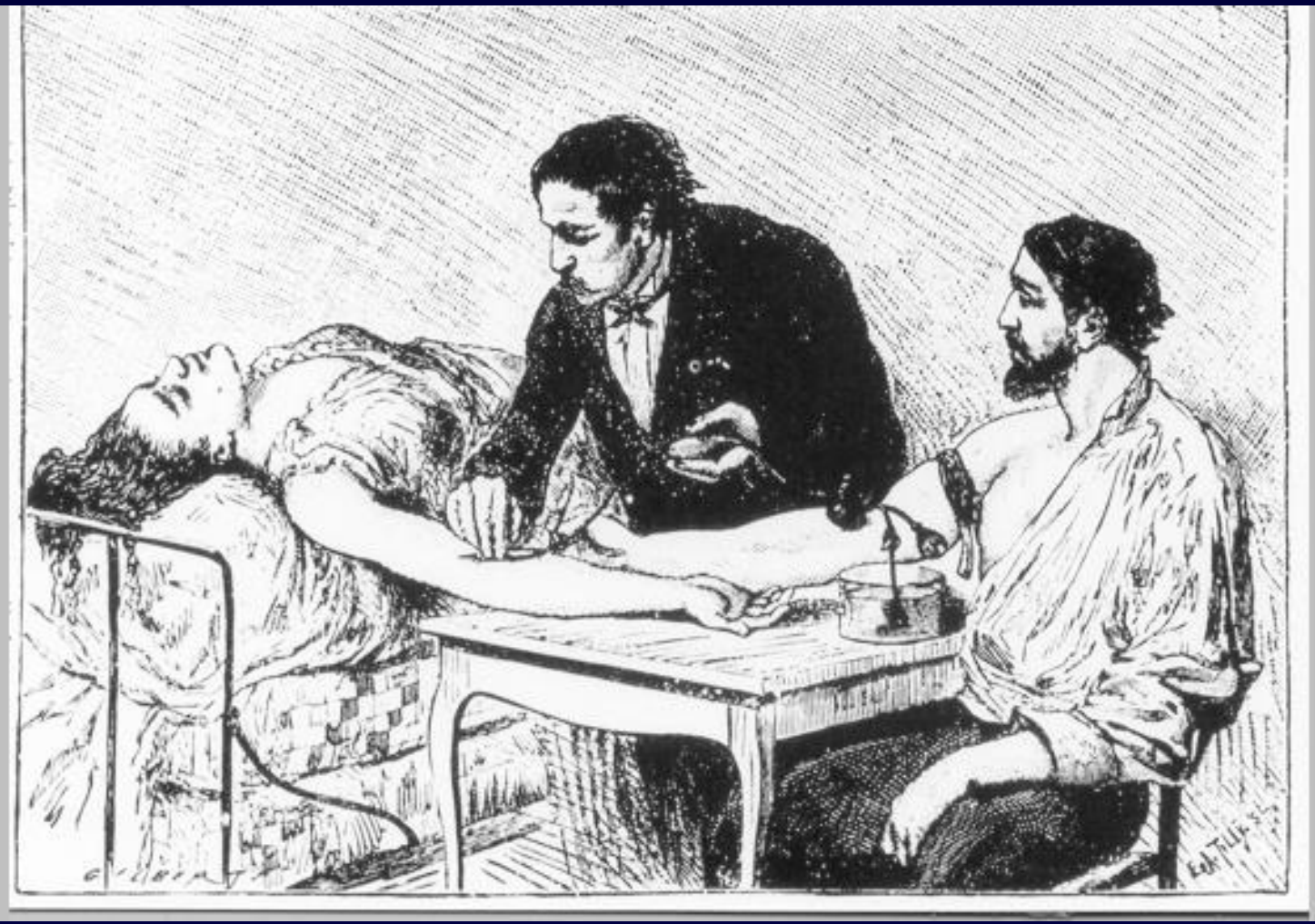
Historical Aspects of Compatibility

- **Pre-laboratory era - “compatibility by chance”**

- The efforts to substitutee blood loss by transfusion recorded centuries ago
- Because of the use of animal blood or human (but often ABO incompatible) the results were unsatisfactory (as in case of Pope Innocent VII in 1492)
- From 1667 to 1829 blood transfusions were illegal
- 1829 ... James Blundell succesfully transfused human blood in a case of postpartum haemorrhage

  .. but the results still were unpredictable and transfusions were used only as a last resort


4
3
2
1



Historical Aspects of Laboratory Compatibility Testing (1)

Overview of major contributions:

- **I.**

1900 - Landsteiner's discovery of A,B and O groups

= Beginning of Immunohaematology and Transfusion Medicine

1902 - Group AB (Decastello and Sturli)

(Independent parallel discovery of the four groups /I-II-III-IV/ by a Czech doctor Jan Jánský)

1900-1944 - Compatibility based on the knowledge of ABO status of donor and recipient and on test methods detecting „in-vitro" agglutination or haemolysis in a simple saline system

= Prevention of Fatal Transfusion Reactions - Intravascular Haemolysis Due to AB0 Incompatibility

Historical Aspects of Laboratory Compatibility Testing (2)

- **II.**

1939 - Rh system described by Levine and Stetson

= Prevention of Alloimmunization Against RhD

- **III.**

1945 - Agglutination enhancement with bovine albumin (Diamond et al)

1945 - Antiglobulin Test (Coombs et al)

1947 - Enzyme Test (Morton and Pickles)

1974 - LISS antigen- antibody interaction enhancement (Low and Messeter)

= Prevention of „In Vivo“ Red Cell Destruction Caused by Incomplete (IgG) Antibodies

Historical Aspects of Laboratory Compatibility Testing (3)

- IV

Last two decades:

- attempts to increase the sensitivity of serologic methods

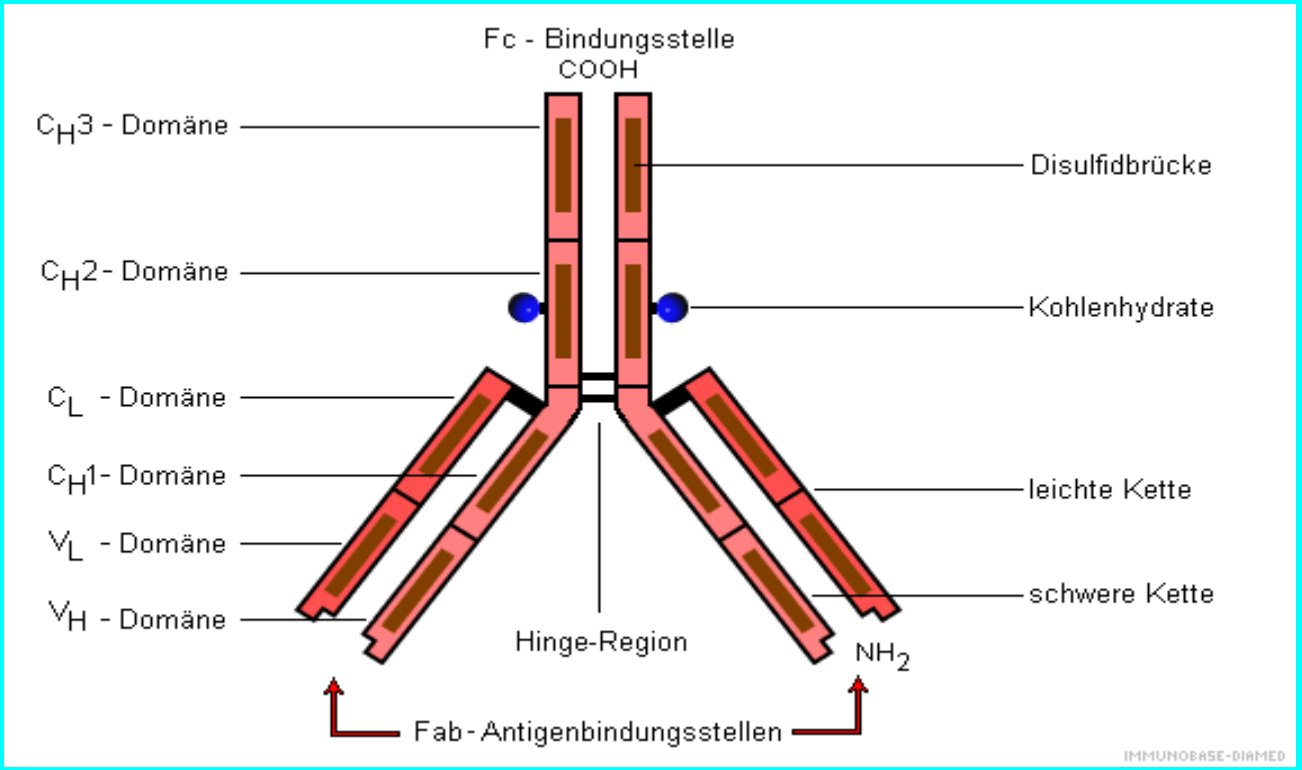
1980 - Lalezari et al. - Polybrene Test

1984 - Plapp et al. - Solid Phase Test

1987 - Nance and Garratty - Polyethylene-Glycol (PEG) Test

1990 - Lapierre et al.: Gel Agglutination Test

= Increased Sensitivity, Reproducibility and Reliability of Serologic Methods

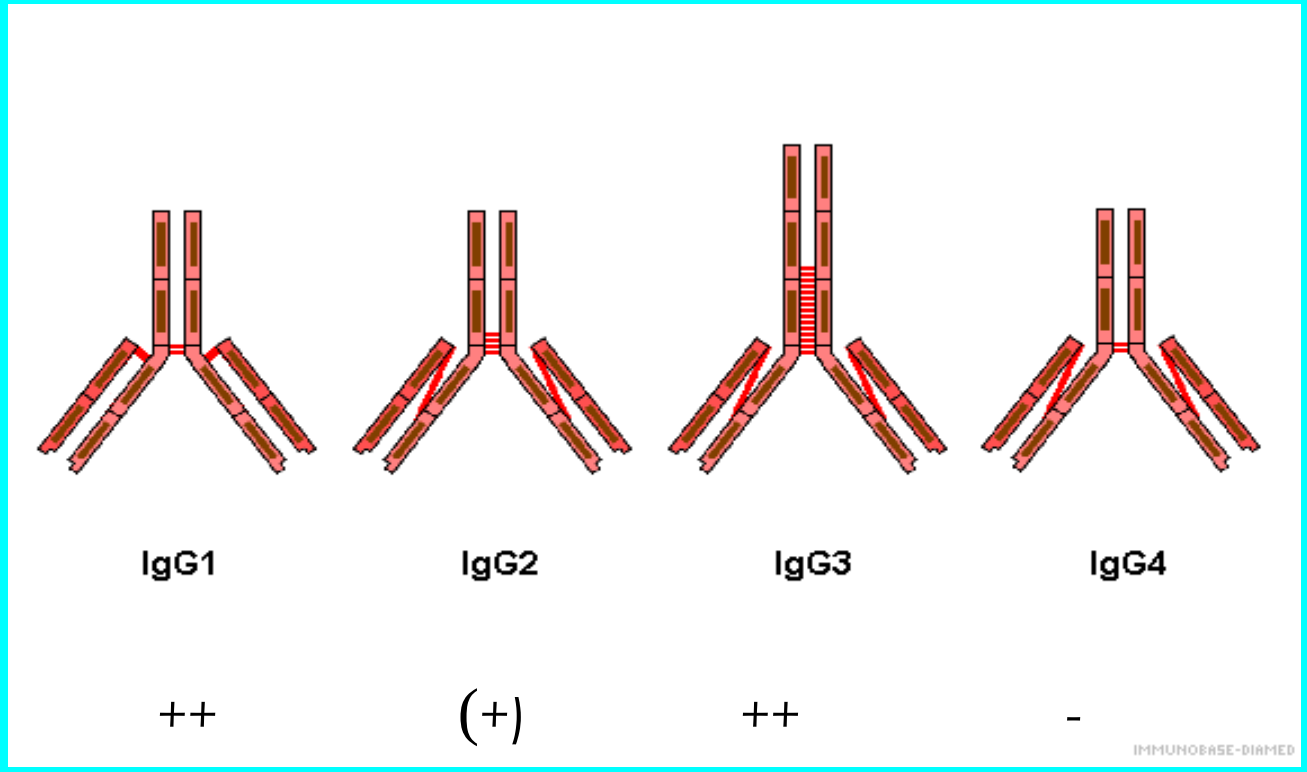


CLASS IgG - 80-90% of imunoglobulins in serum (7-17g/l)

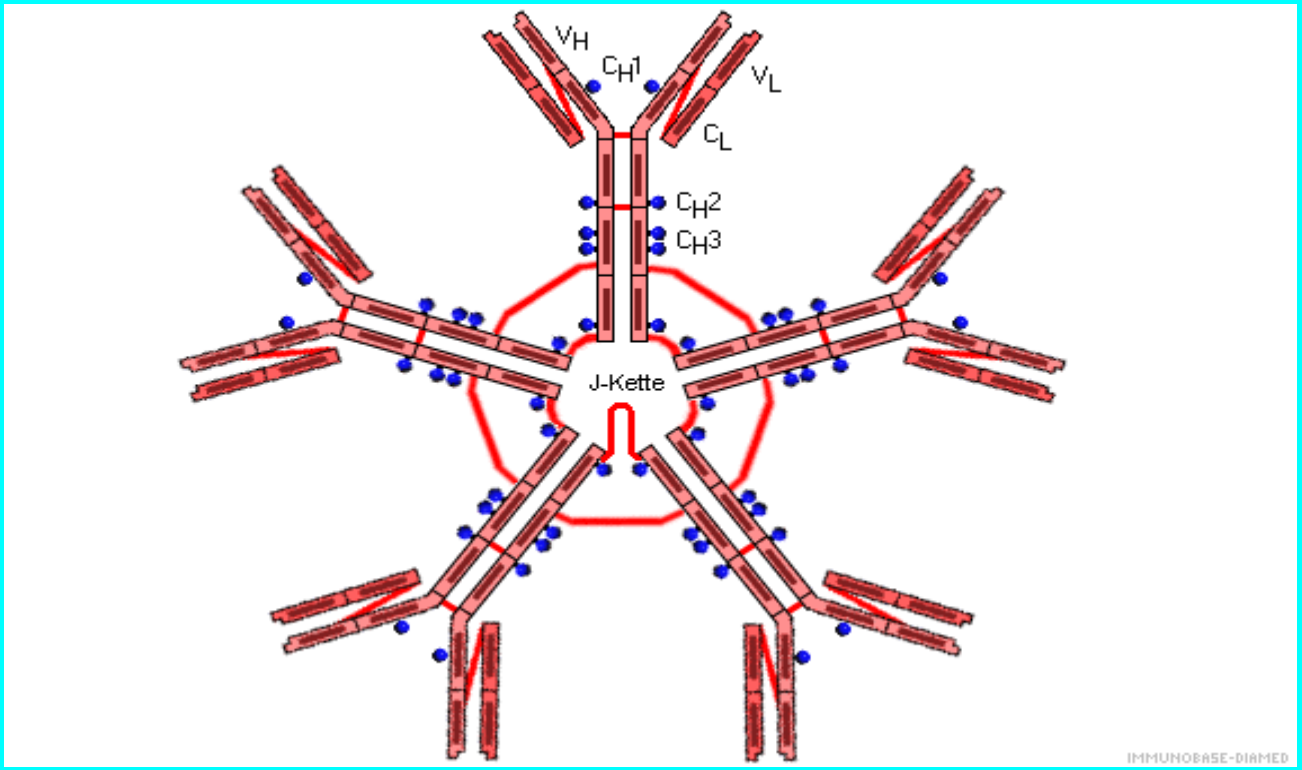
- crossing placenta

- fixing complement (except of interaction with Rh antigens)

Subclasses of IgG



Efficiency of fagocytosis induction and complement fixation

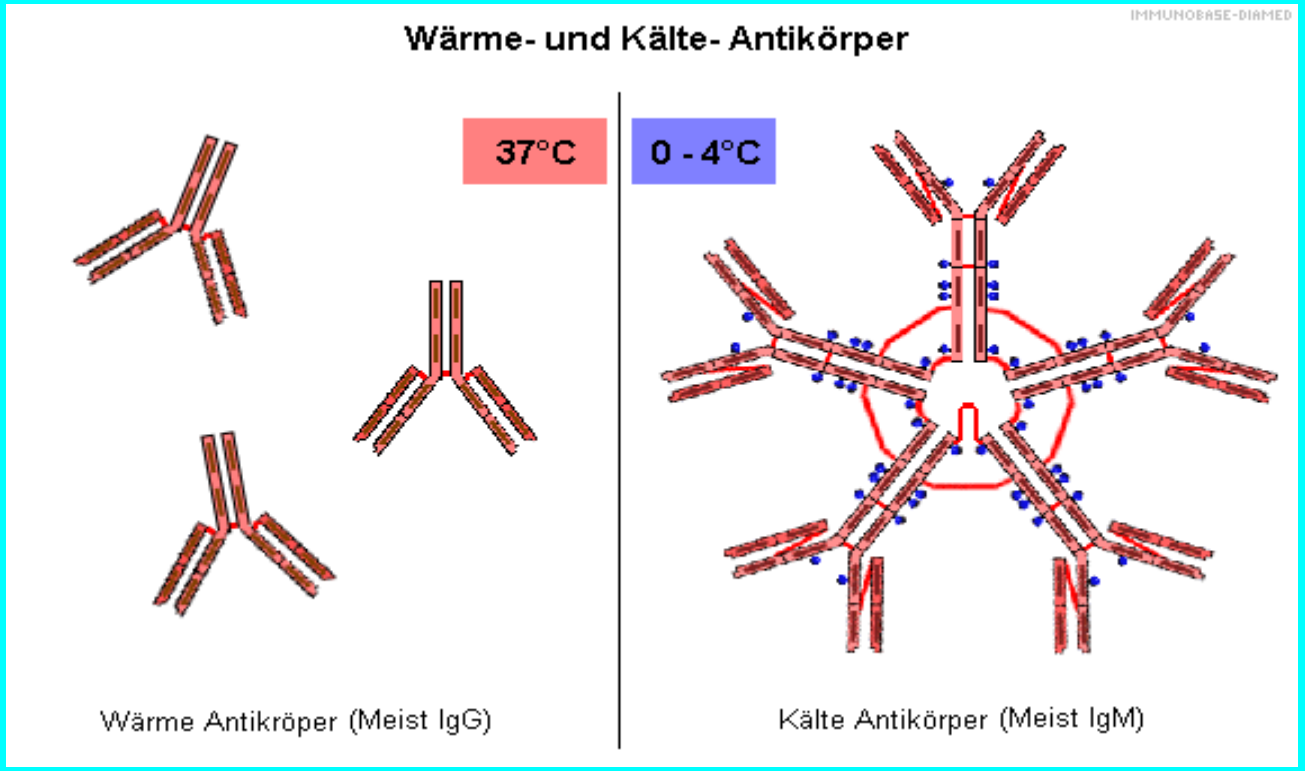


CLASS IgM - serum concentration: 1-2,8 g/l

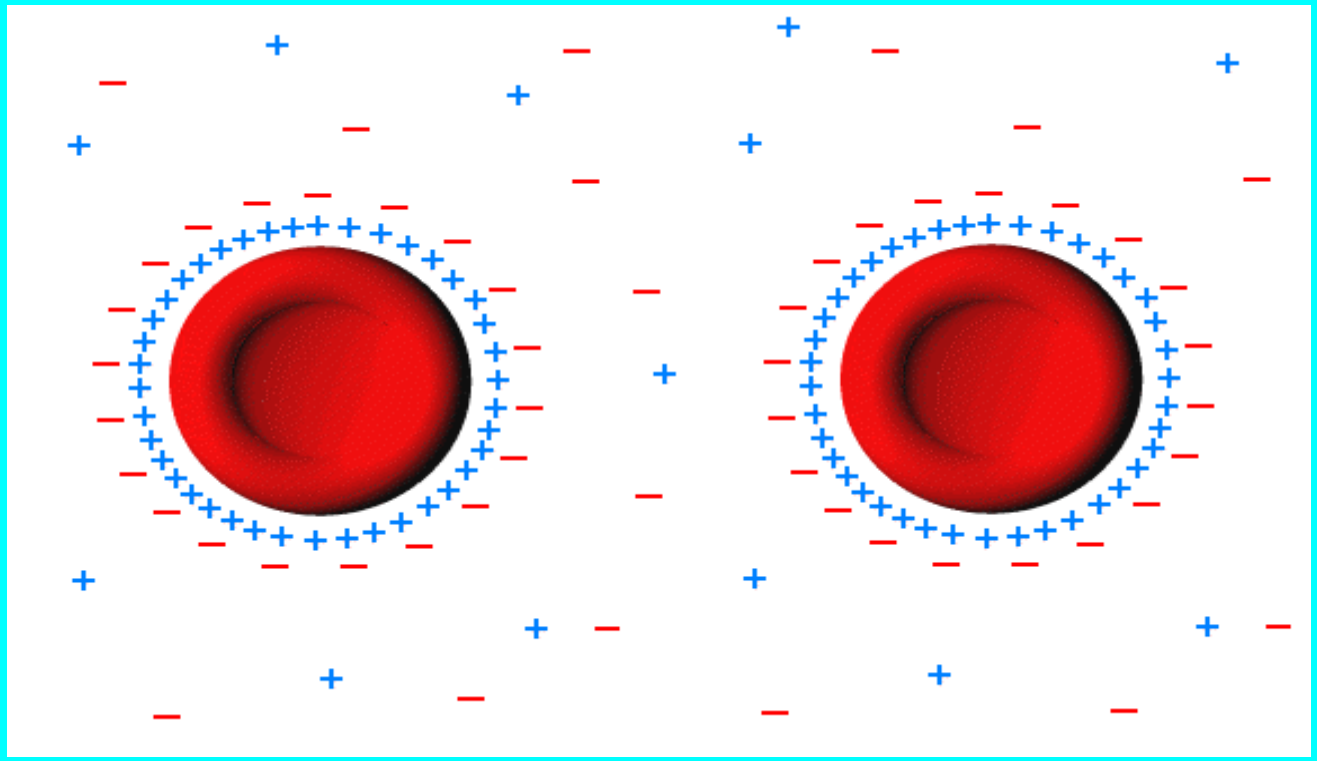
- fixing complement strongly (up to MAC)

- does not cross placenta

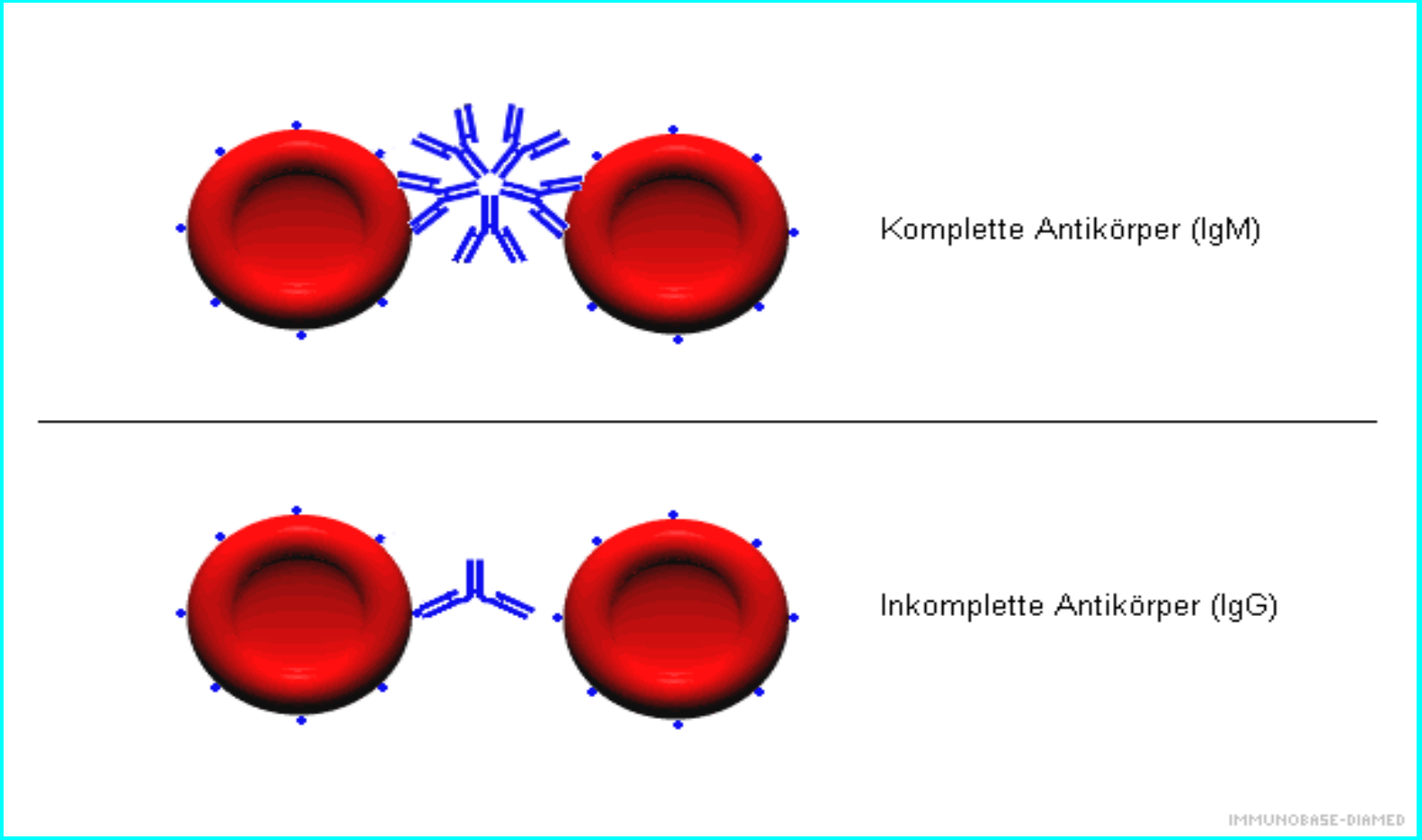
„Warm“ and „Cold“ Antibodies



RBC surface is negatively charged

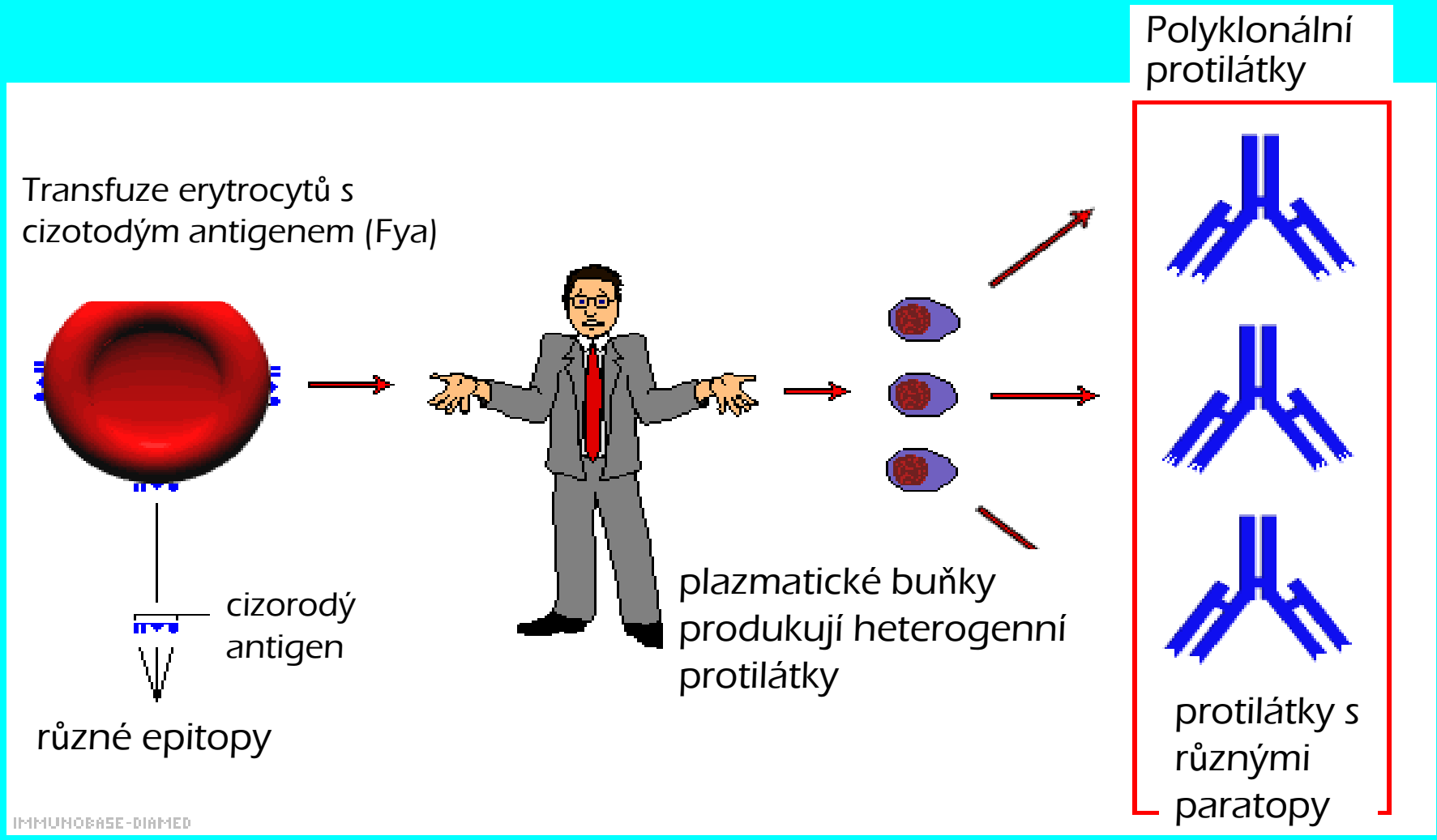


Complete antibody (IgM) - causing agglutination of erythrocytes

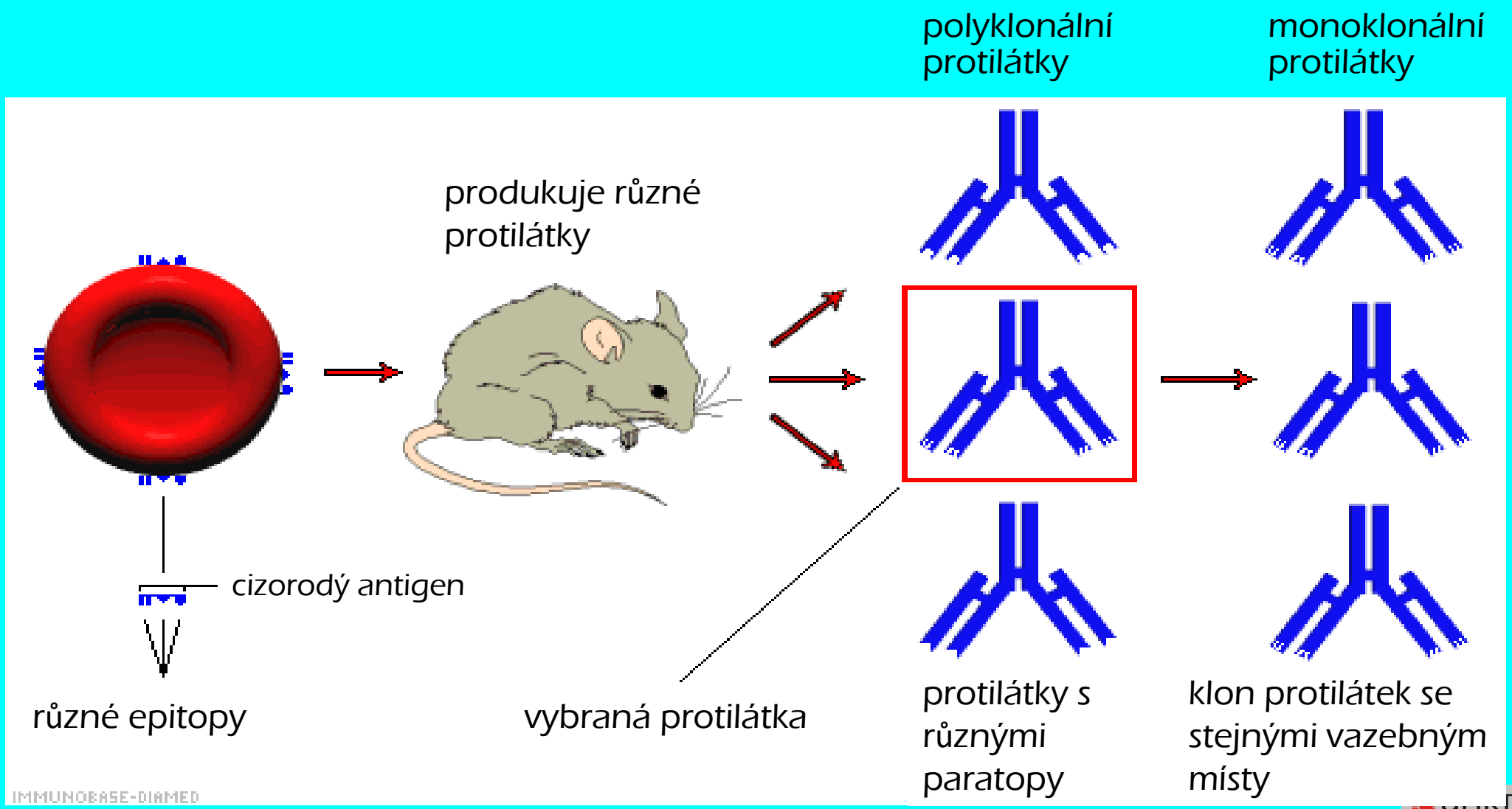


Incomplete antibody (IgG)- not able to agglutinate erytrocytes

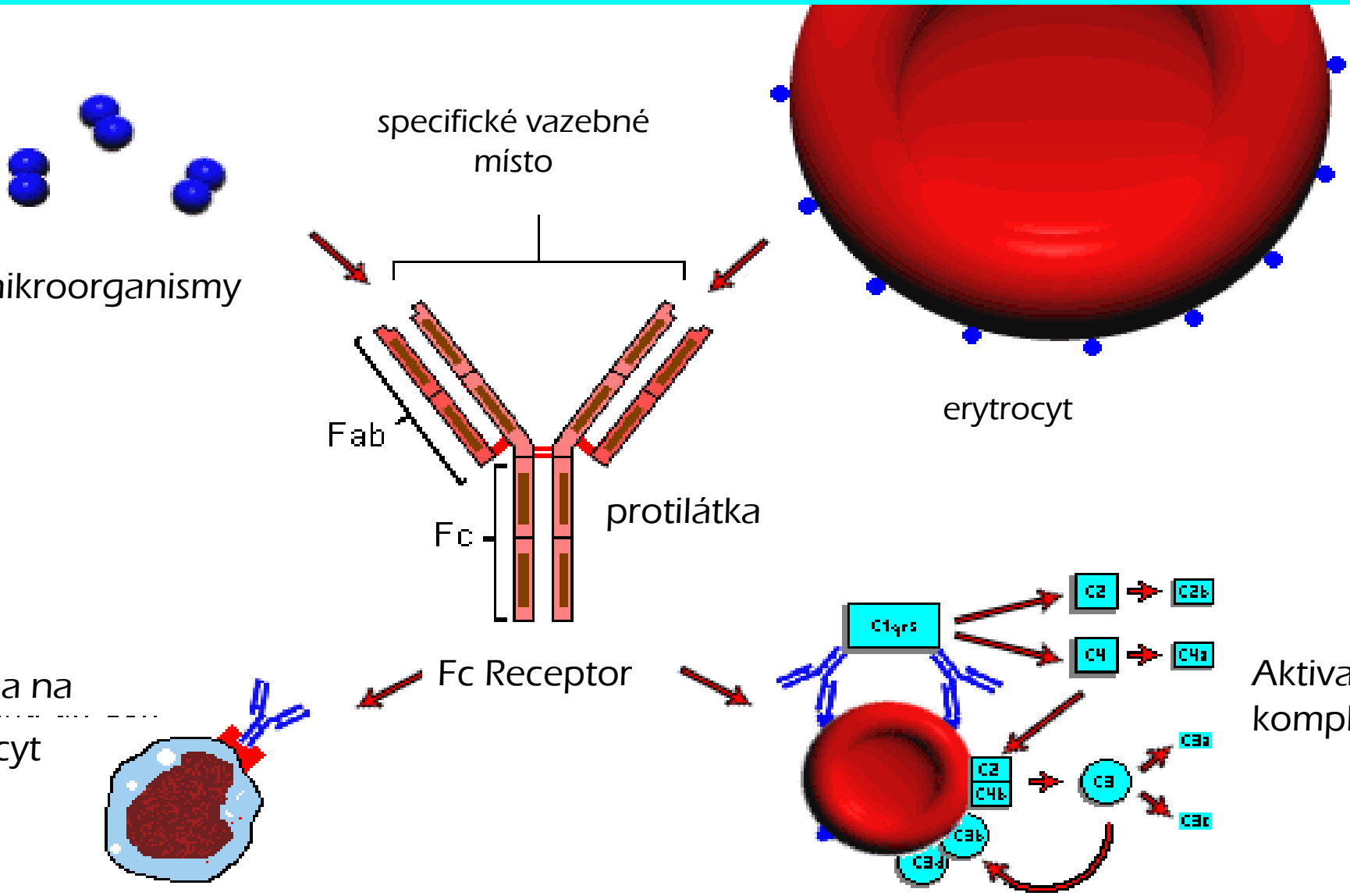
Polyclonal antibodies



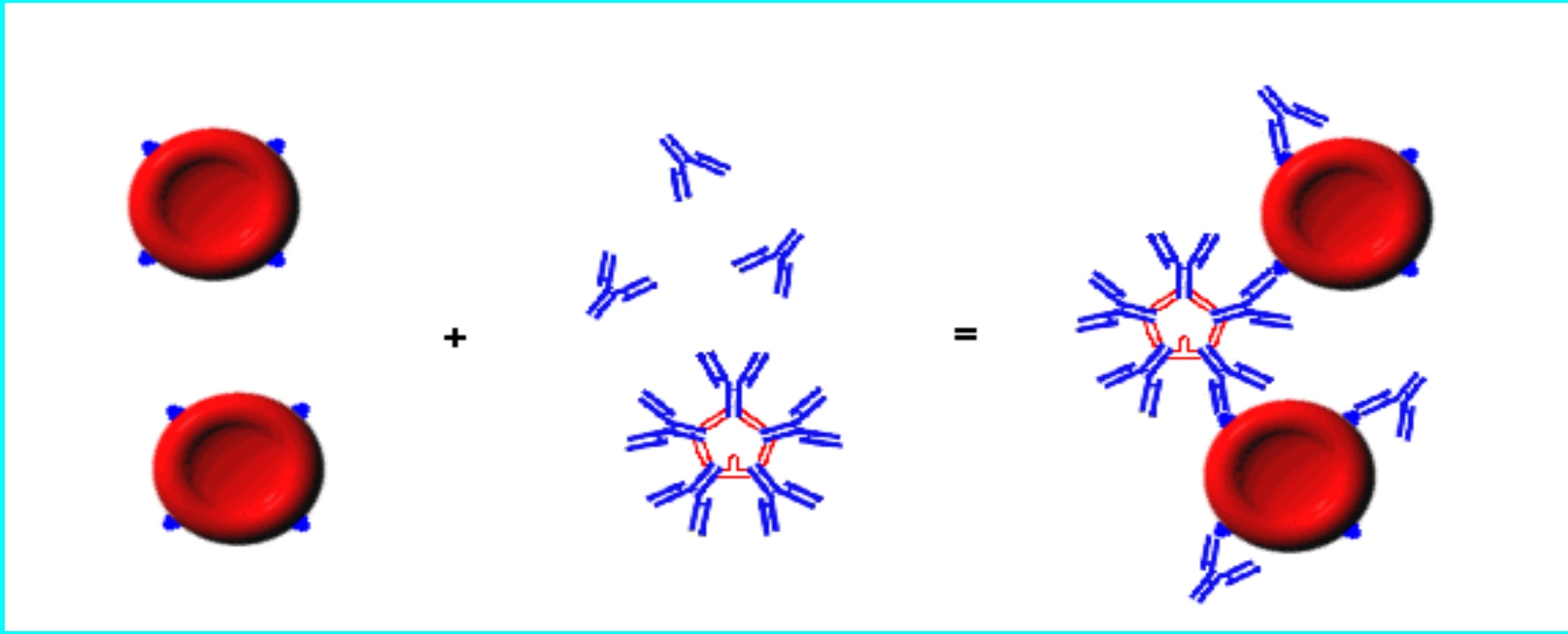
Monoclonal antibodies



Antibody - functions



Antigen - antibody reactions



Erytrocyt s antigenem

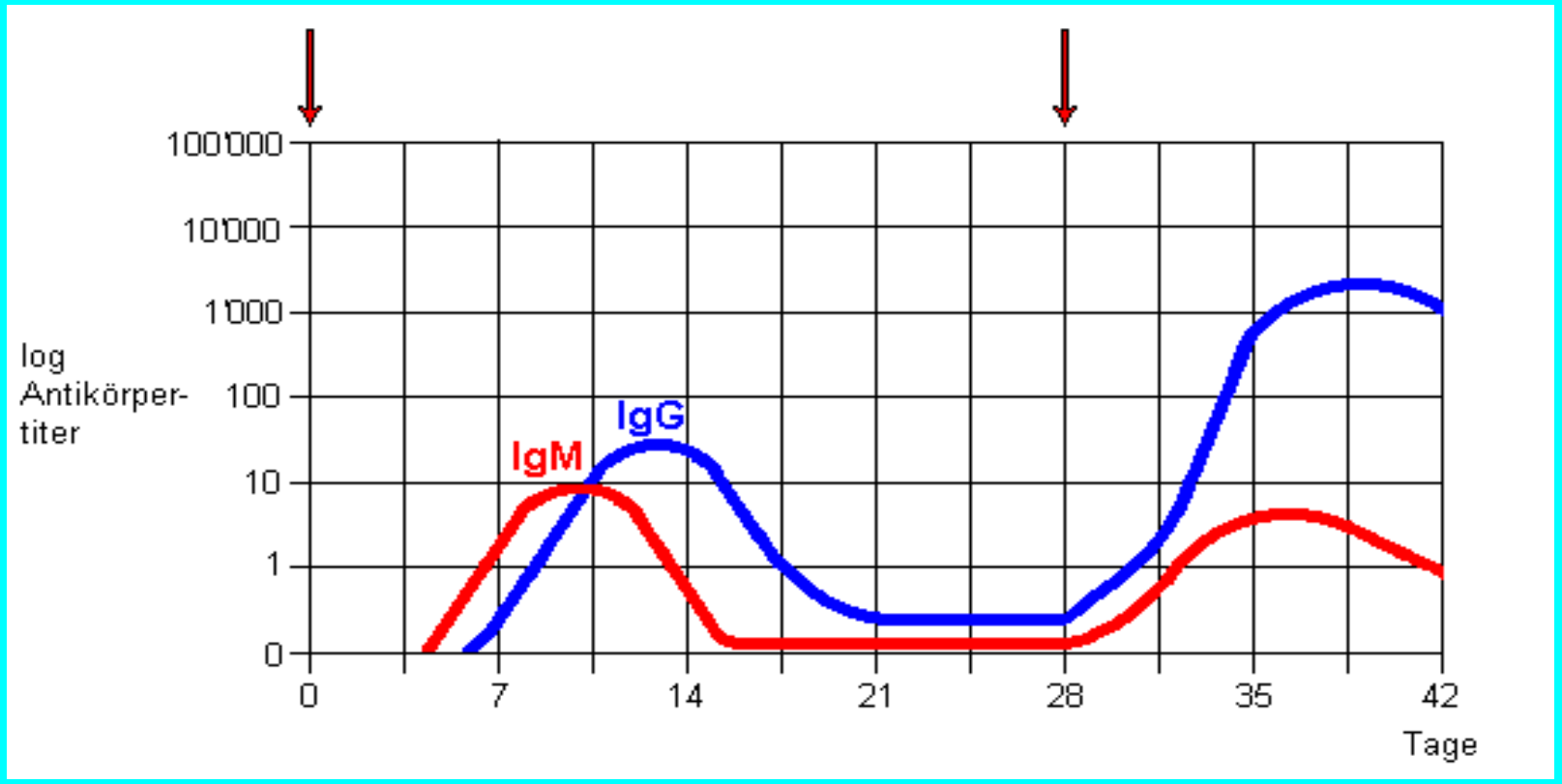
Protilátka proti antigenu

- vazba na erytrocyt
- aglutinace erytrocytů
- ev. aktivace komplementu

Primary and secondary antibody response

First antigen presentation

Second antigen expozition



Methods in erythrocyte immunohematology

- serologic tests
  - antibody detection in serum/plasma
  - antigen detection (phenotyping)

- DNA techniques - genotyping

Serologic tests

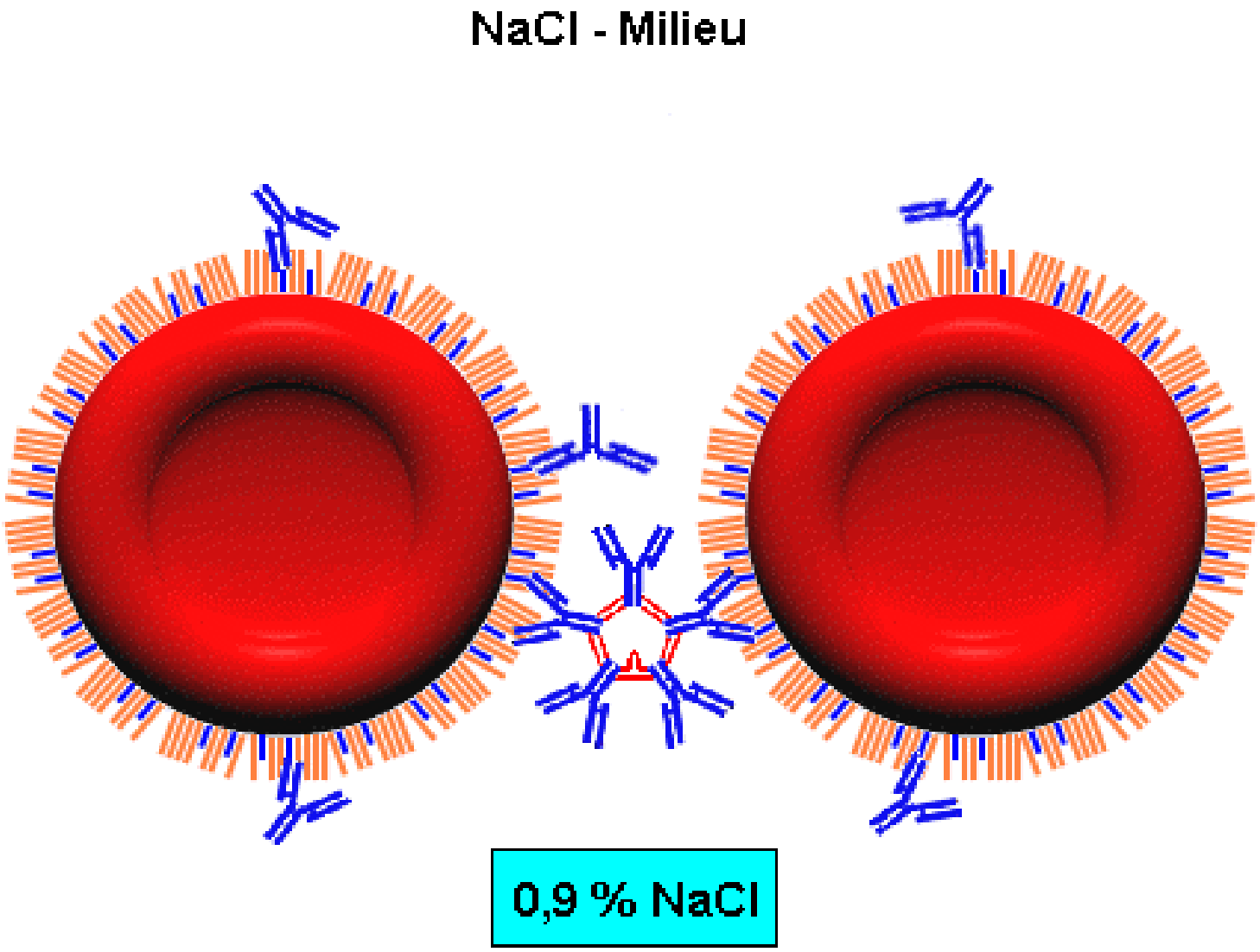
- saline test („direct agglutination“ test)
  - IgM
- Coombs test (Antiglobulin Test)
  - IgG
  - reactions with secondary antibody (AGH) = anti-IgG + anti- C3d

  - direct
  - indirect
- enzyme test

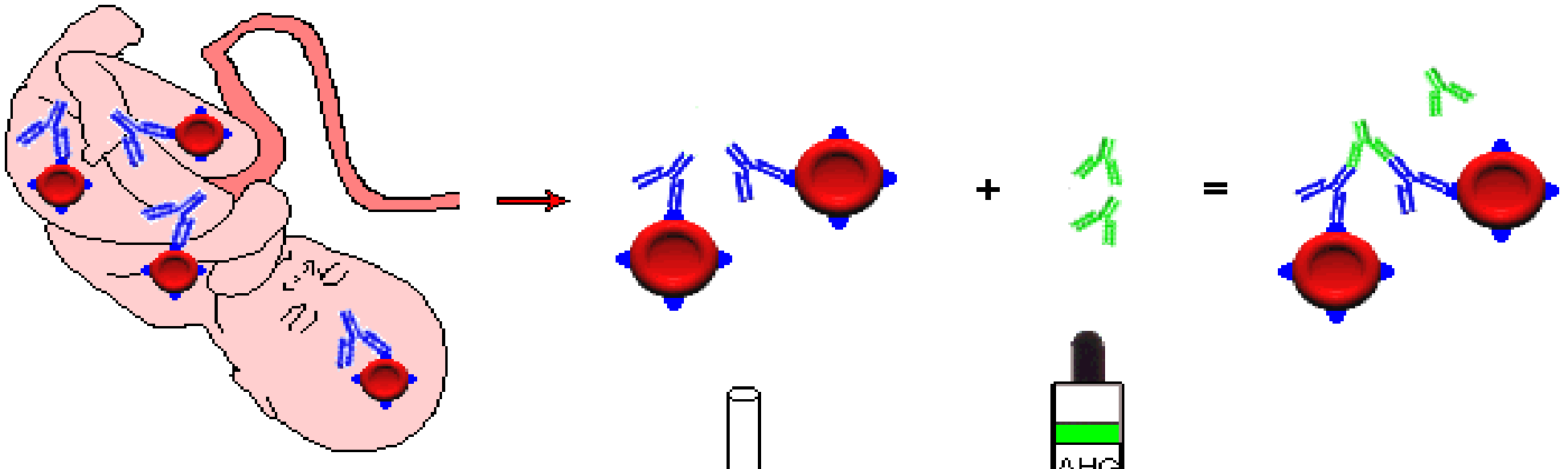
Increasing of sensitivity of IgG tests

- LISS (Low Ionic Strenght Solution)
- polyethylene-glycol (PEG)

Saline test



Direct Coombs test (DAT)



MhN des Kindes

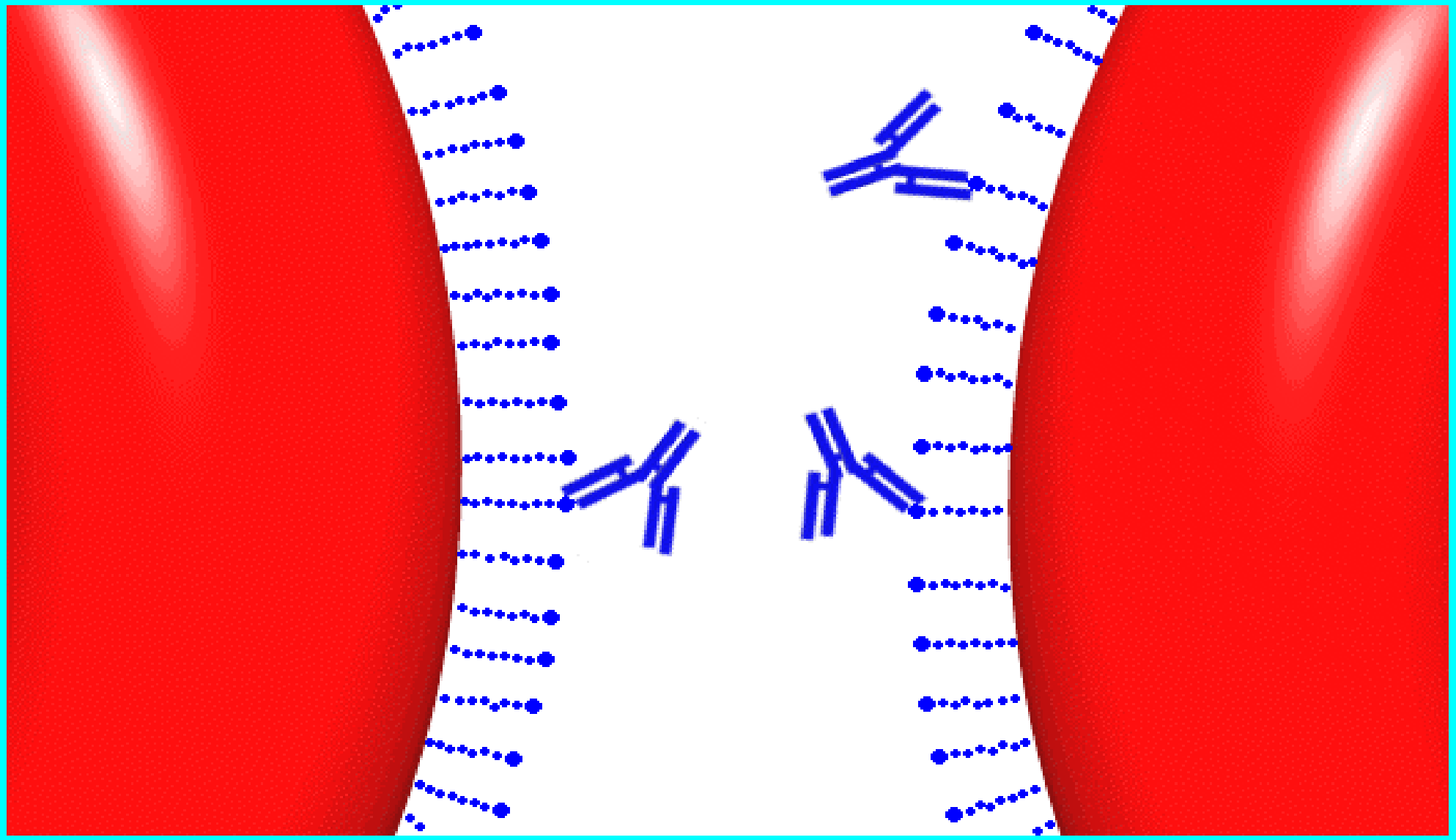
In vivo Beladung der Erythrozyten durch mütterliche Allo-Antikörper

Blutprobe
3x ausgewaschen

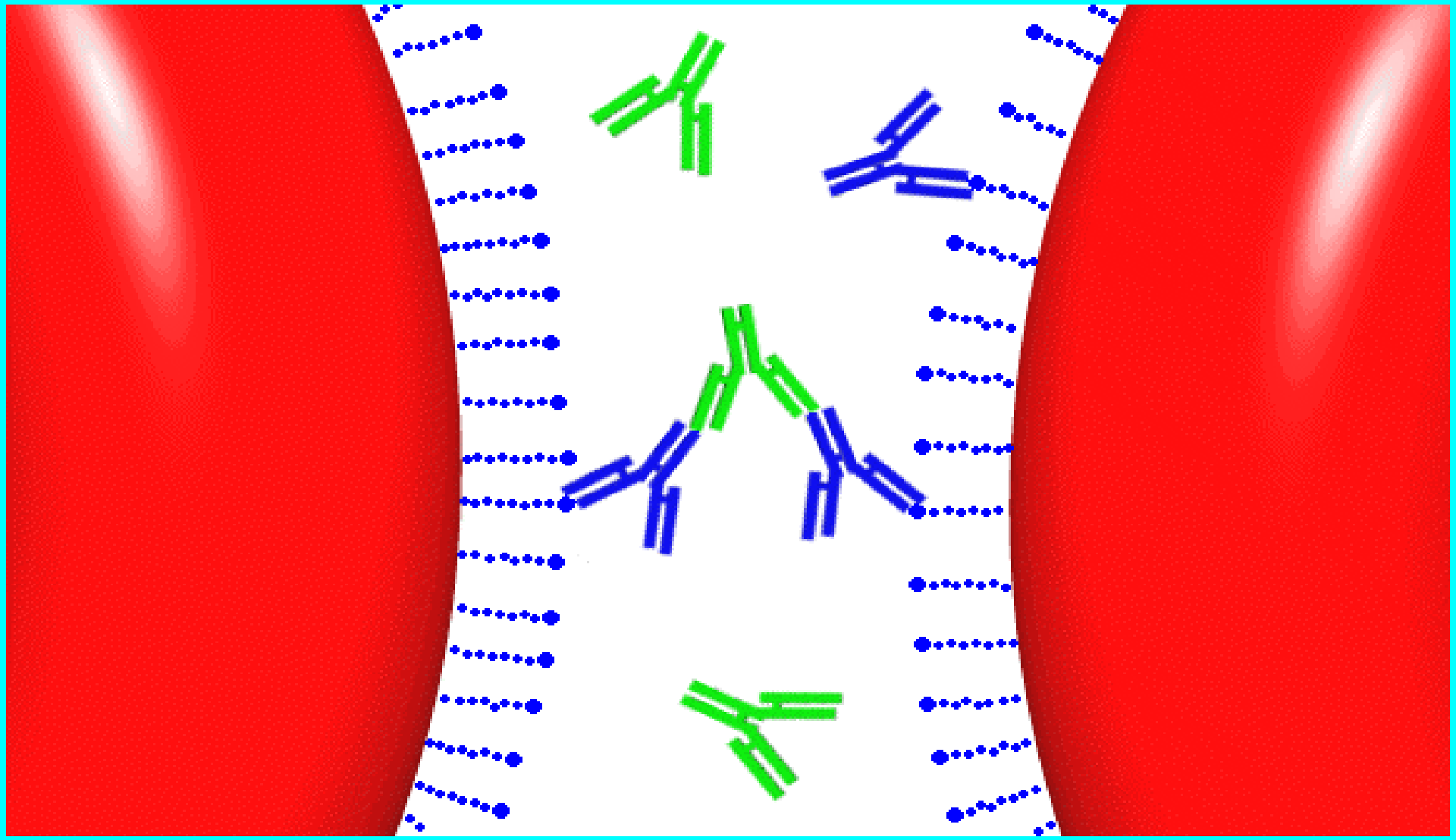
AHG-Serum

Agglutination

Direct Coombs test (DAT)



Direct Coombs test (DAT)



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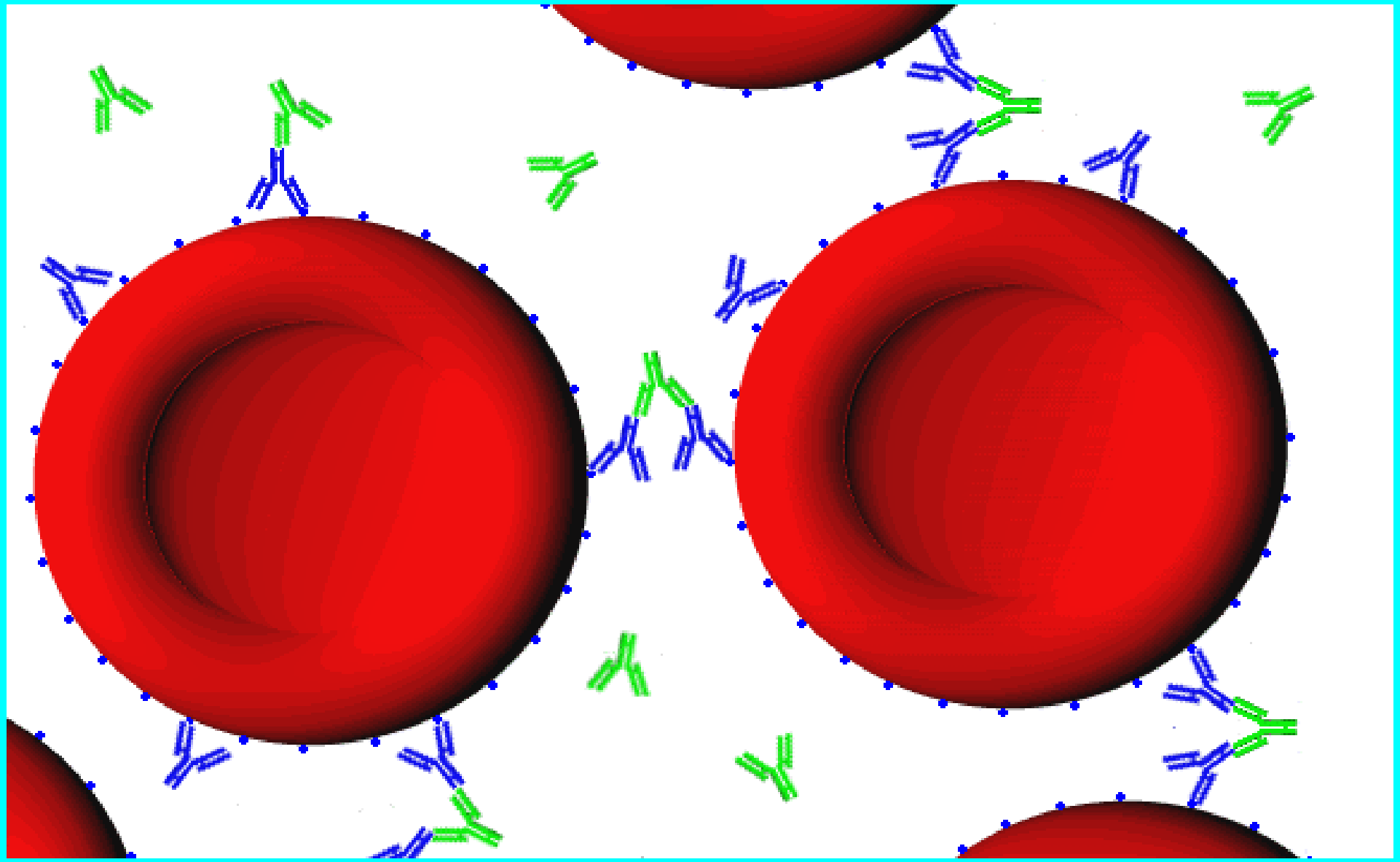
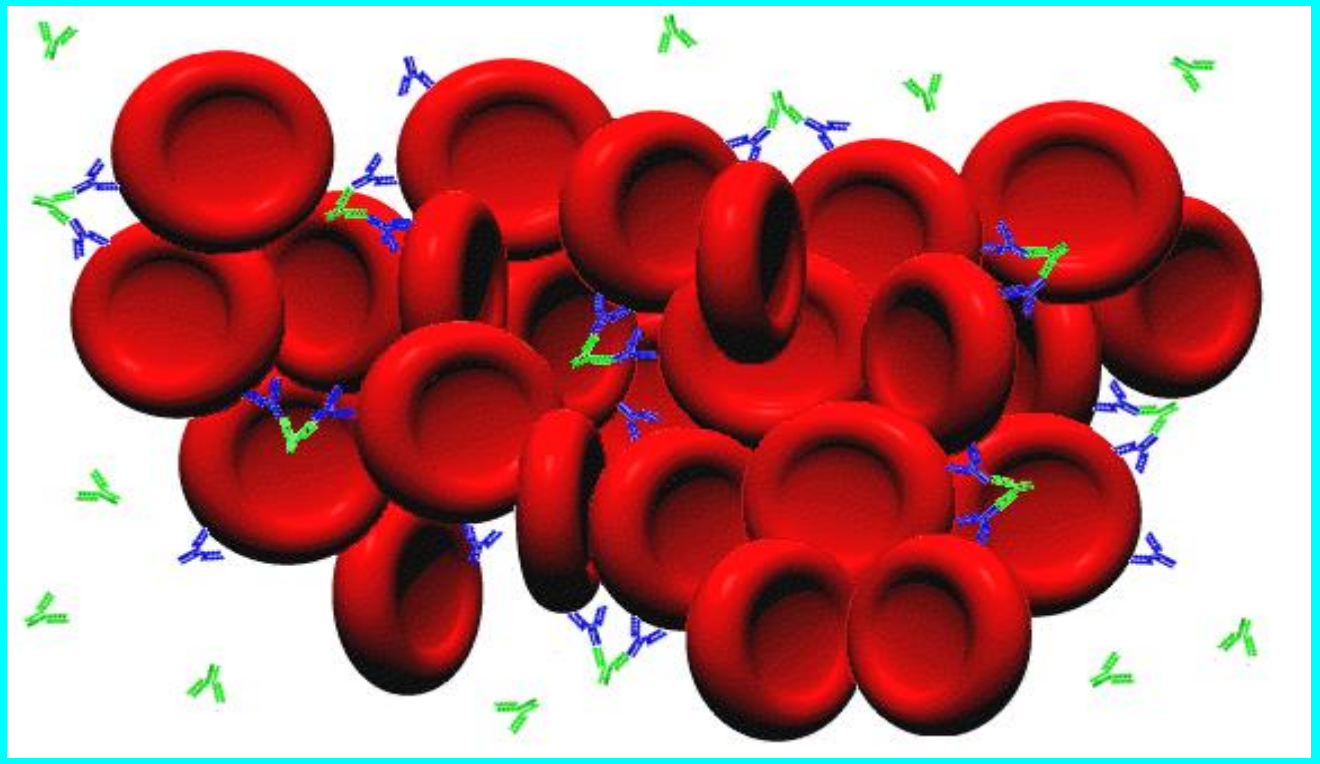
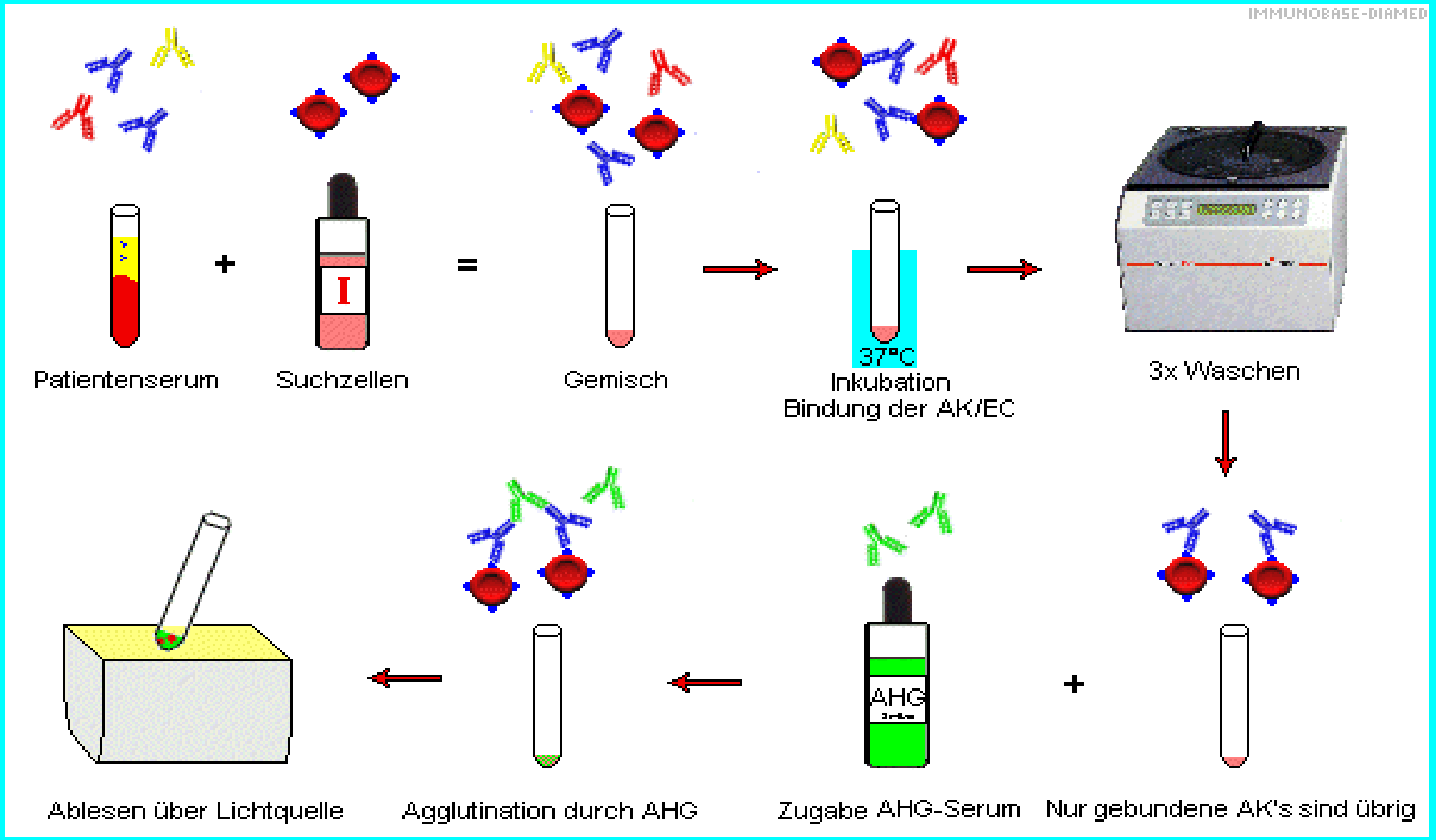


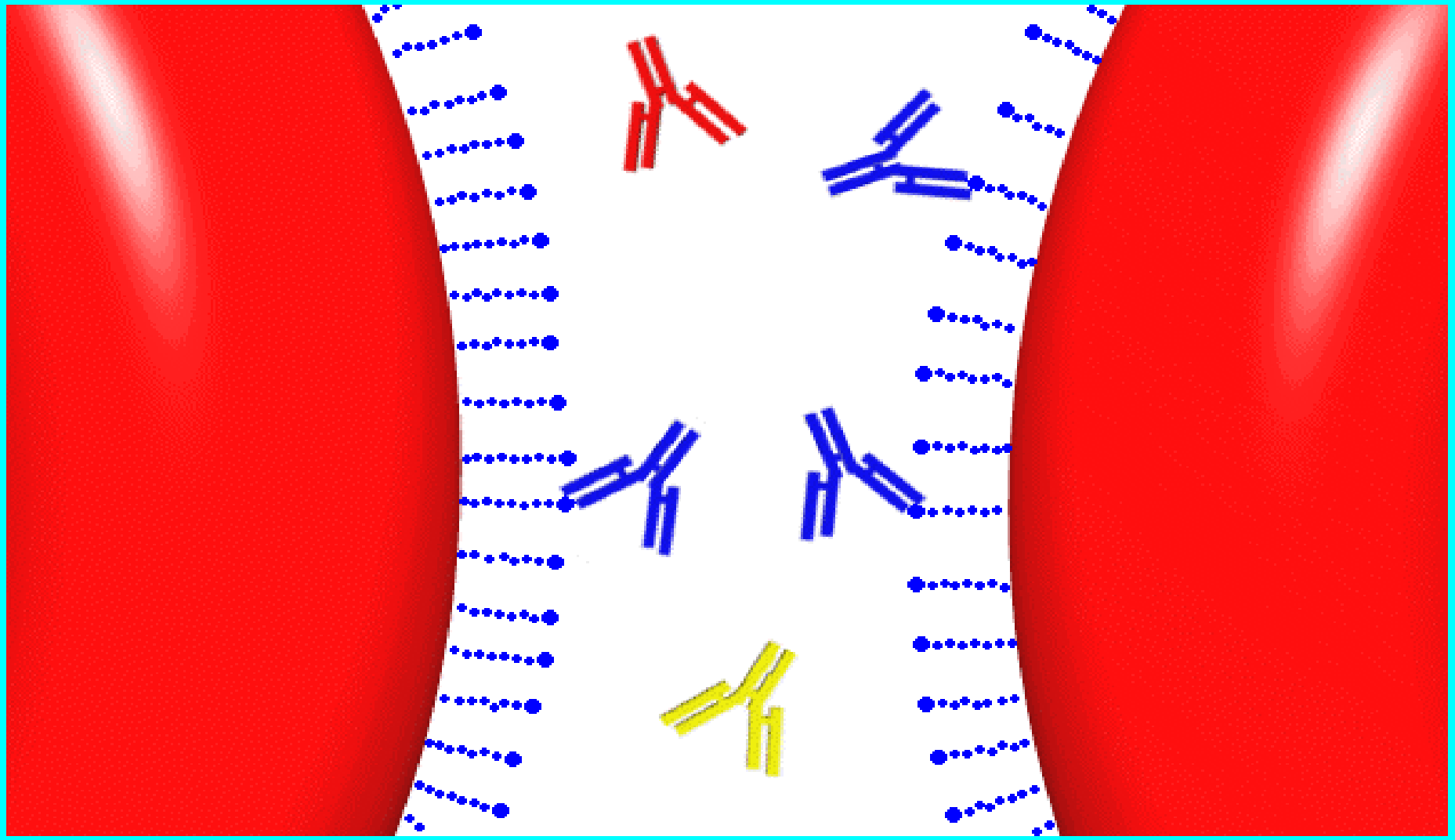
Direct Coombs test (DAT)



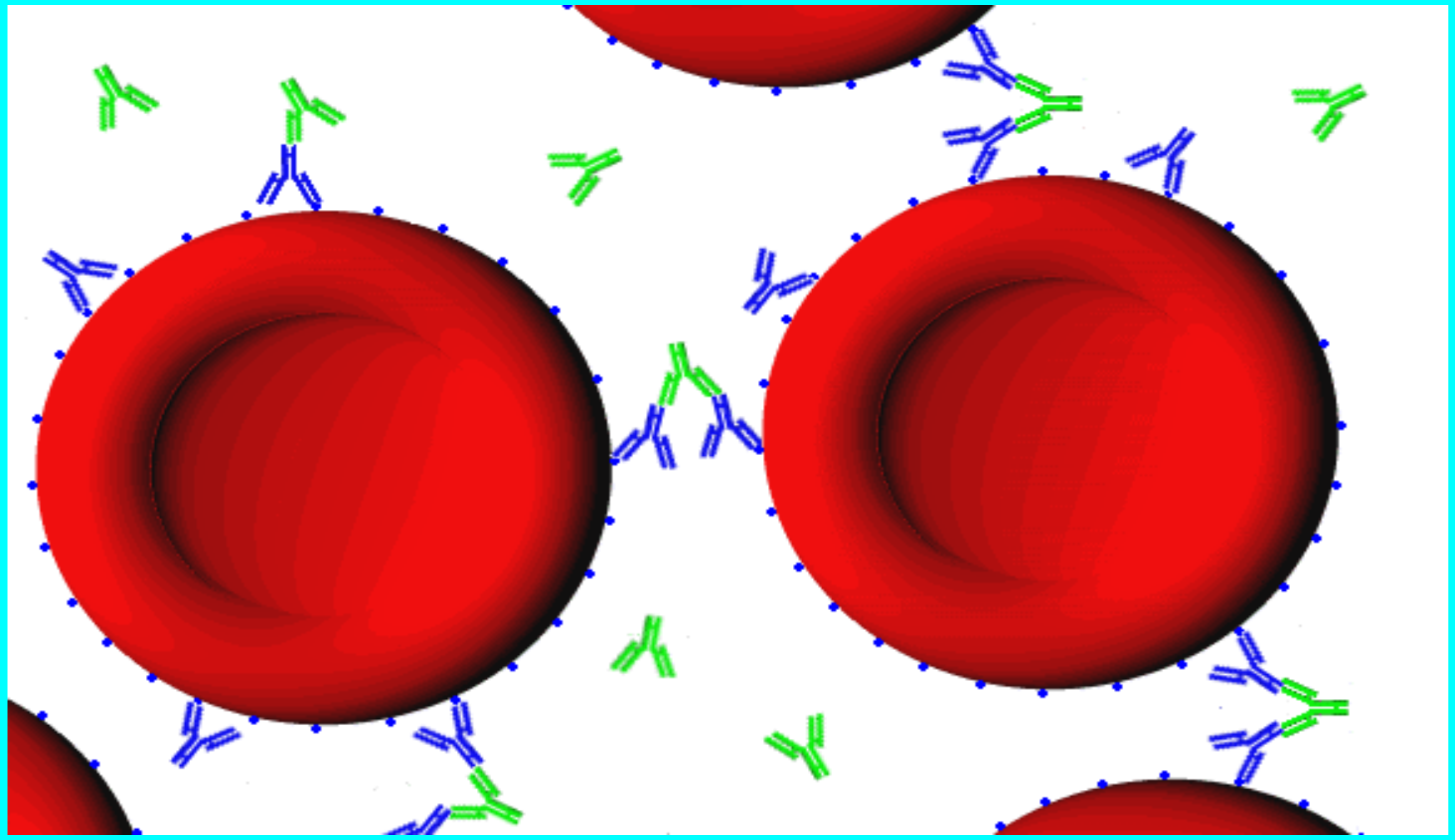
Indirect Coombs test



Indirect Coombs test (IAT)

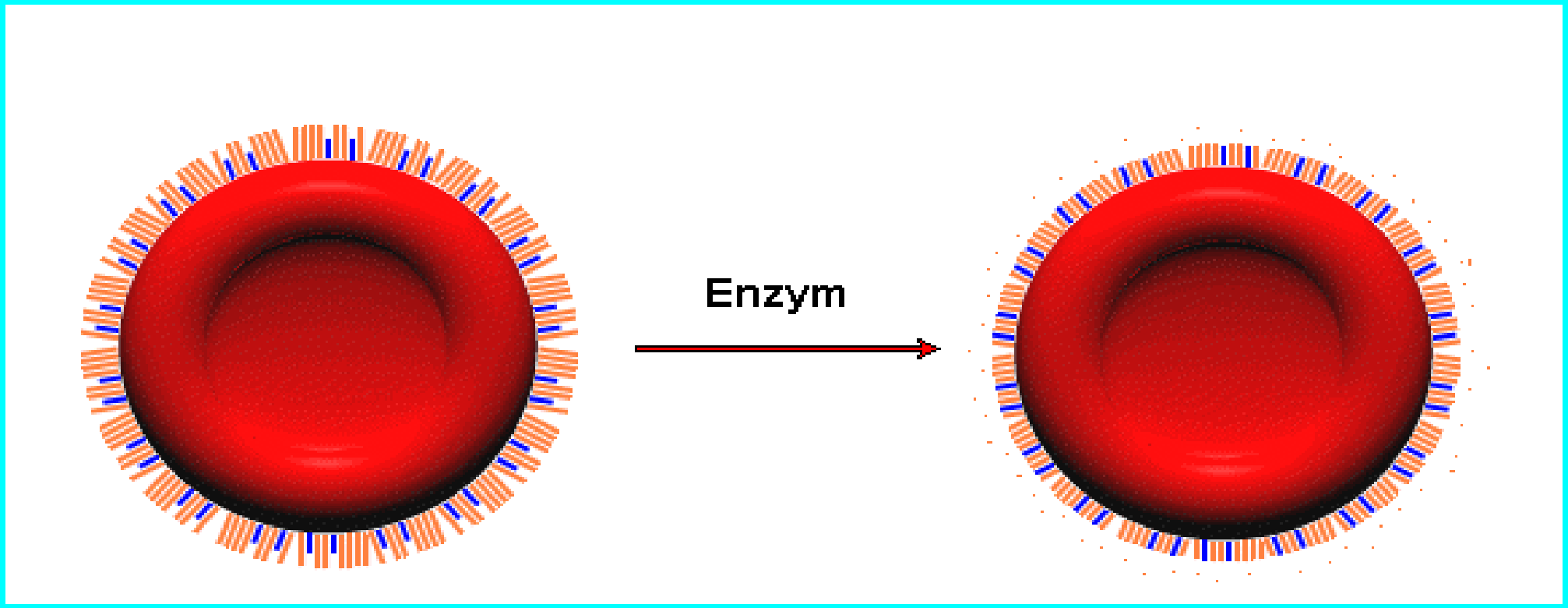


Indirect Coombs test (IAT)

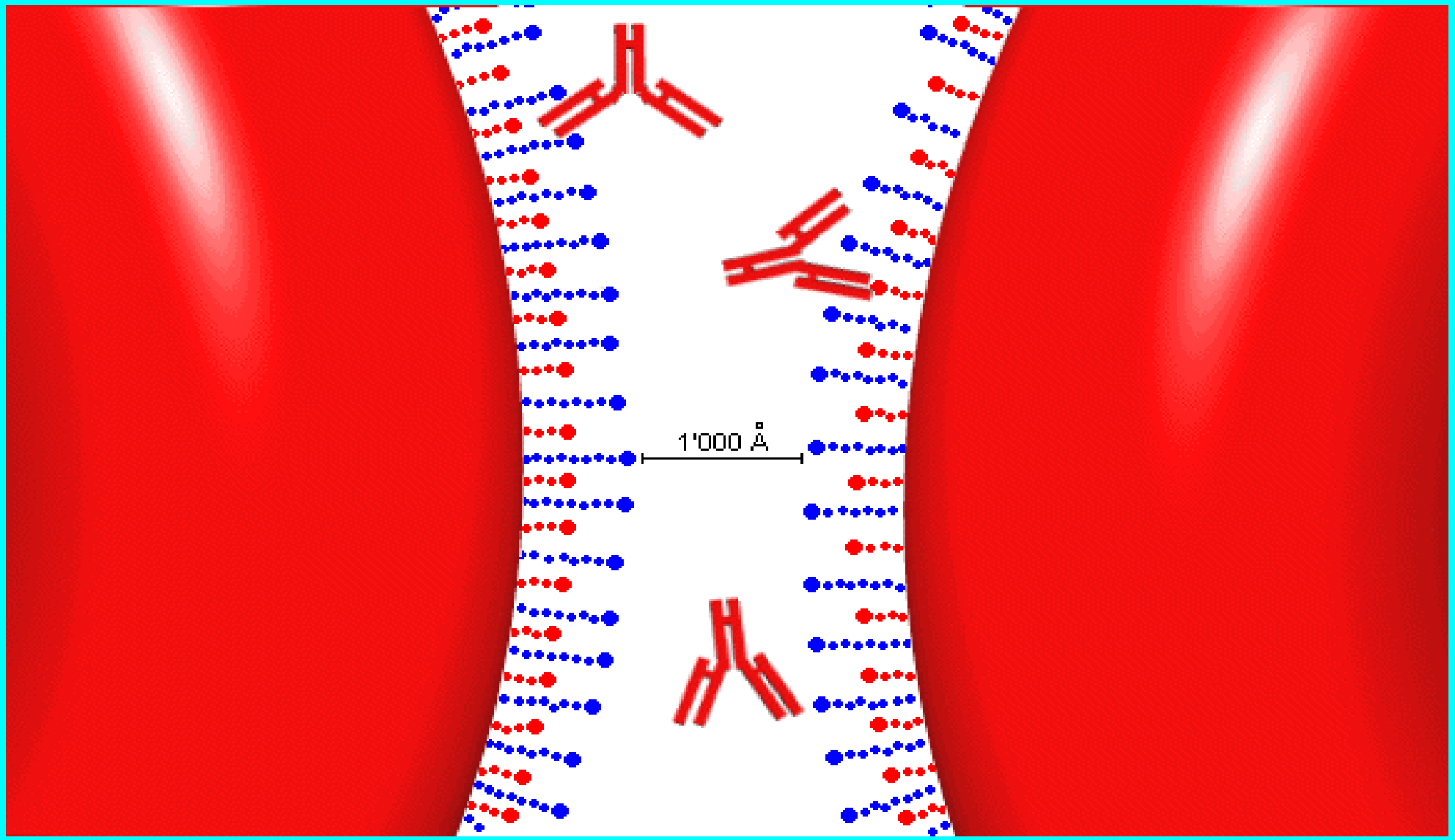


Enzyme test

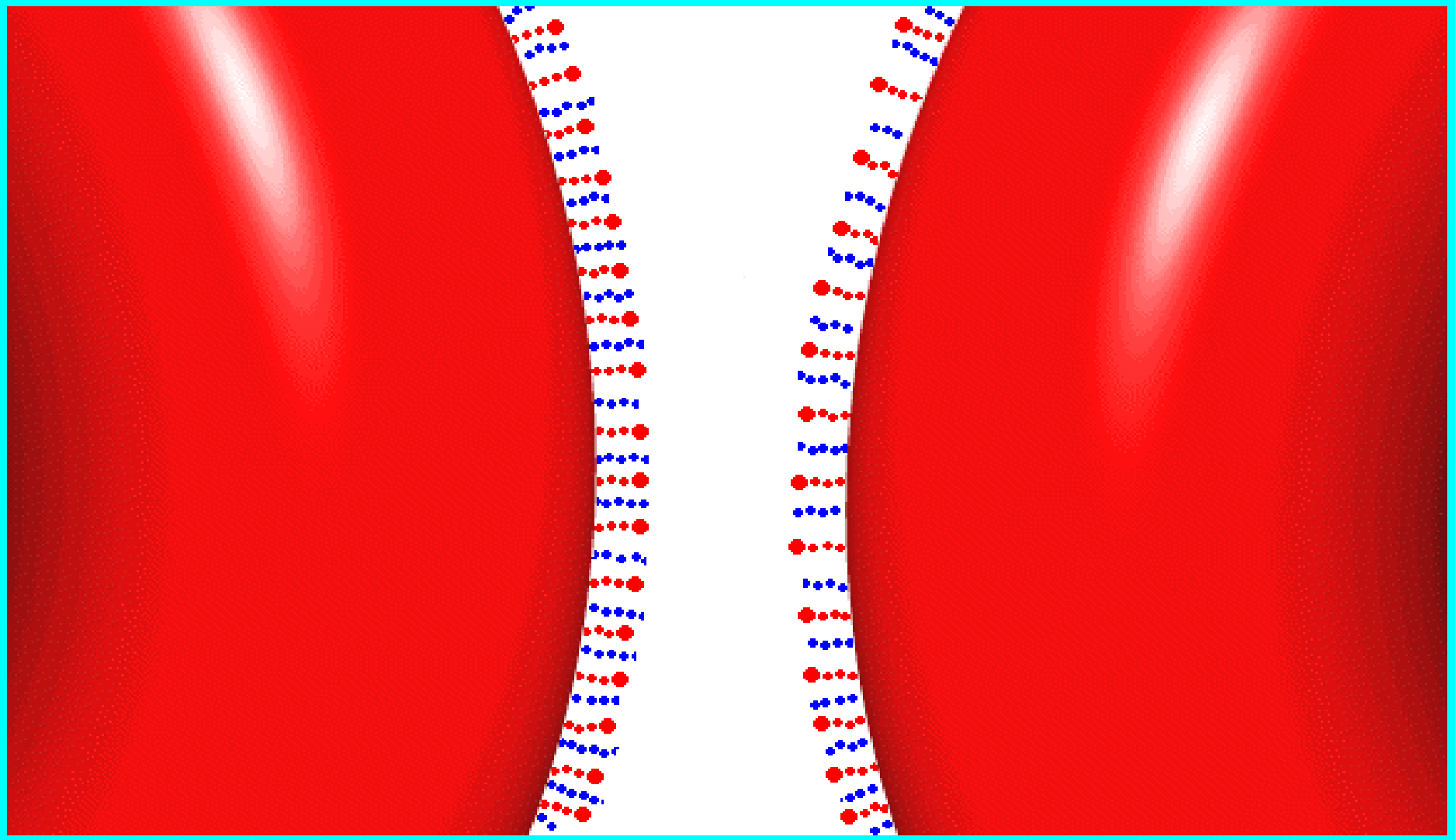
- protease treatment (bromelin, papain, ficin)
- decrease of negative charge of the cell membrane
- destruction of some antigens (MNSs, Duffy)



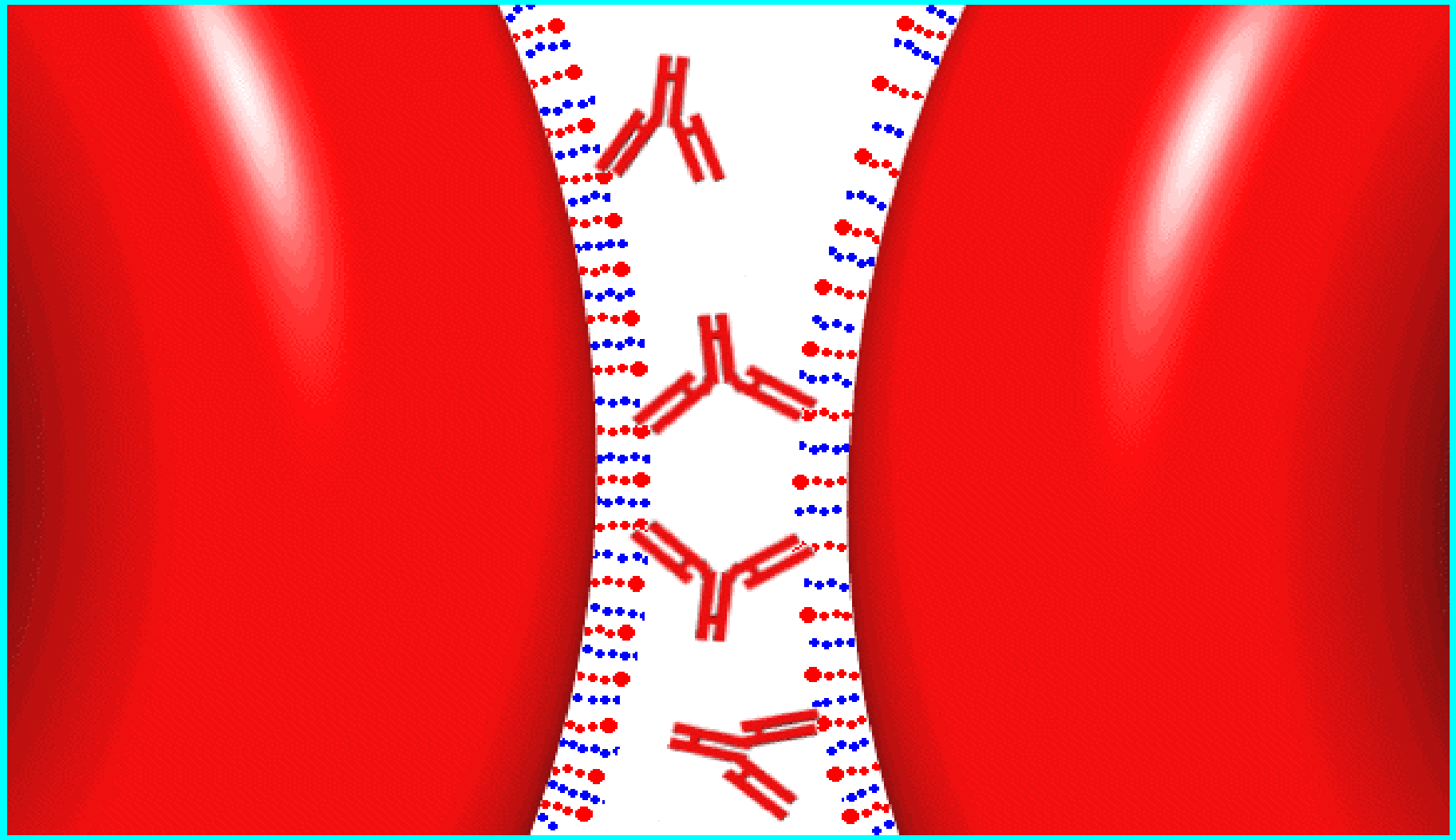
Enzyme test



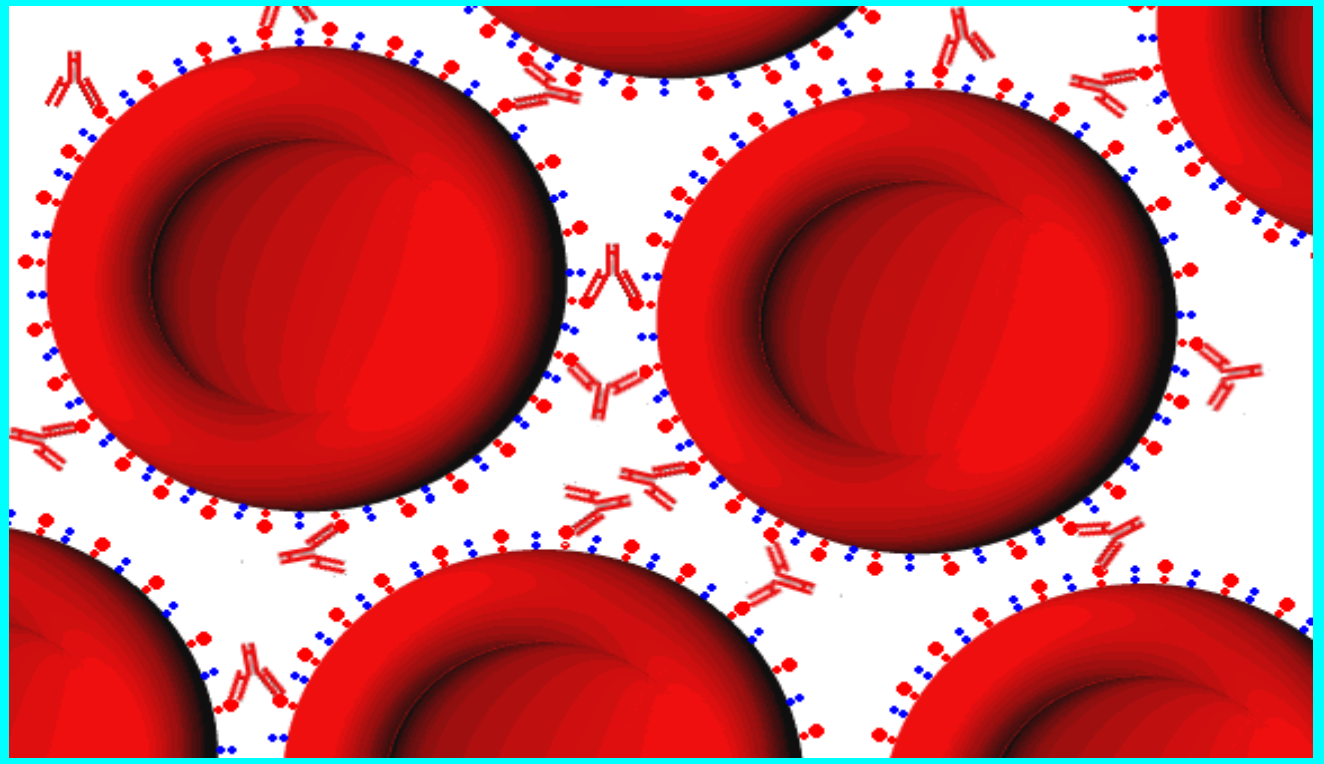
Enzyme test



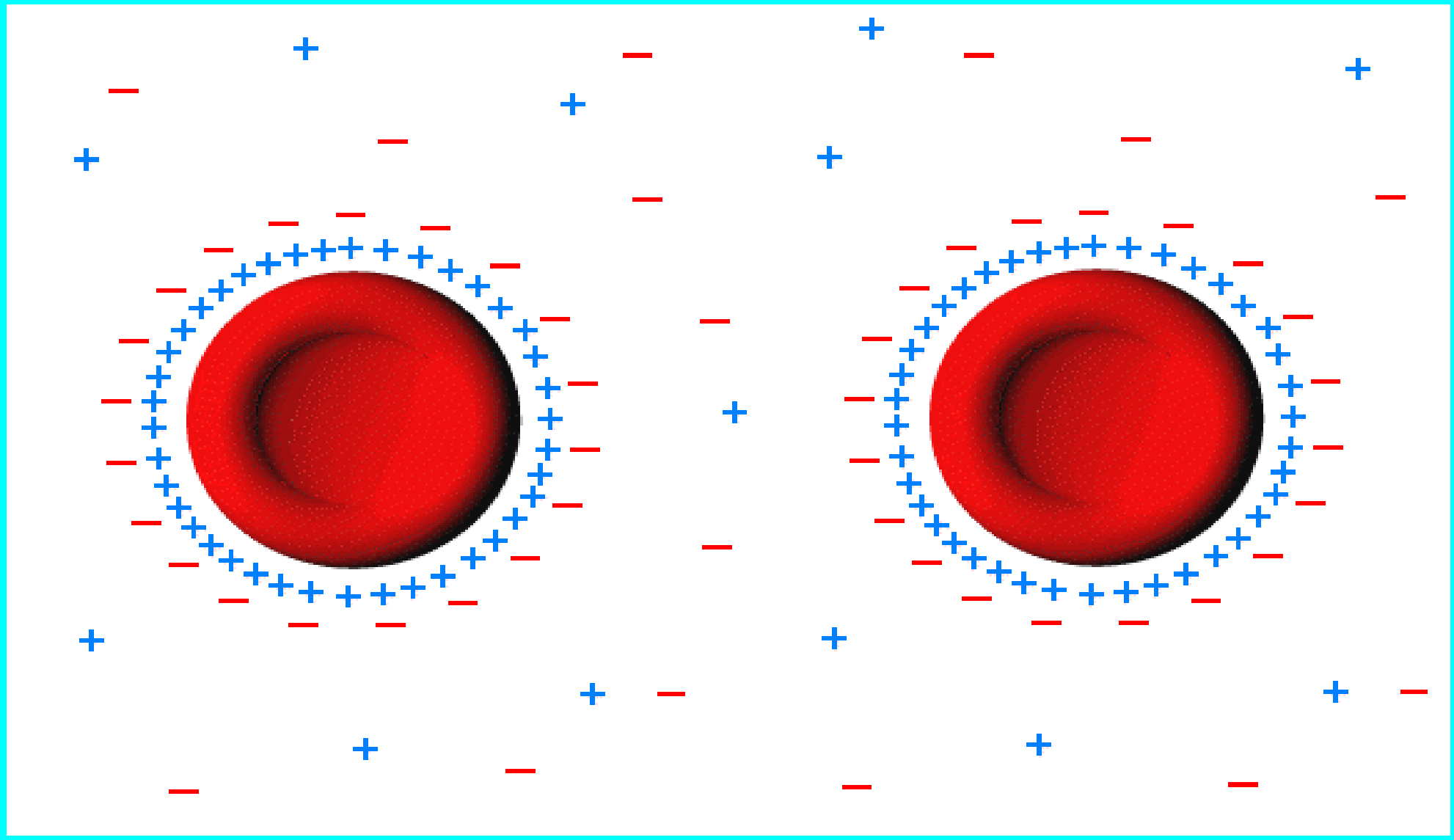
Enzyme test

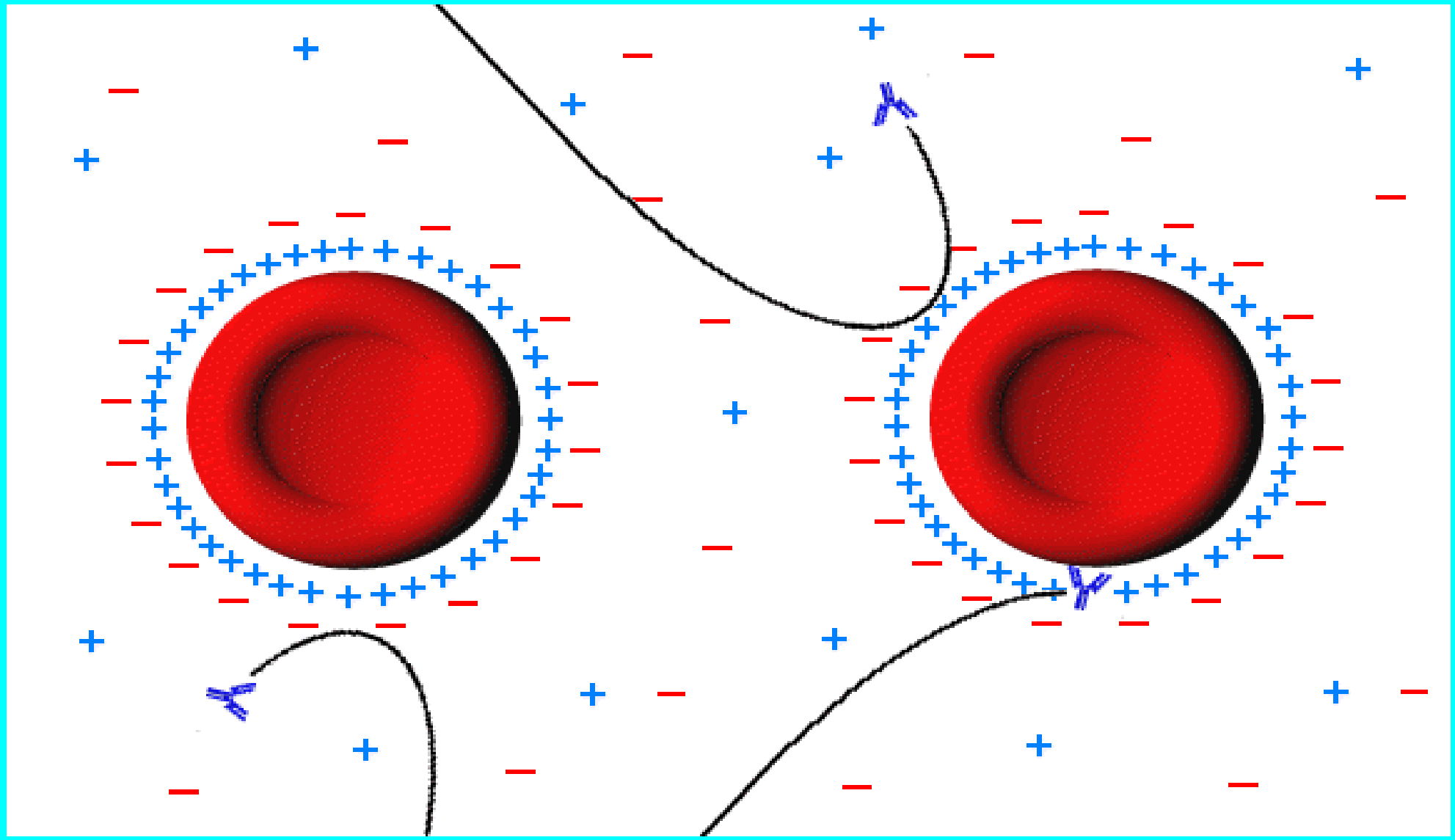


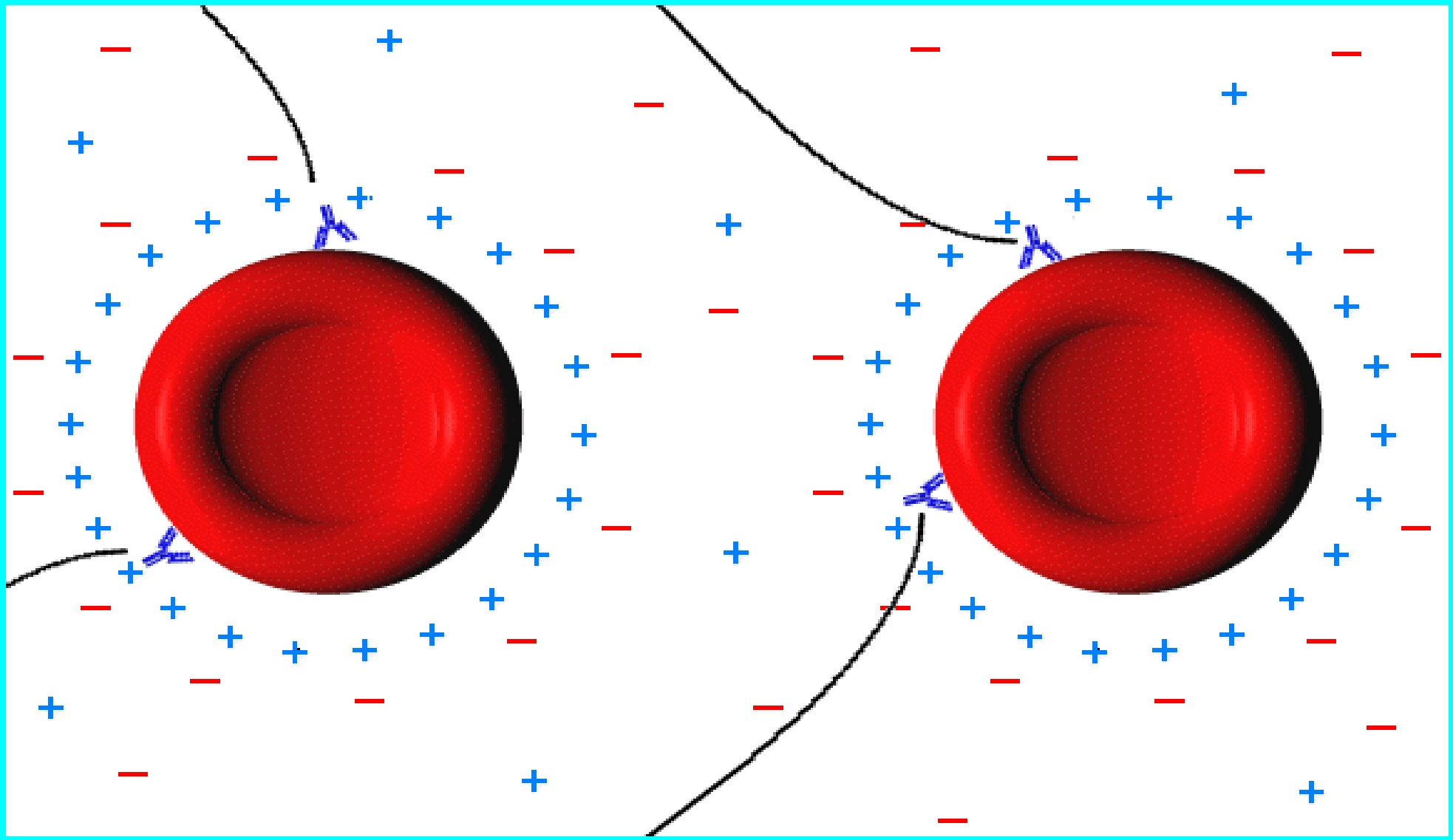
Enzyme test



LISS test



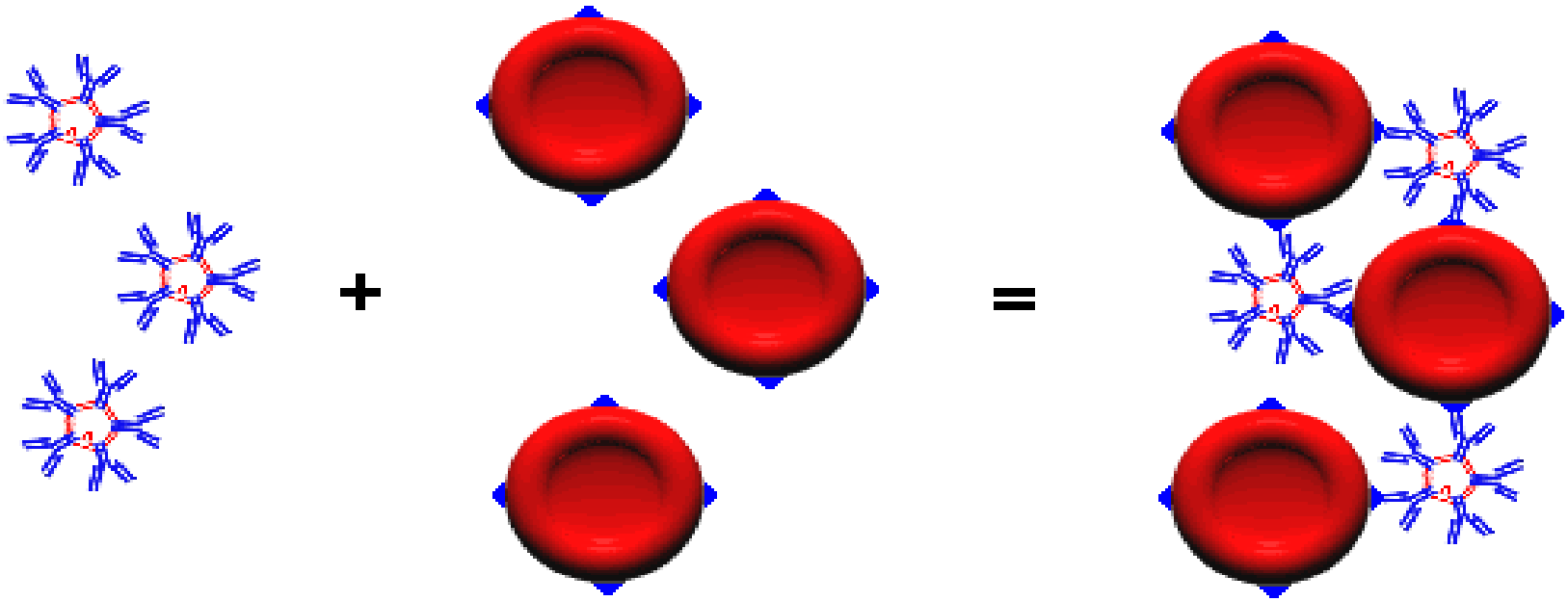




TECHNIQUES

- tube test
- solid phase test
- gel test (column agglutination)

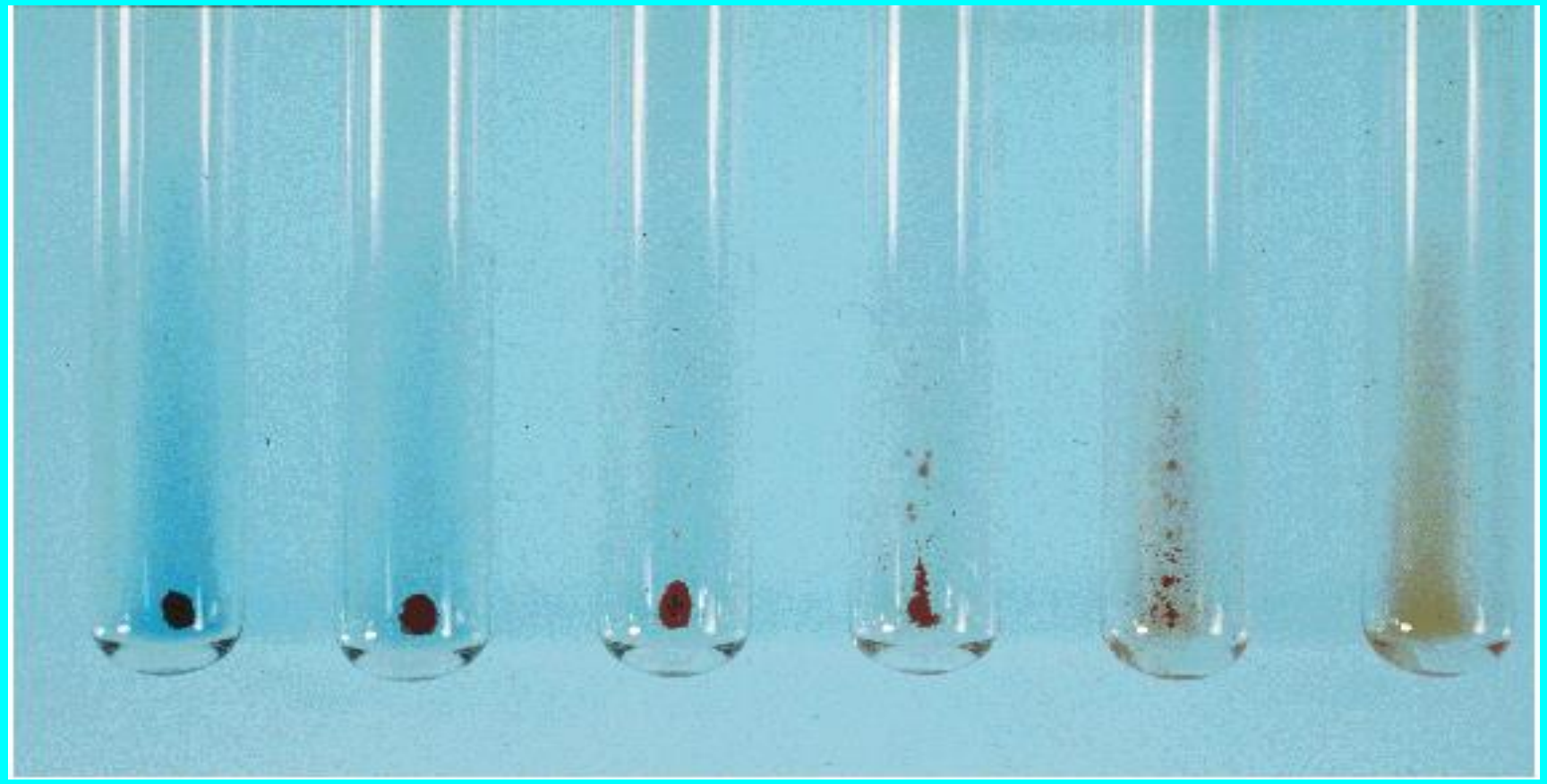
Tube test

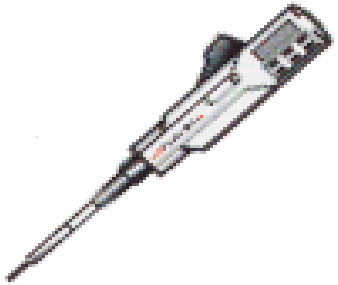


Beispiel:

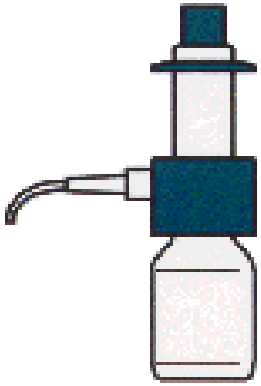
Anti-A Antikörper Erythrozyten mit Antigen A Agglutination

Tube test

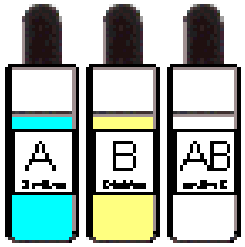




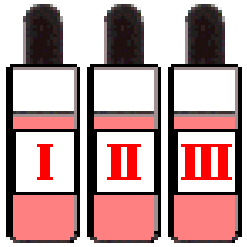
Pipette



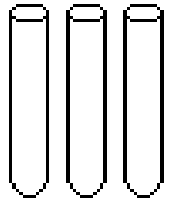
NaCl



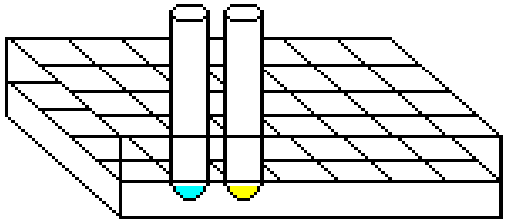
Antiseren



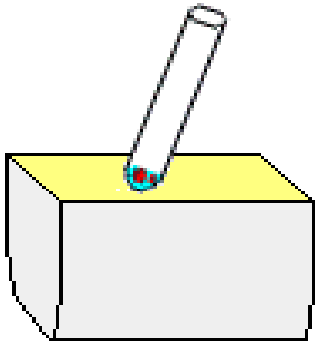
Testzellen



Röhrchen

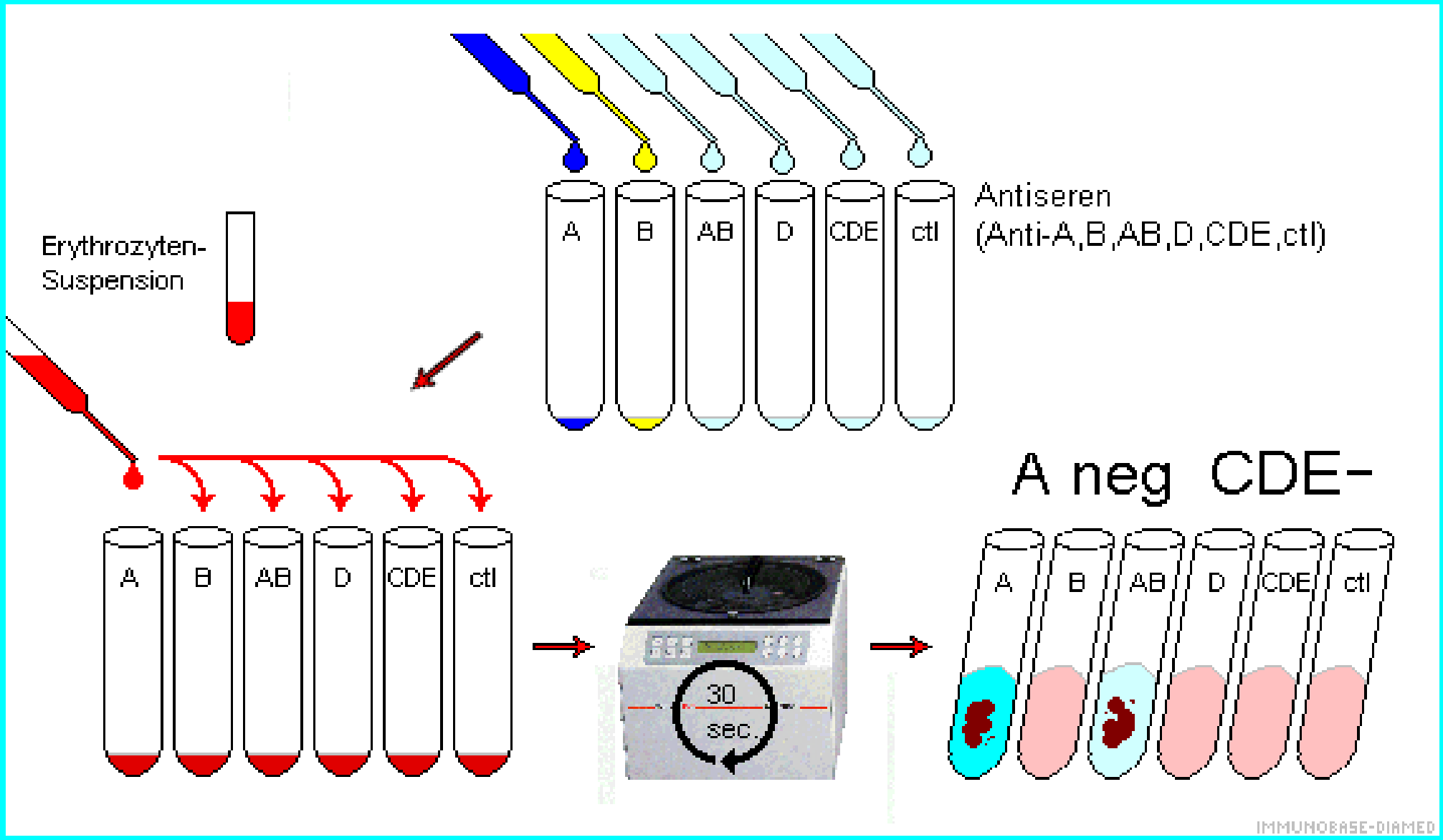


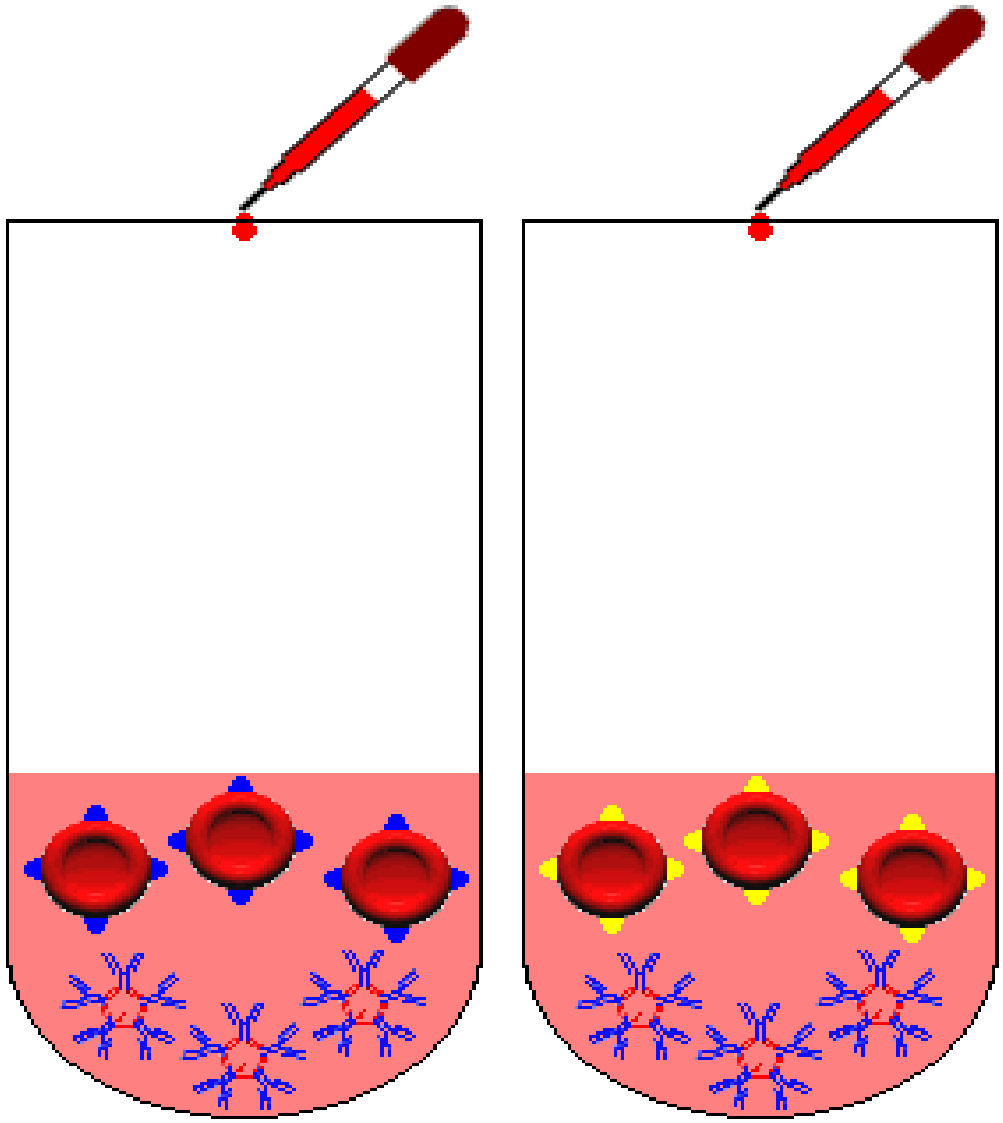
Röhrchengestell

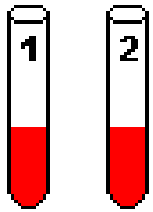


Lichtquelle

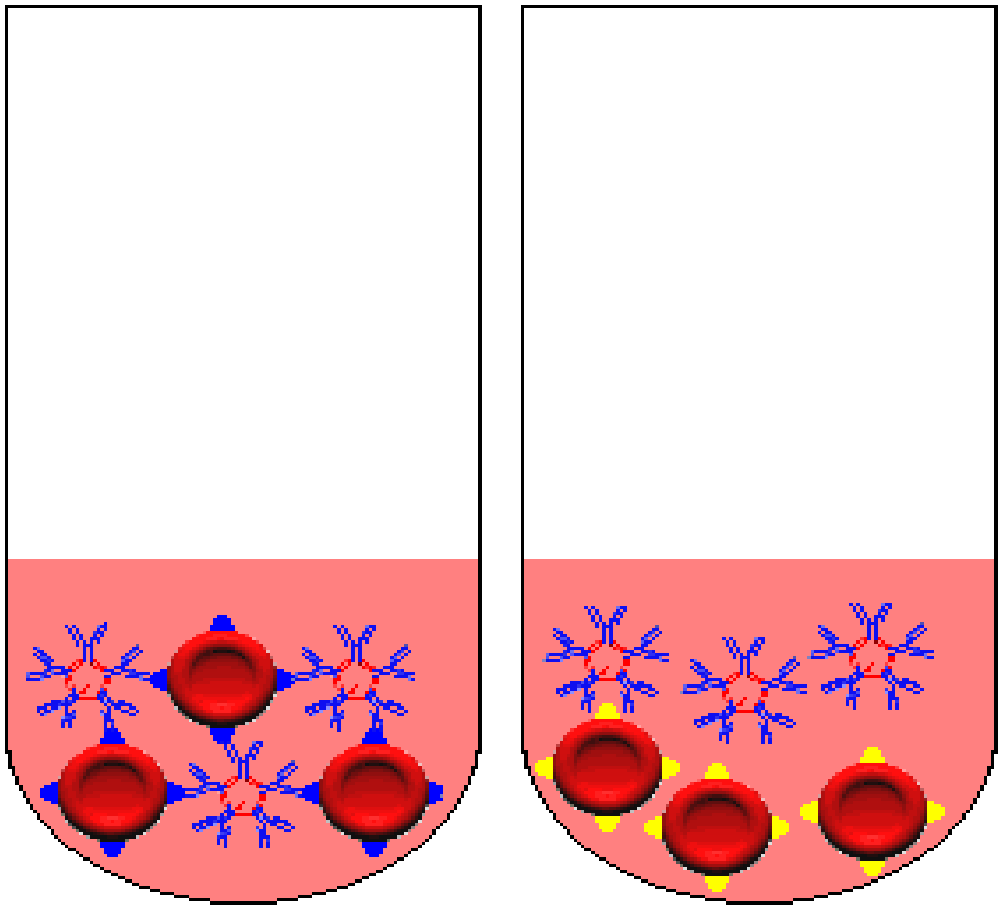
Waschzentrifuge


Erythrozyten-
Suspension
A
B
AB
D
CDE
ctl
Antiseren
(Anti-A,B,AB,D,CDE,ctl)
A neg CDE-
30
sec.
IMMUNOBASE-DIAMED

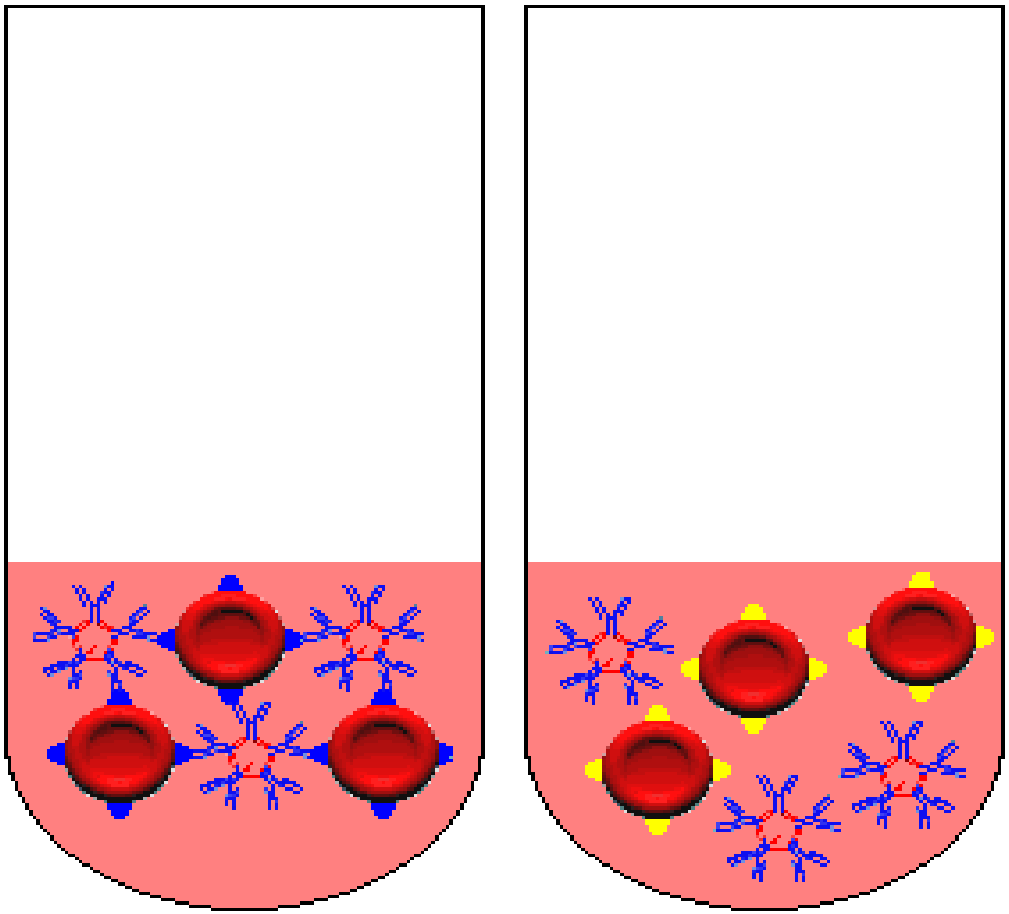


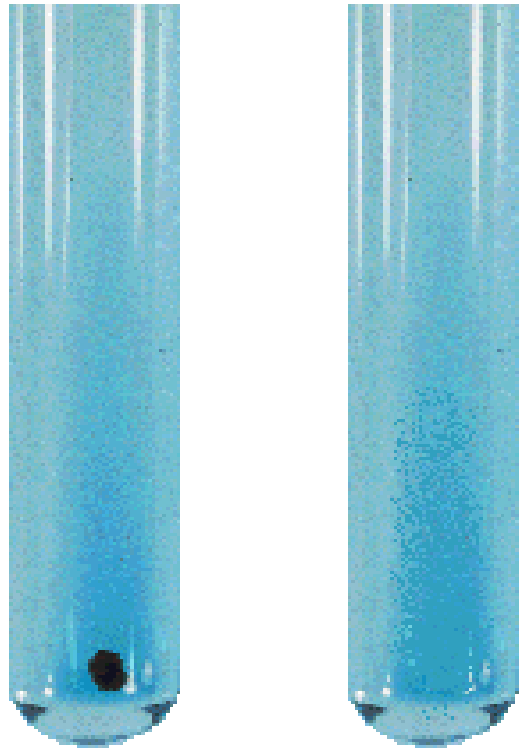


Erythrozyten
Suspension



Zentrifugation

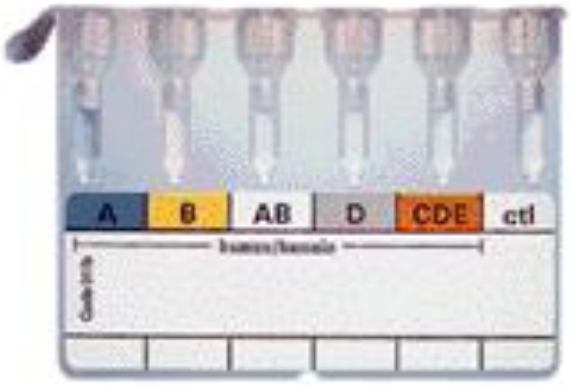




Agglutination

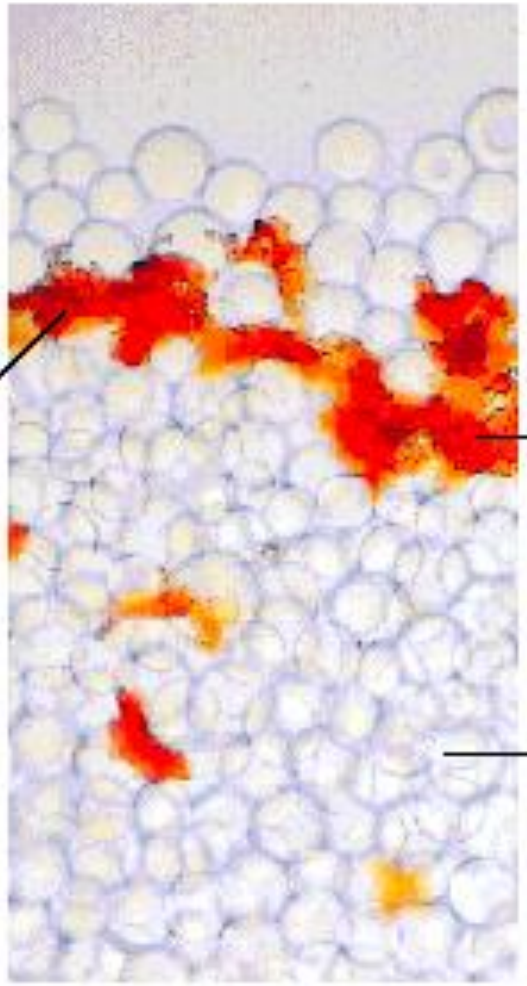
Keine Agglutination

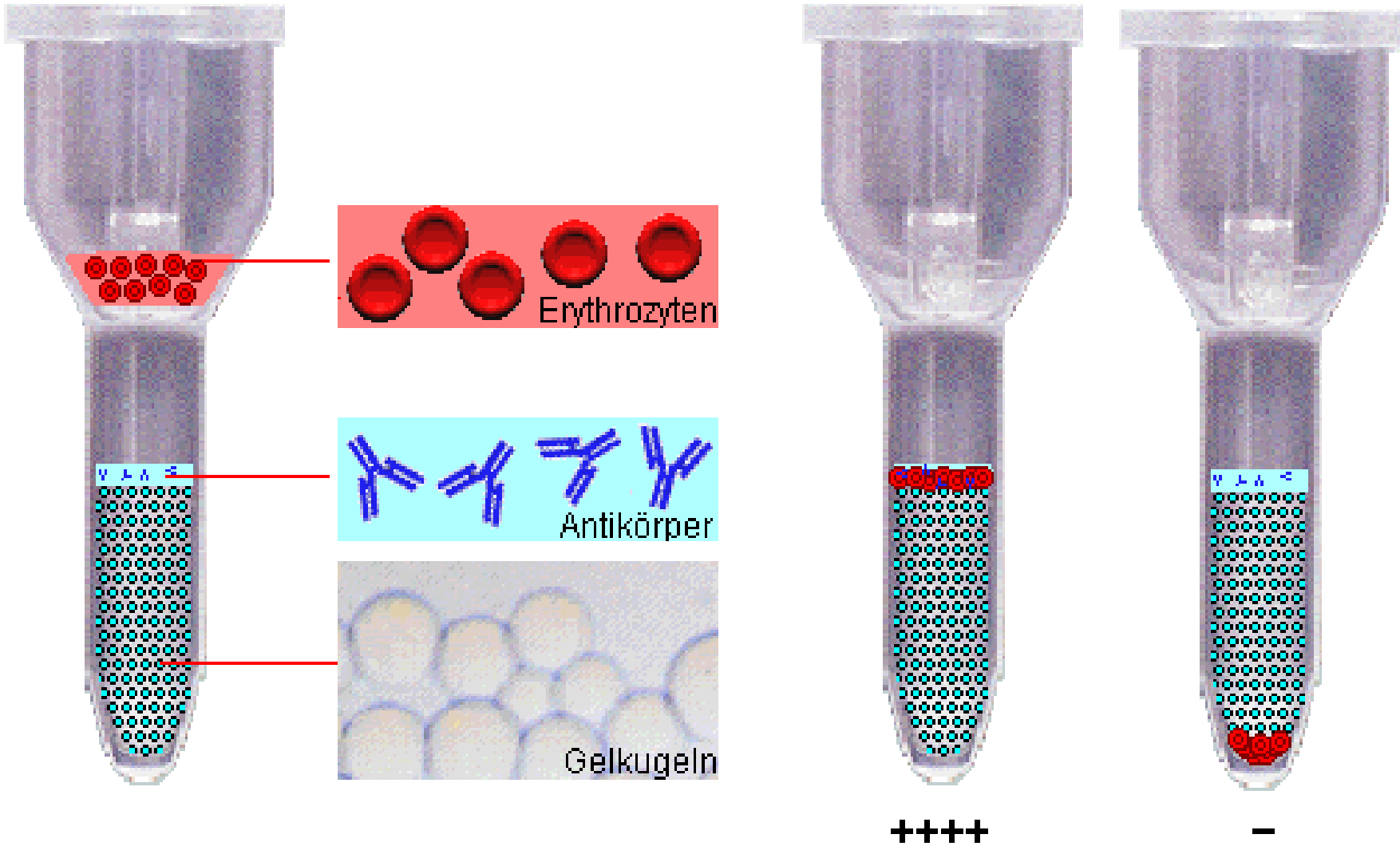
Gel test - column agglutination



Gelkarte zur Antigen-Blutgruppenbestimmung

Gelküvette

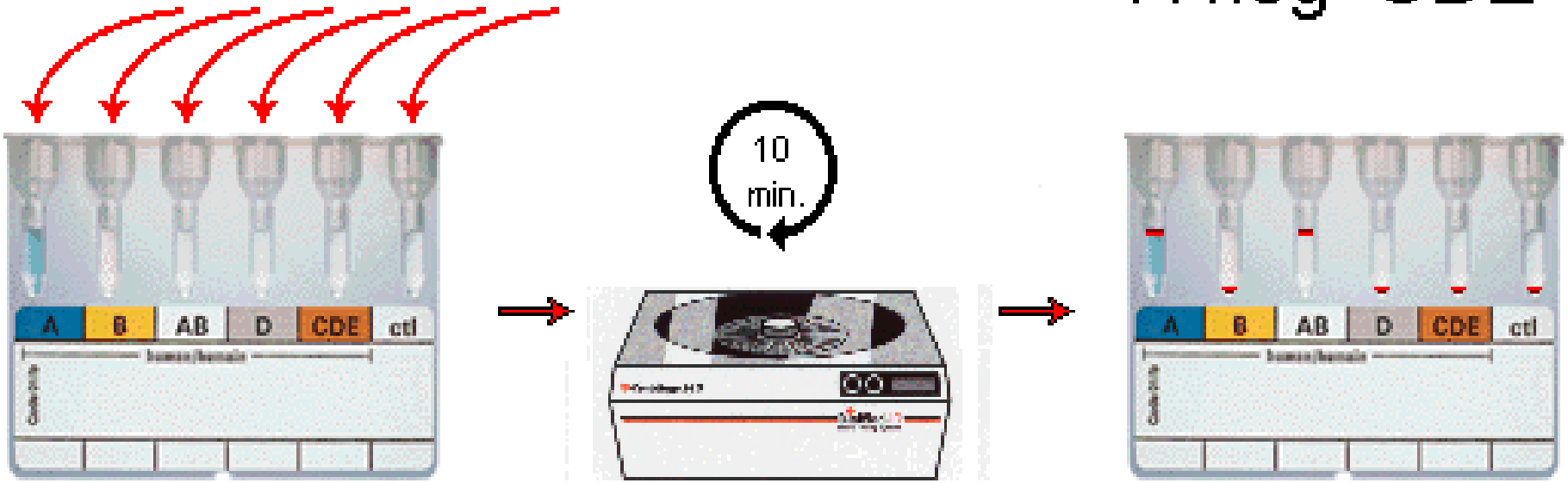



Erythrozyten
Antikörper
Gelkugeln
++++
–

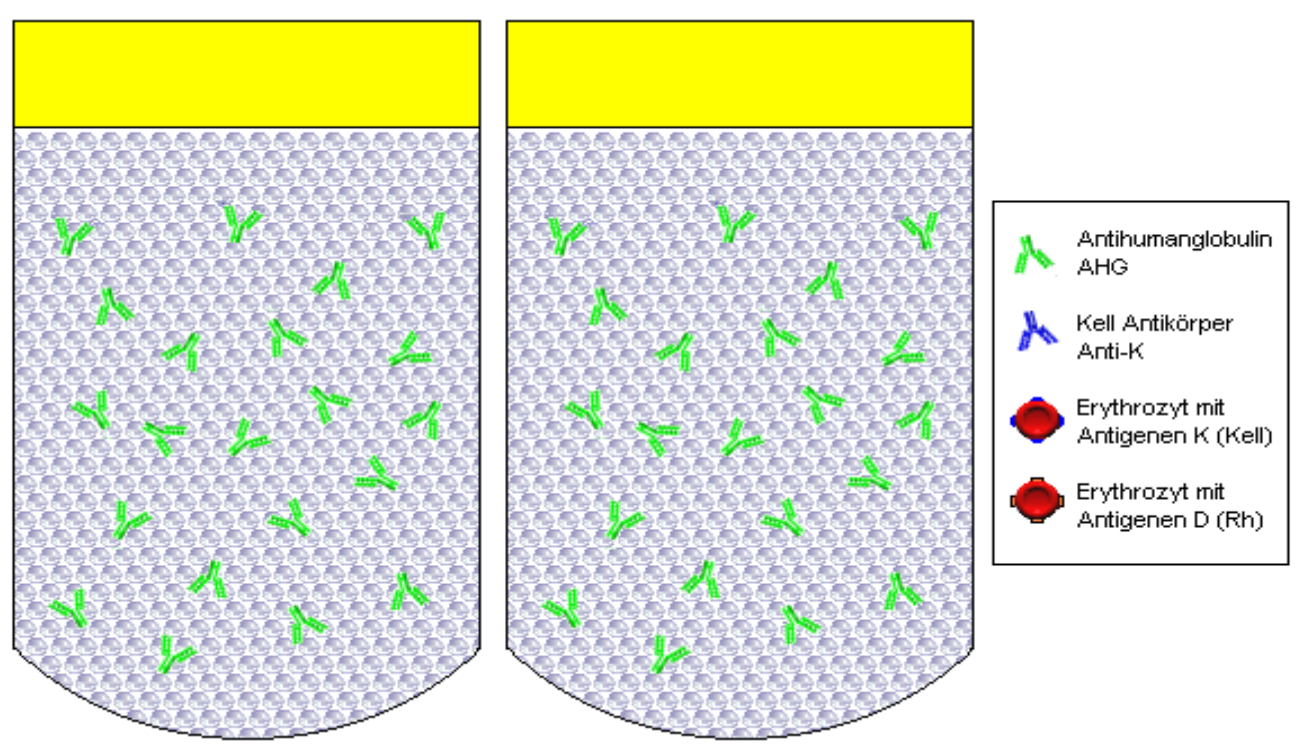
Erythrozyten-
Suspension

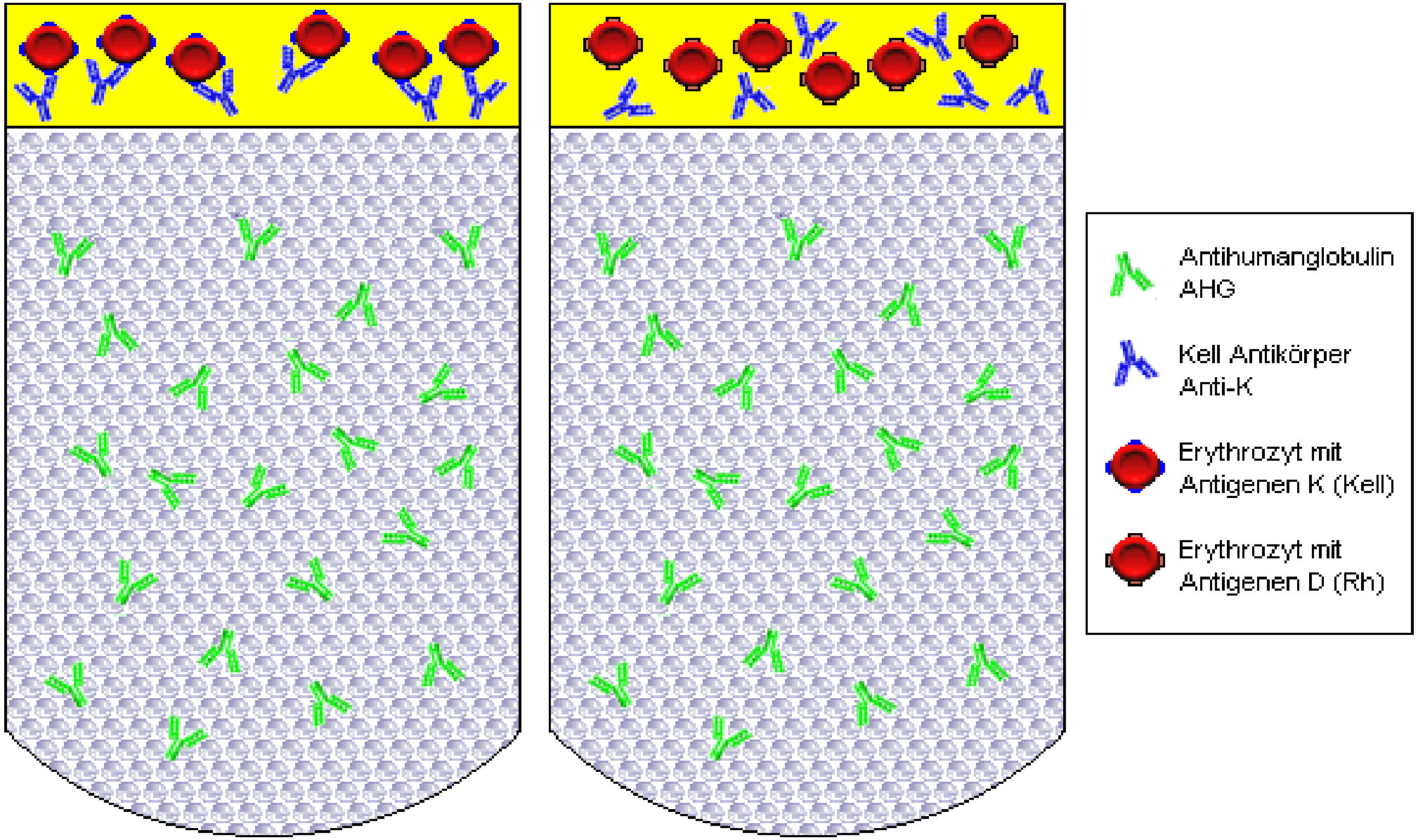
10 ul

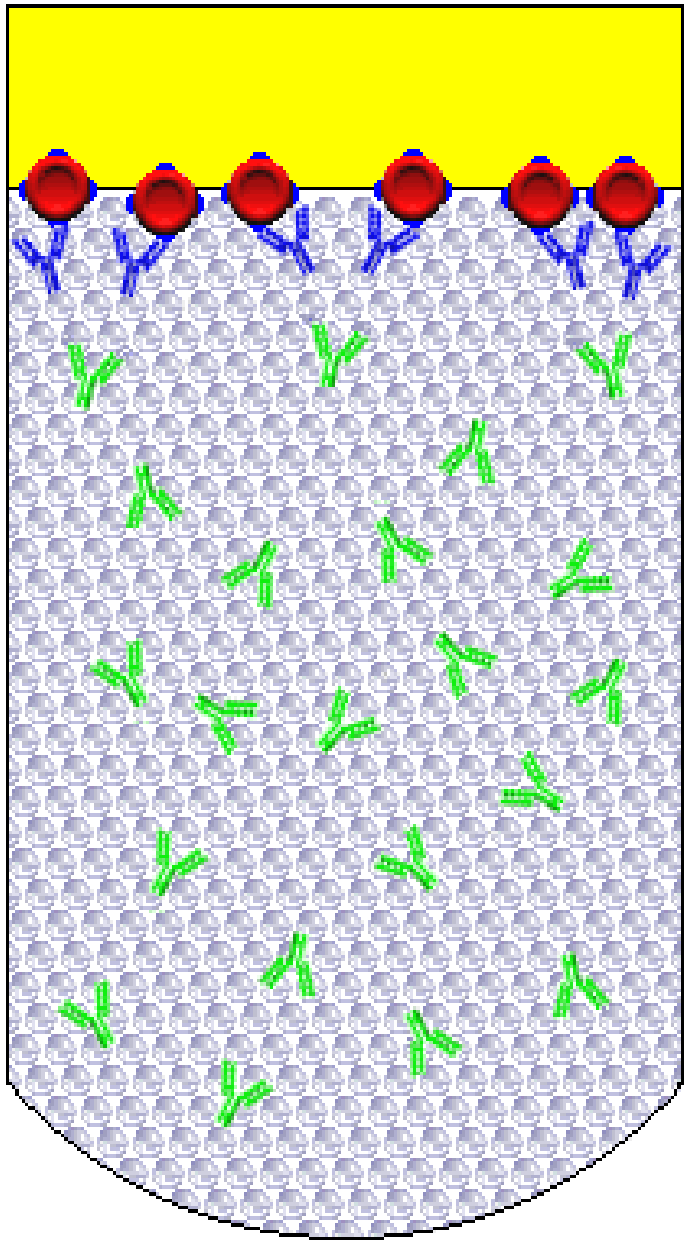
A neg CDE-

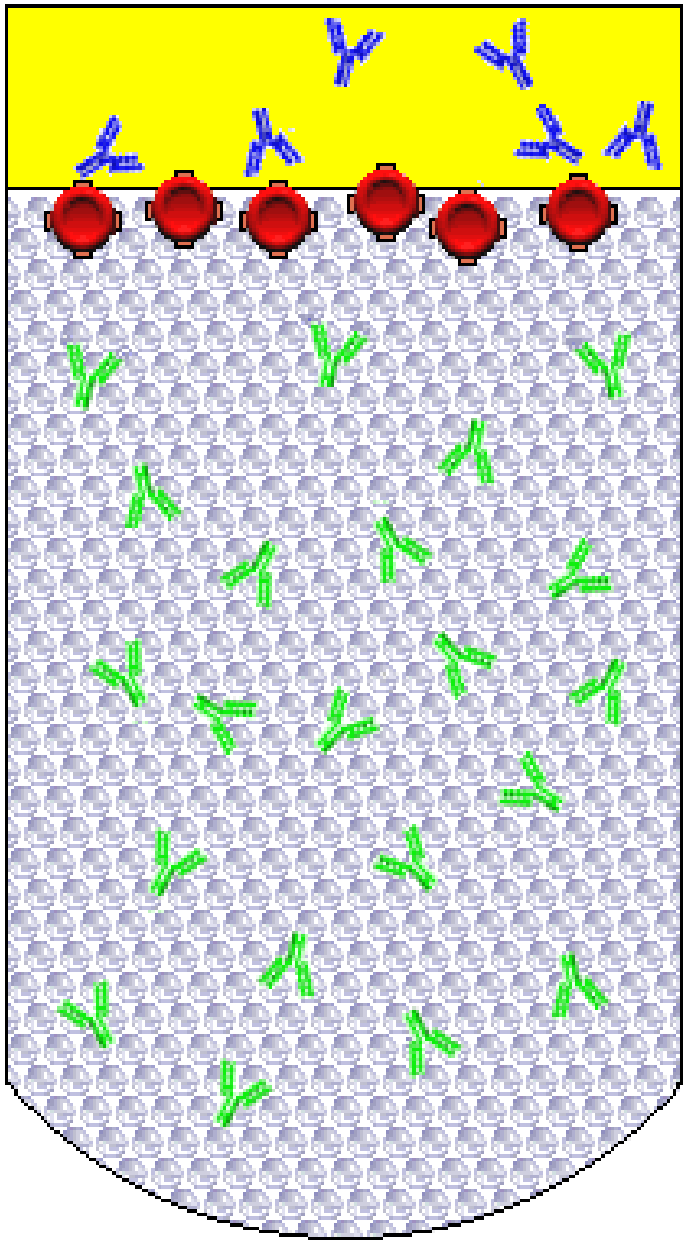


Indirect Coombs test in gel




Antihumanglobulin
AHG
Kell Antikörper
Anti-K
Erythrozyt mit
Antigenen K (Kell)
Erythrozyt mit
Antigenen D (Rh)



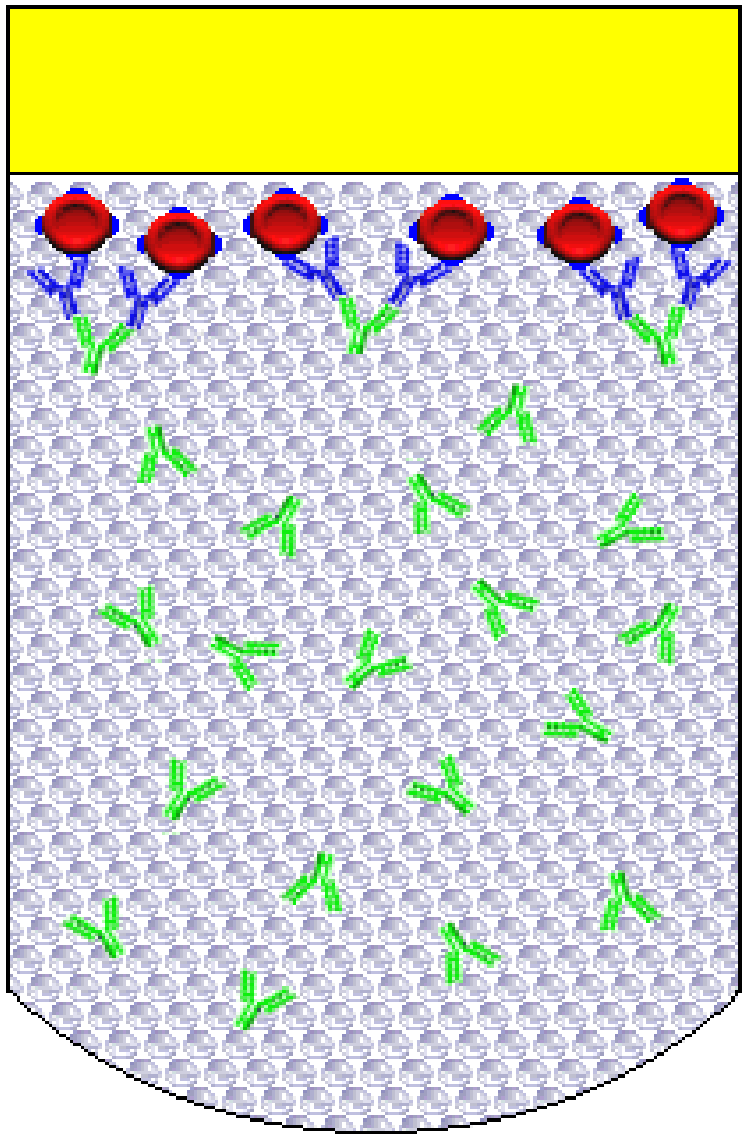


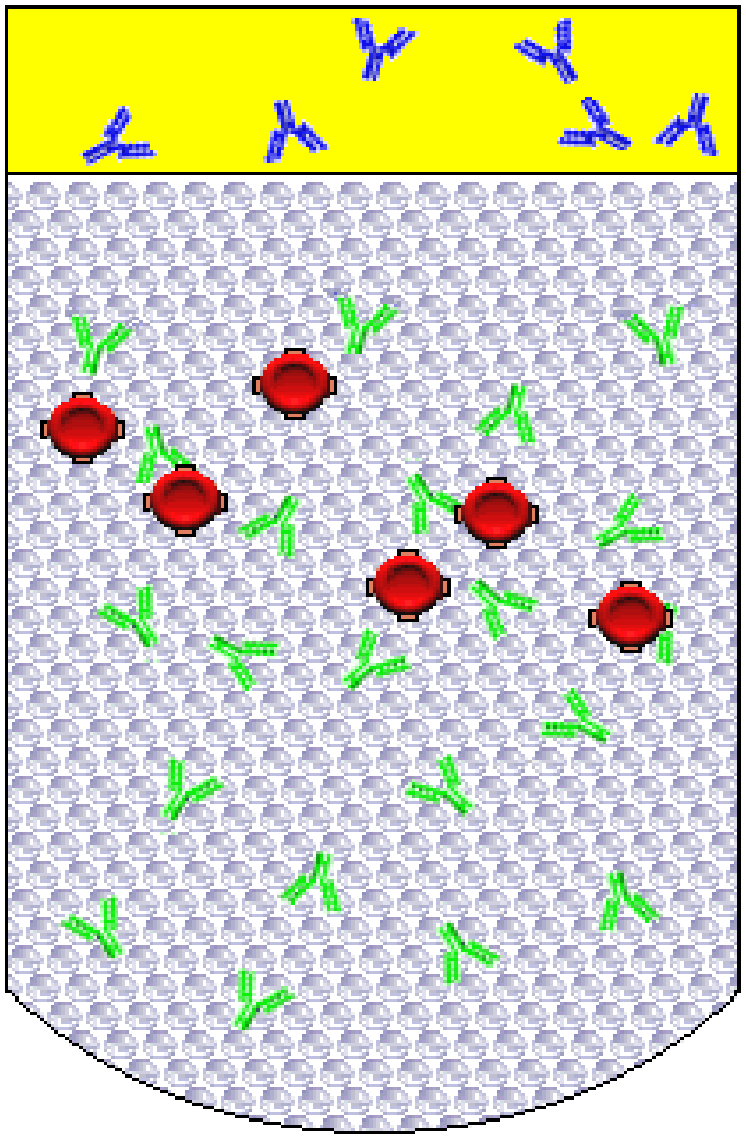
Antihumanglobulin
AHG

Kell Antikörper
Anti-K

Erythrozyt mit
Antigenen K (Kell)

Erythrozyt mit
Antigenen D (Rh)



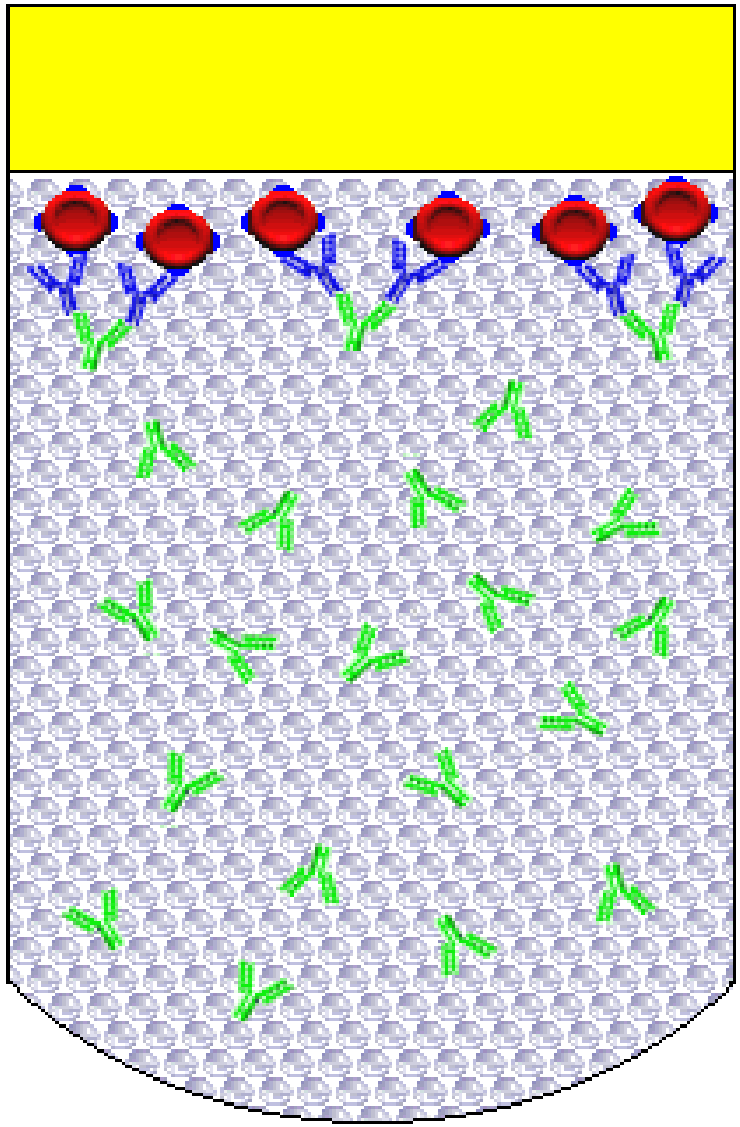


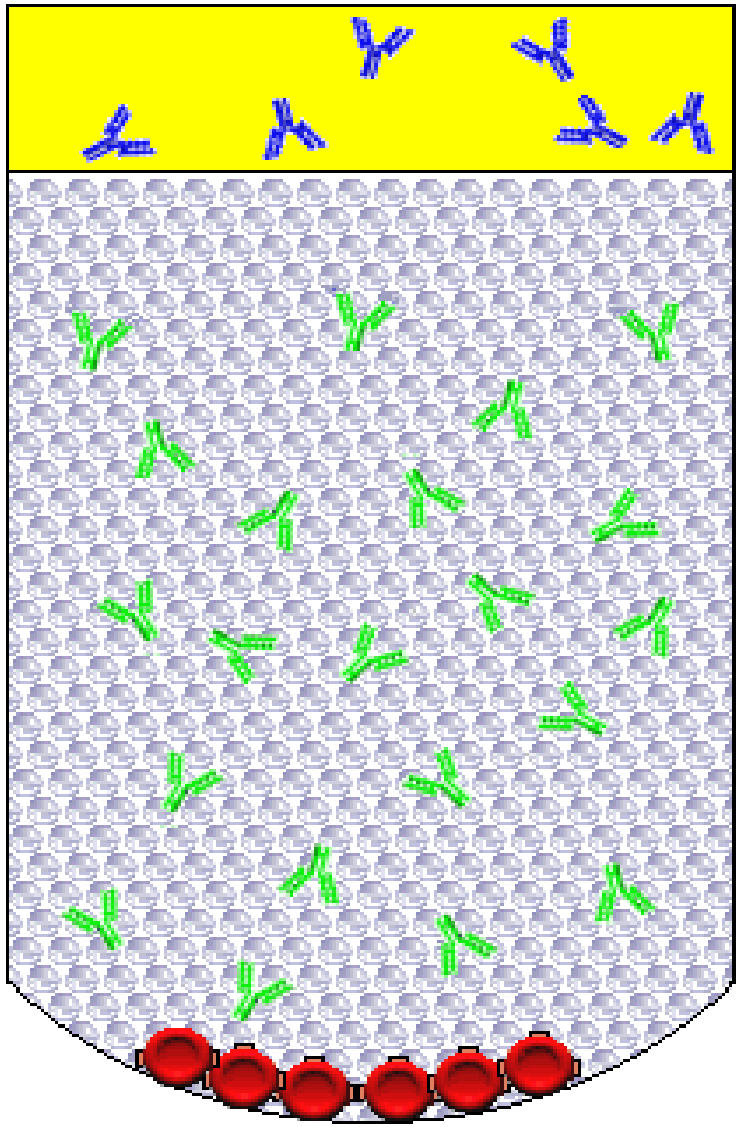
Antihumanglobulin
AHG

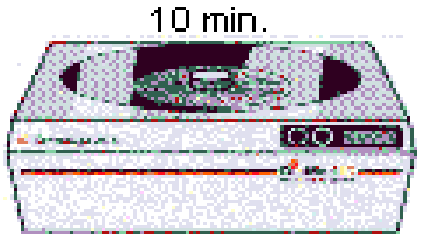
Kell Antikörper
Anti-K

Erythrozyt mit
Antigenen K (Kell)

Erythrozyt mit
Antigenen D (Rh)







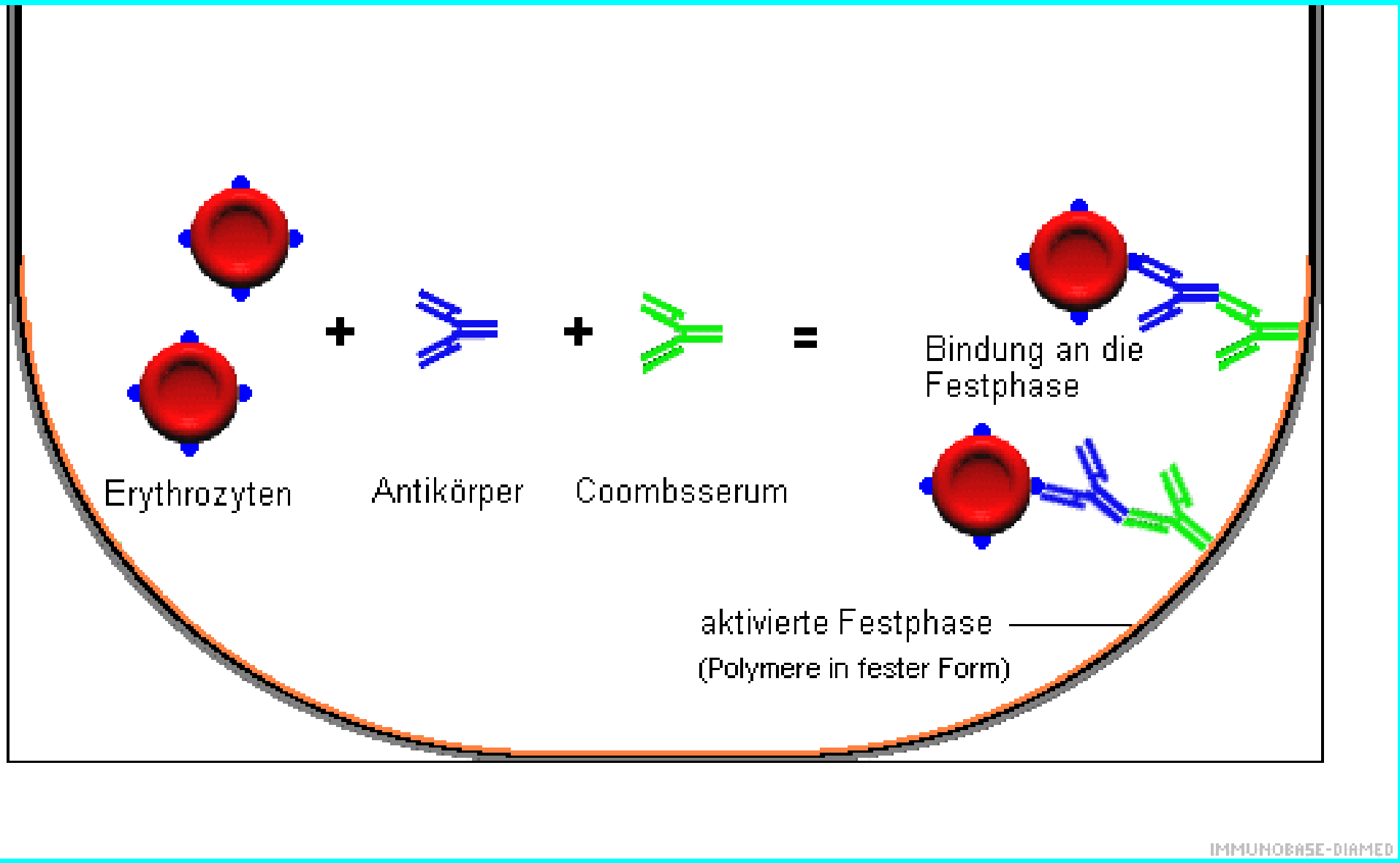
Antihumanglobulin
AHG

Kell Antikörper
Anti-K

Erythrozyt mit
Antigenen K (Kell)

Erythrozyt mit
Antigenen D (Rh)

Solid Phase Coombs Test



AB0 test on Solid Phase