

# Immuno-hematological Work-Up for making Safe Transfusion available in Case of Allo-/Autoantibodies



Deutsches Rotes Kreuz   
DRK-Blutspendedienst  
Baden-Württemberg | Hessen  
gemeinnützige GmbH

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 **EuBIS**  
European Blood Inspection System  
Initiated under the Public Health Programme of the EC  
Directorate General SANCO - GA No. 2006202



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# Pathomechanism of immune haemolysis

## 1. Opsonisation



extravasal haemolysis

Spleen, Liver

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**KAN**  
GÜVENLİĞİ  
• VE HEMOVİJİLAN

Makrophage  
RHS

TÜRK  
KIZILAY

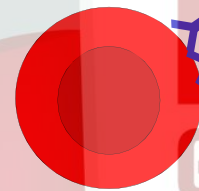
International  
Haemovigilance  
Network

# Pathomechanism of immune haemolysis

## 2. complement activation + complement mediated cell lysis

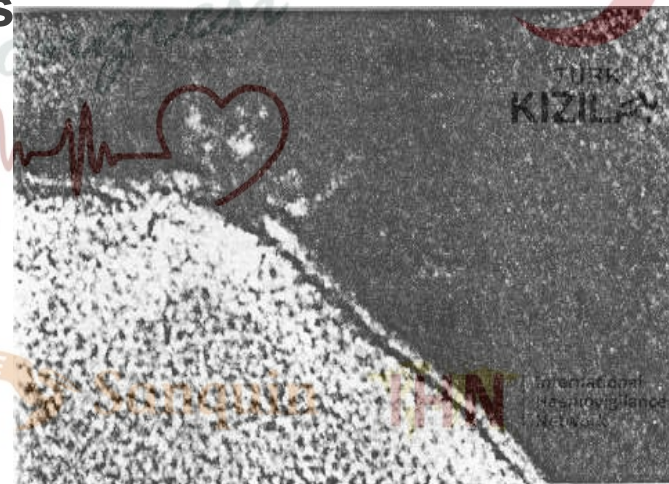


intravasal haemolysis



free  
Haemoglobin

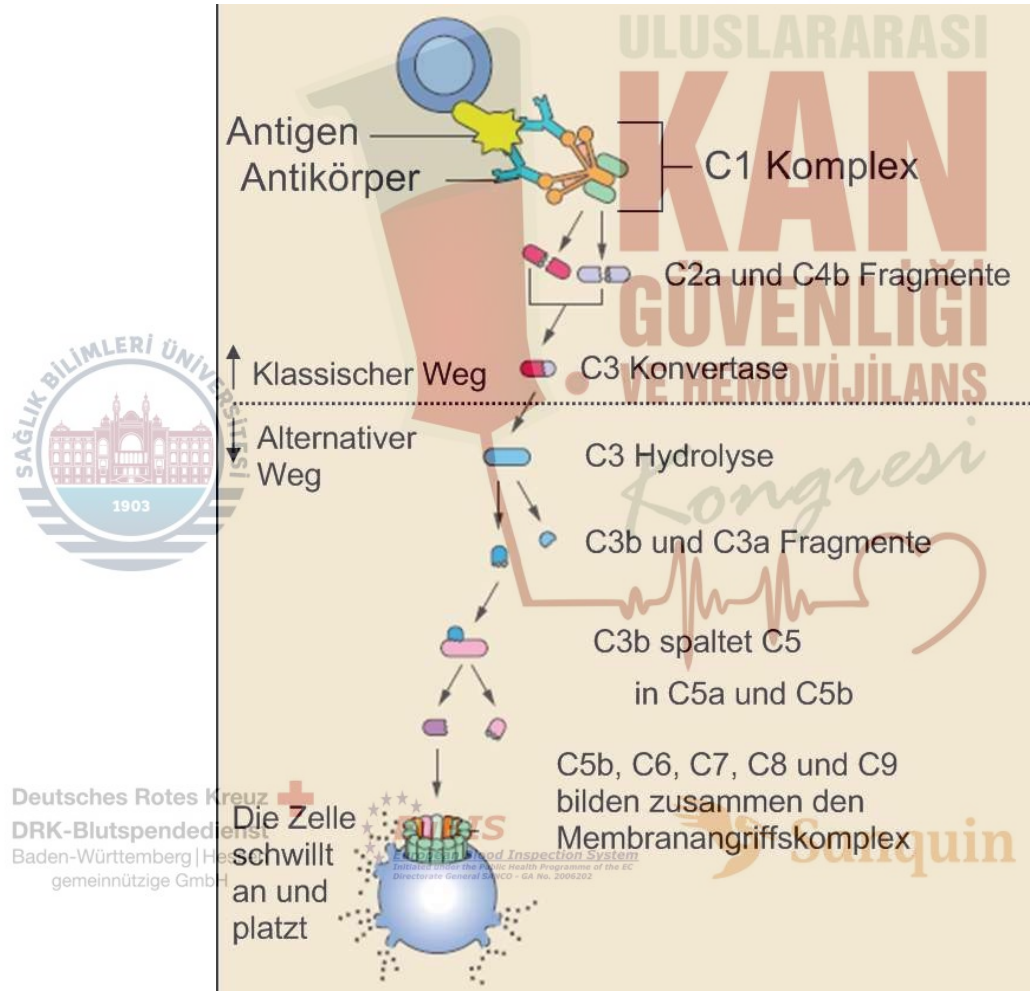
membrane-/  
cellfragments



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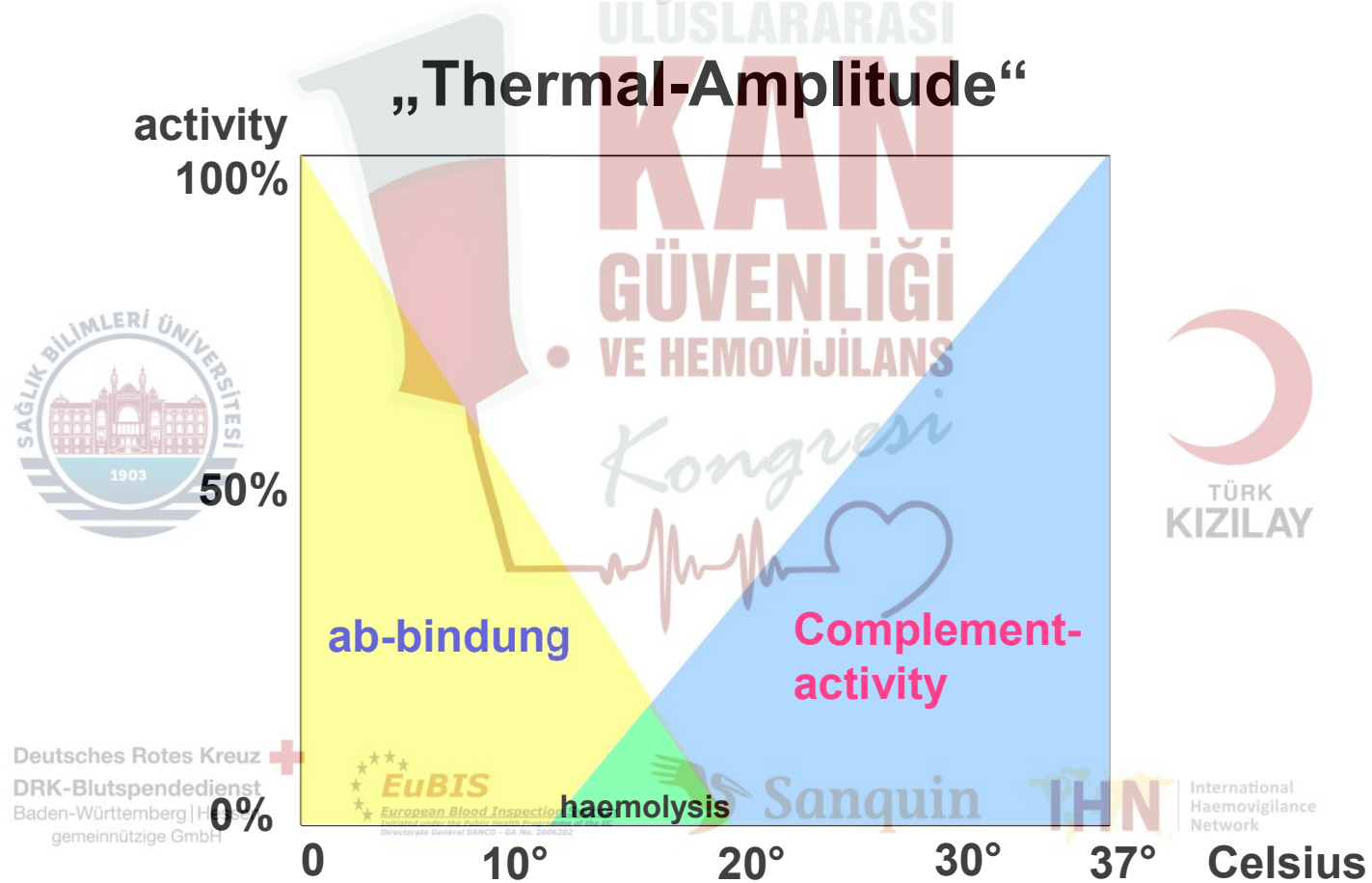
# Complement activation



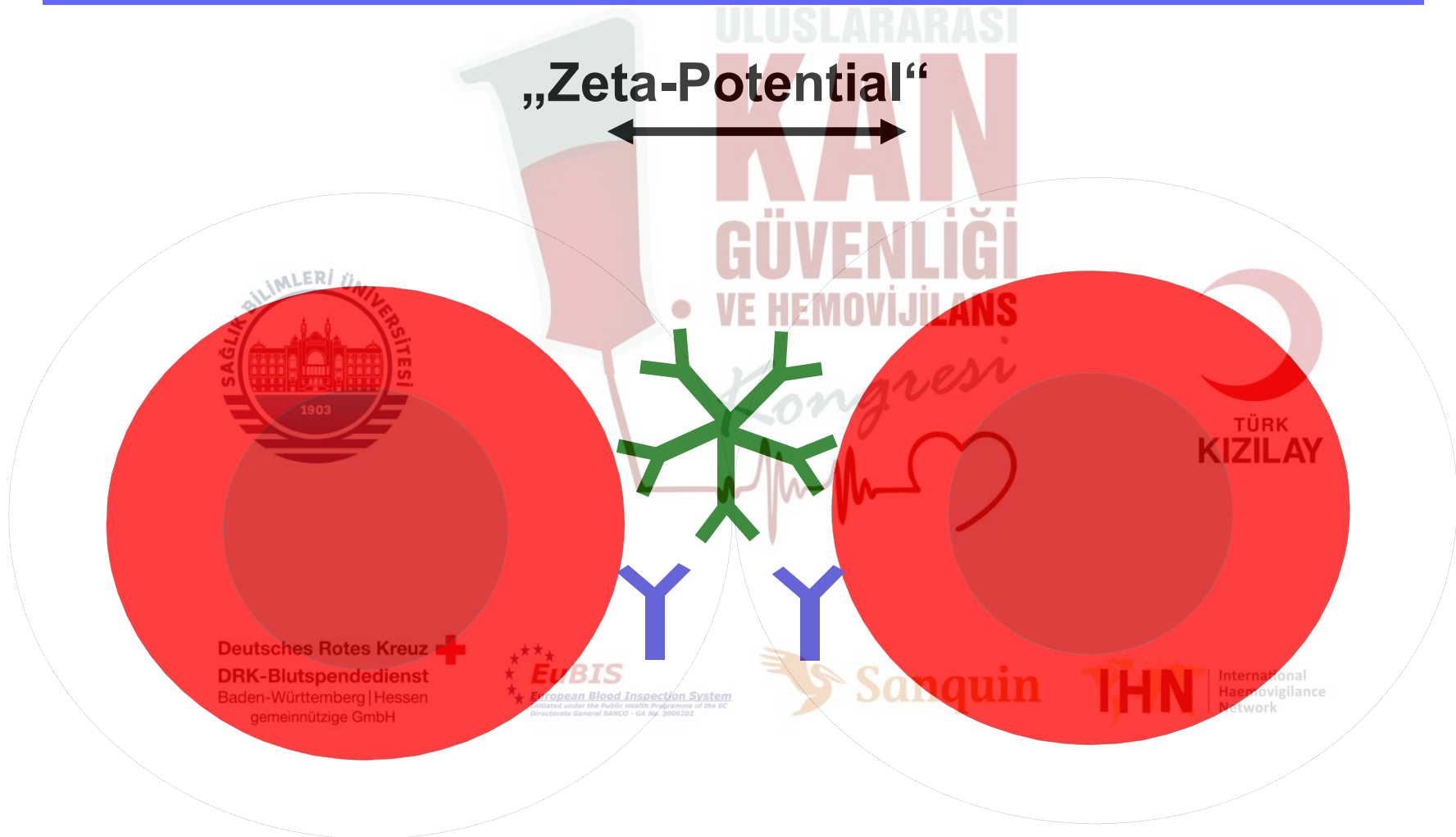
Deutsches Rotes Kreuz  
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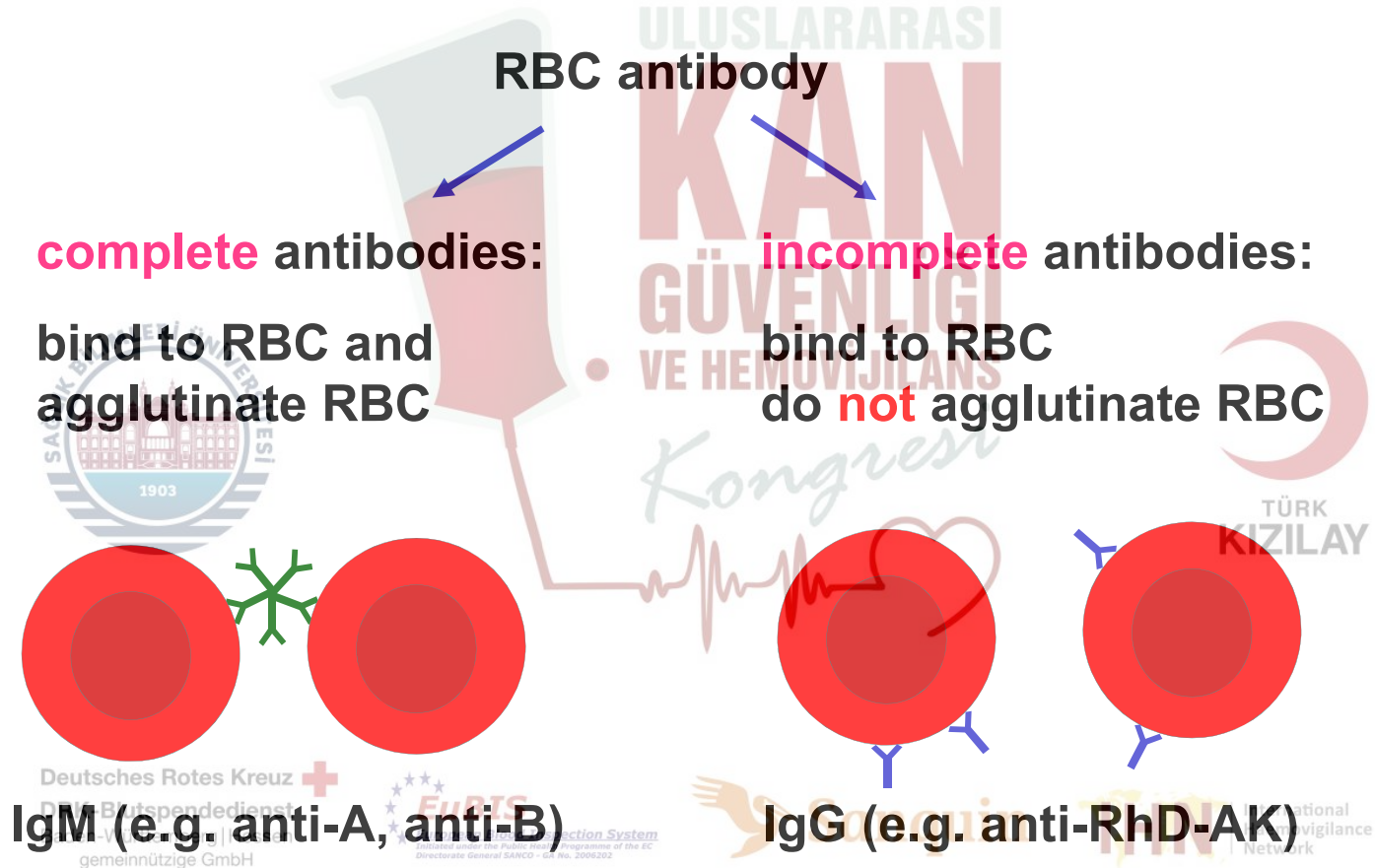
# Under which conditions would „cold reacting“ IgM ab´s lead to complement mediated haemolysis?



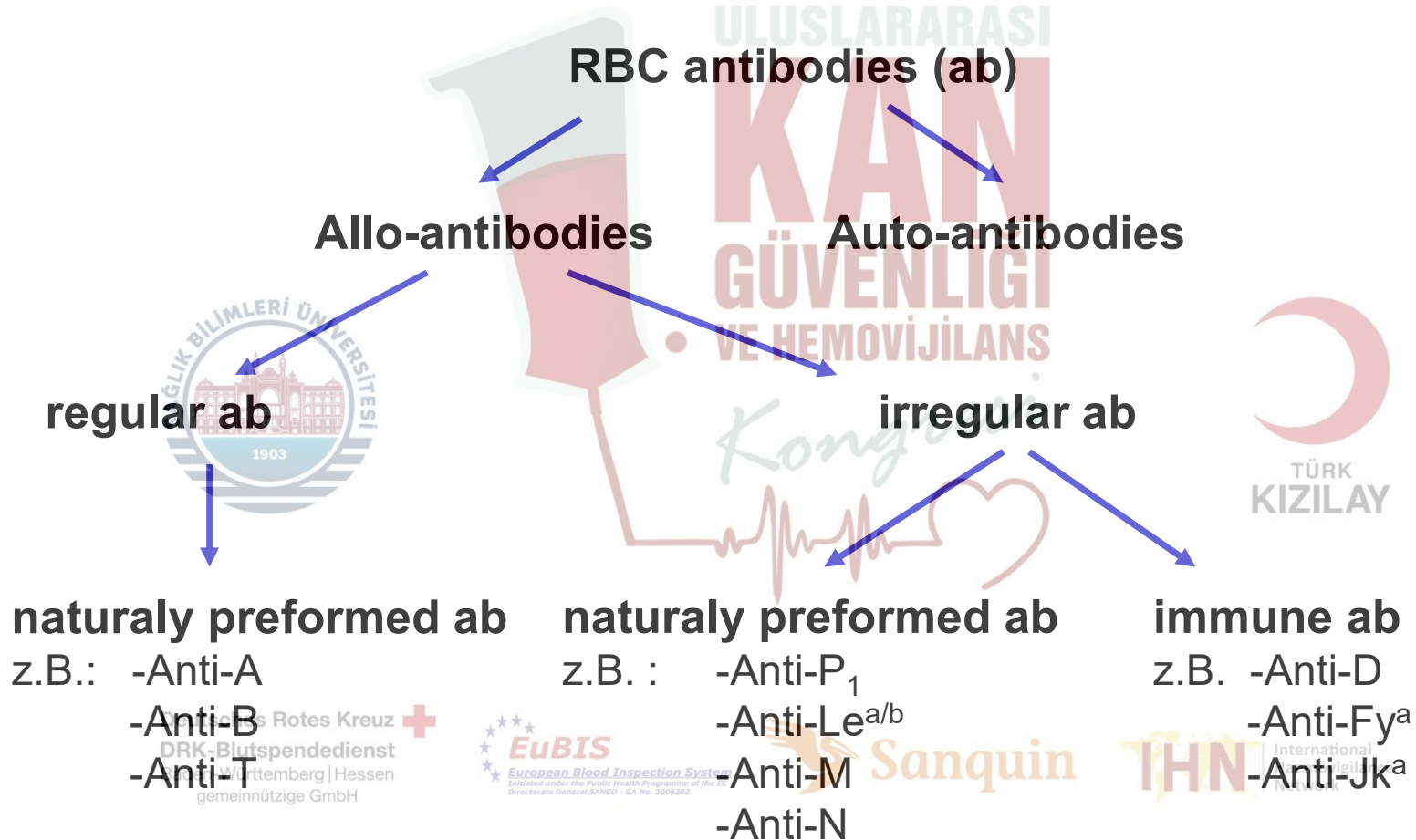
# Complete / incomplete antibodies



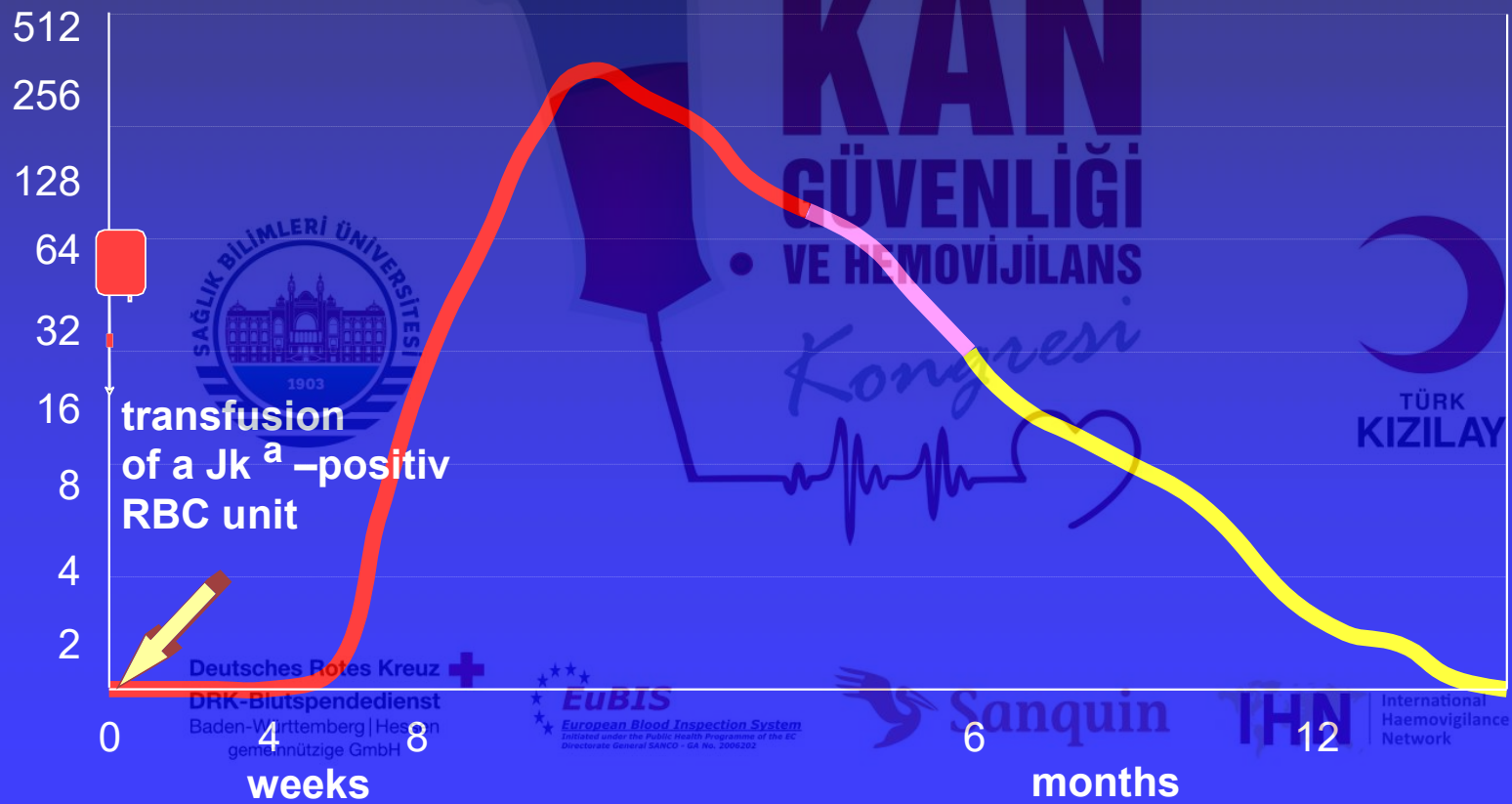
# RBC antibody terminology



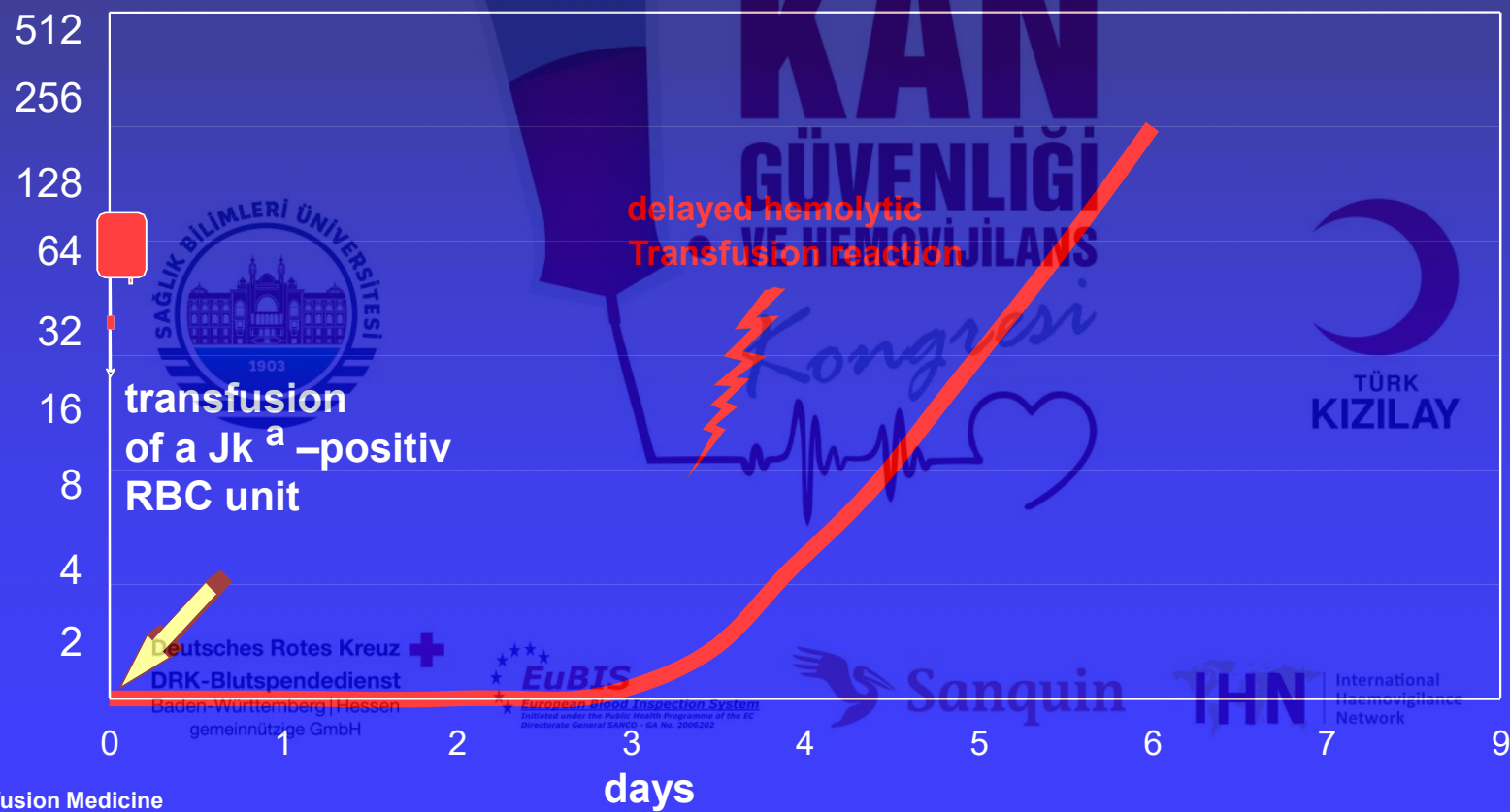
# RBC antibody terminology



# Primary immune reaction (i.e. Anti-Jk<sup>a</sup>)



# Sekundary immune reaction

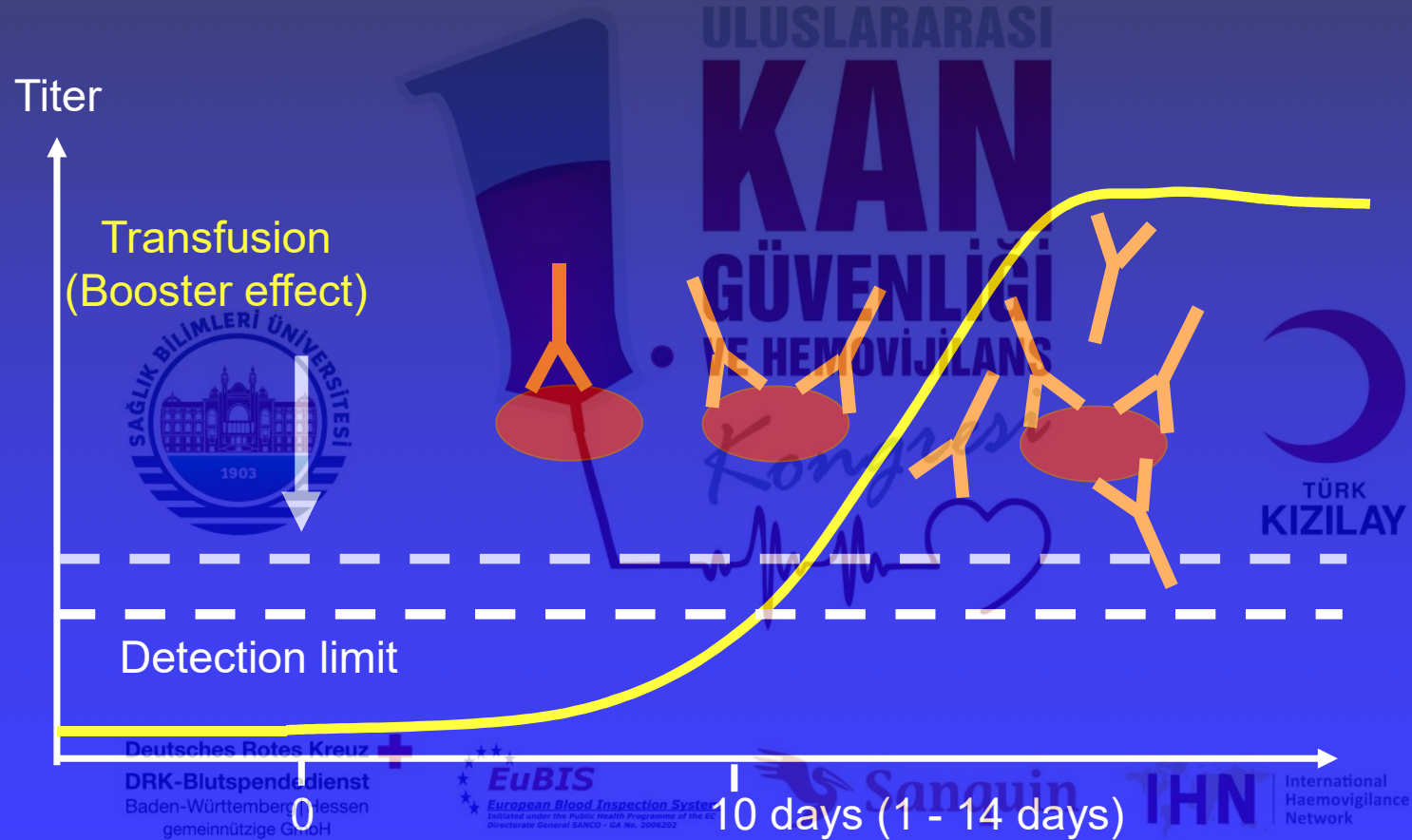


# Delayed hemolytic transfusion reaction immunisation and „Booster-Effect“



# Delayed transfusion reaction

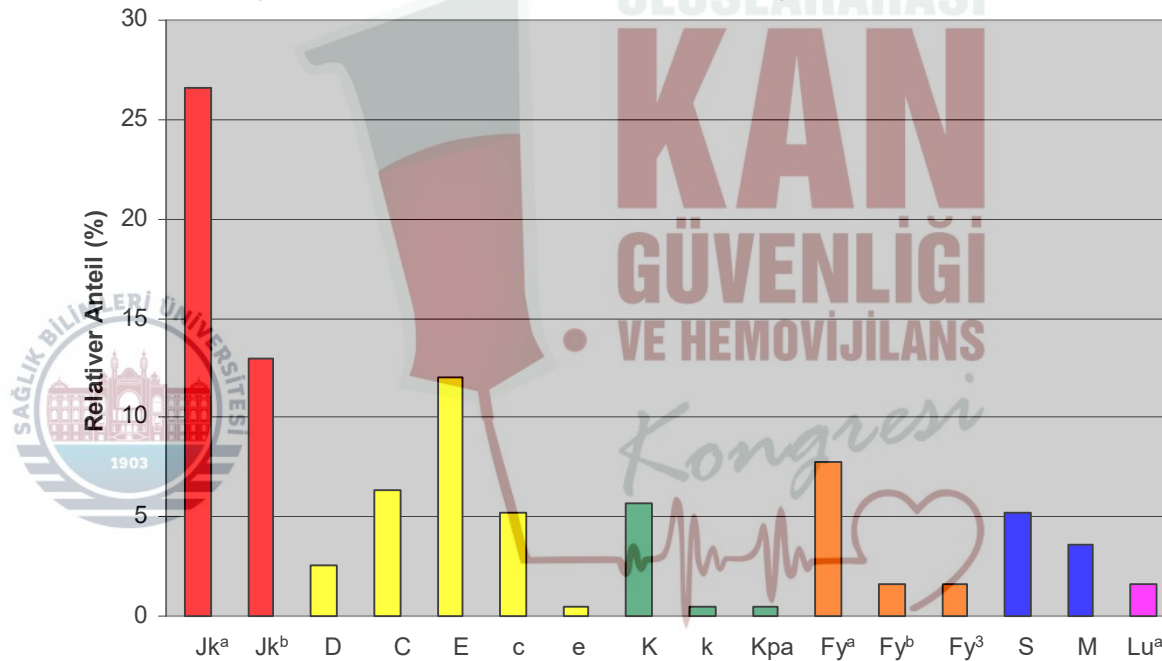
Critical timepoint: DAT positiv, ab screening yet negative



# DHTR

## Delayed Hemolytic Transfusion Reaction

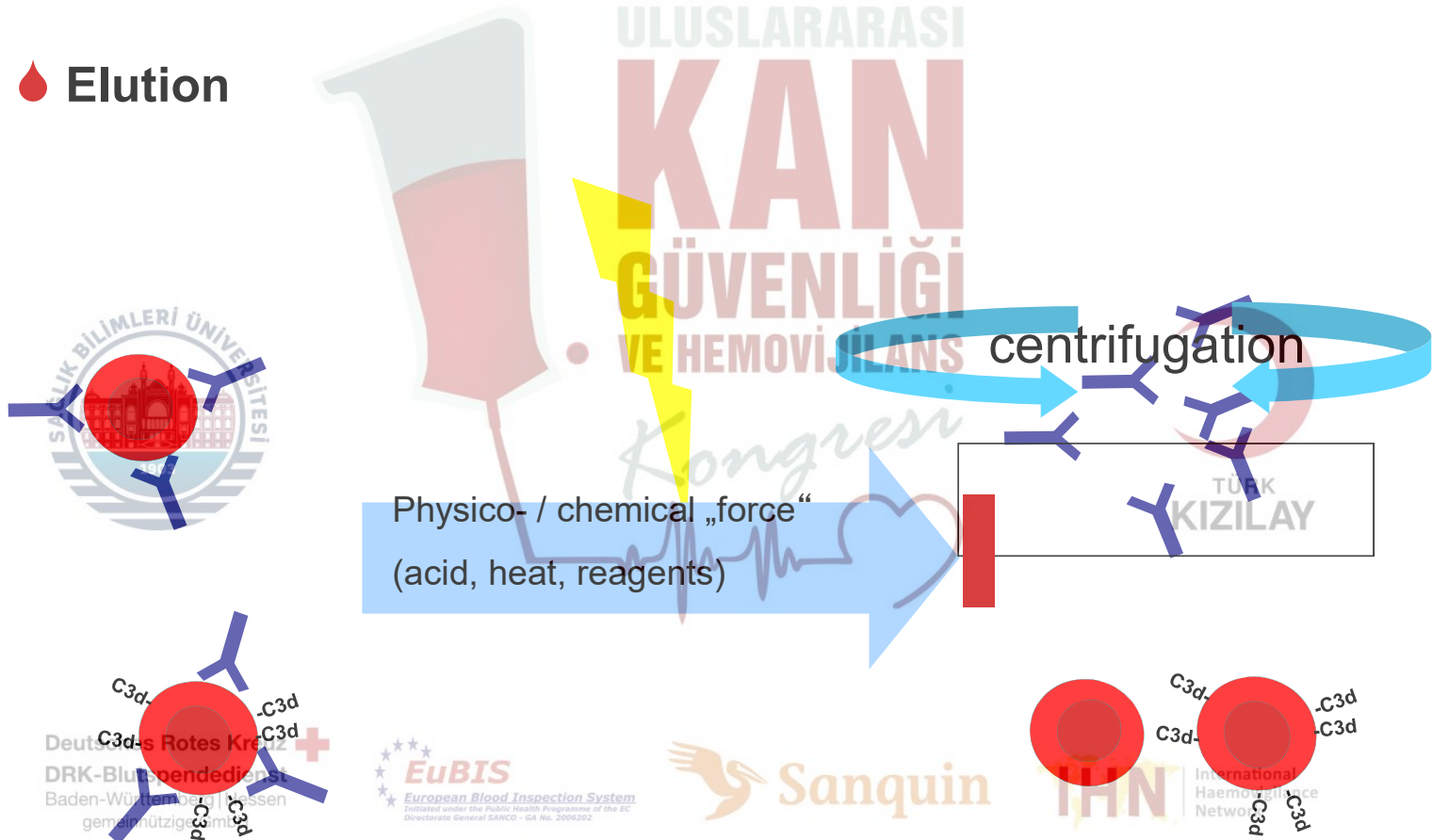
RBC antibodies (Booster-Effect)  
1 : 1.000 - 1 : 4.000 Transfusions  
fatal (< 1 auf 1.000.000 Transfusions)



Häufigkeit von neu nachgewiesenen Alloantikörper-Spezifitäten bei verzögerten hämolytischen Transfusionsreaktionen (Auswertung der Angaben in den Jahresberichten 2006-2009 „Serious Hazards of Transfusion“ (SHOT) ([www.shotuk.org](http://www.shotuk.org)).

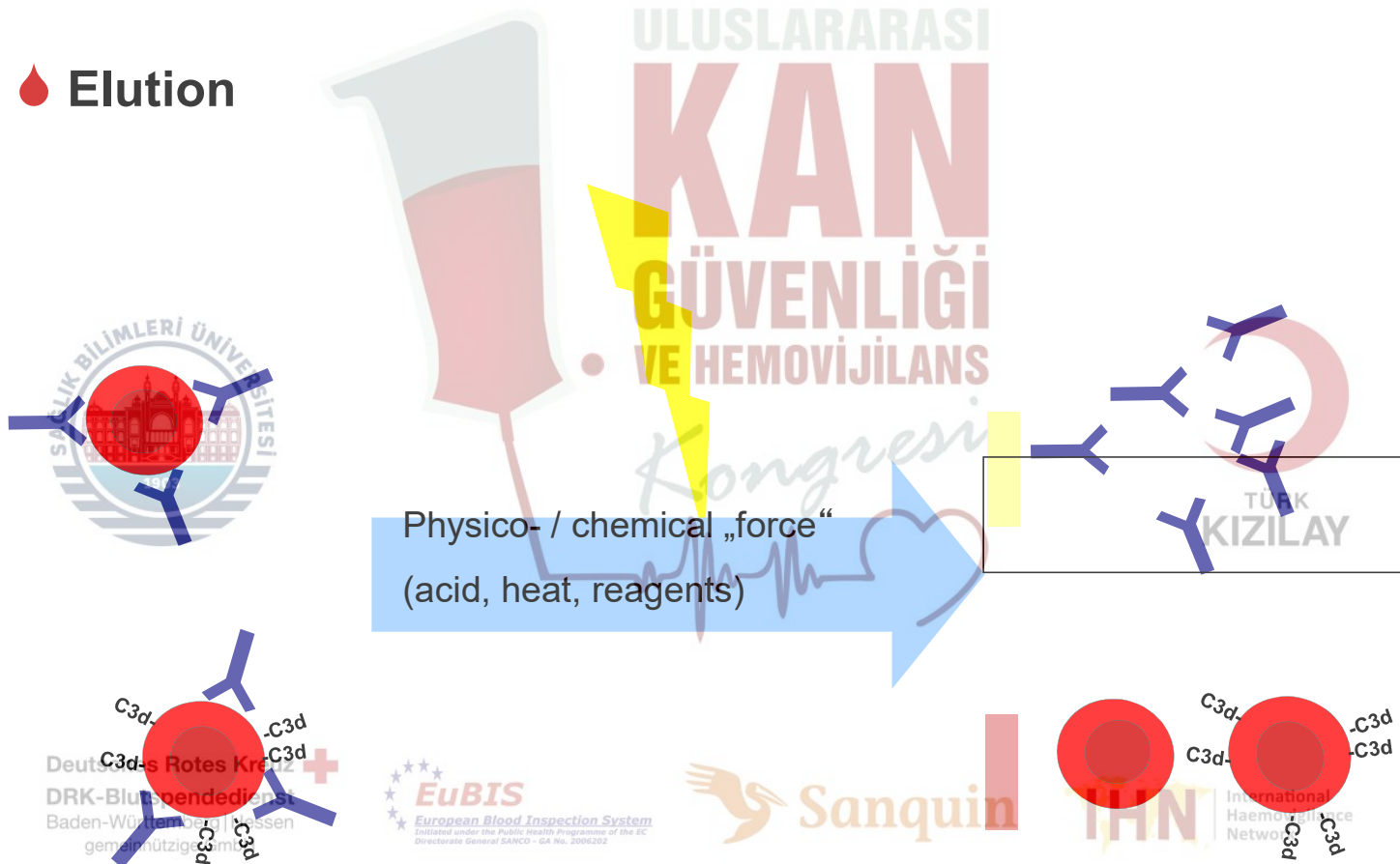
# Elution II

## 🔴 Elution

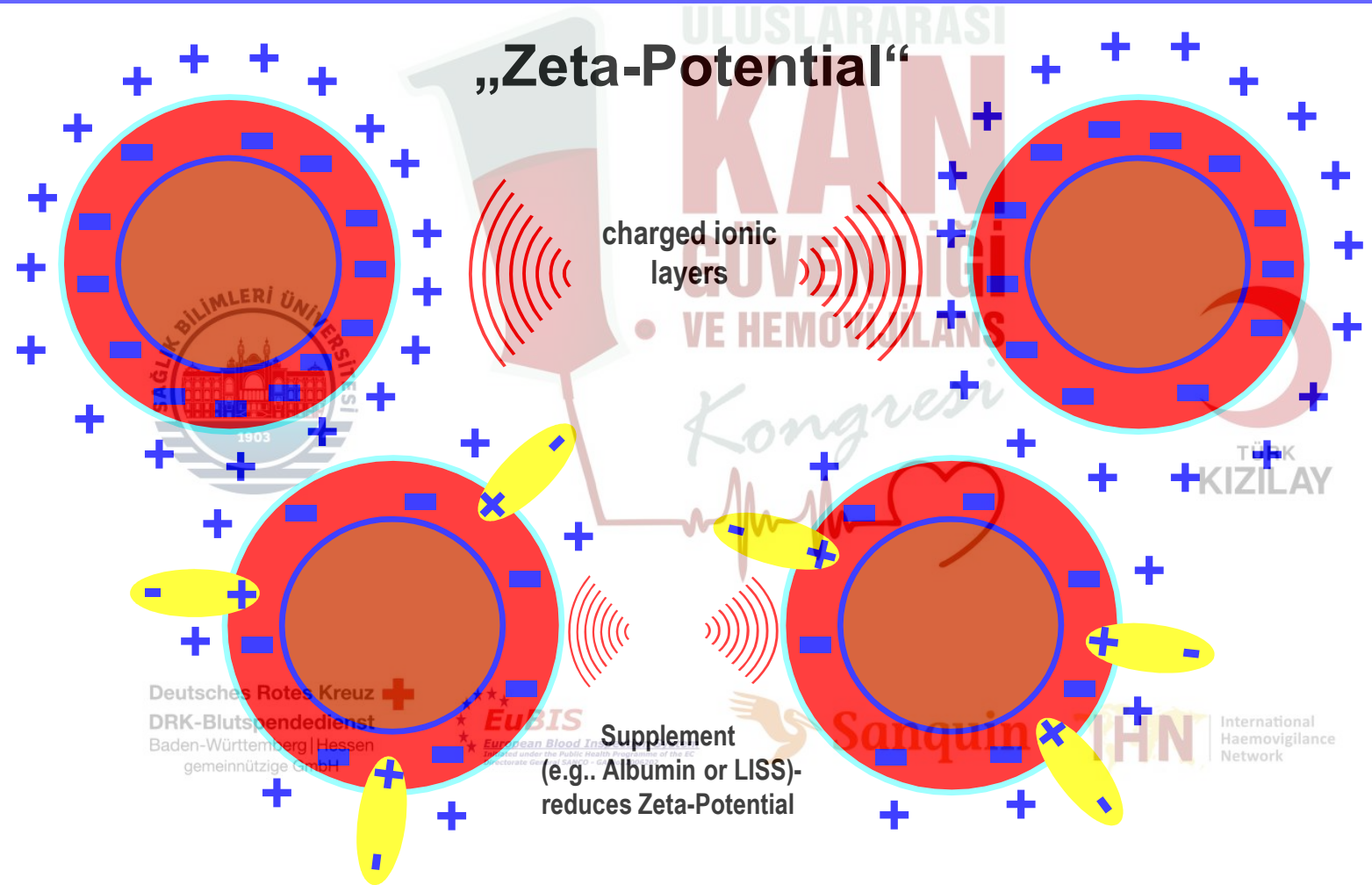


# Elution III

## 🔴 Elution



# RBC reject each other



# Antihumanglobuline test

Moreschi (1908): Anti-Protein-Sera

Coombs (1945): Antihumanglobuline test for the detection of incomplete ab

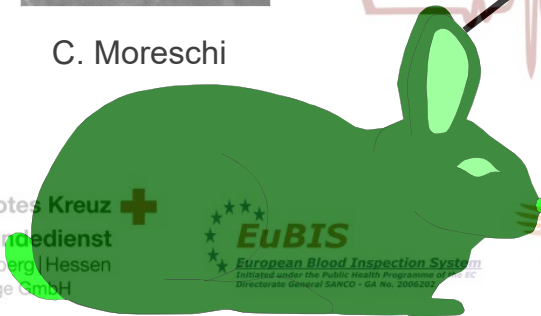


R. R. A. Coombs

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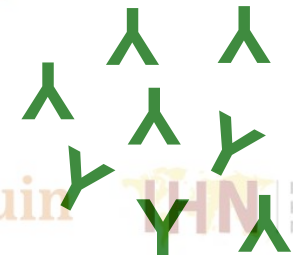
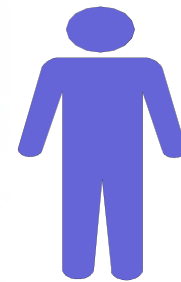


C. Moreschi



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VE HEMOVİJANS  
Kongresi



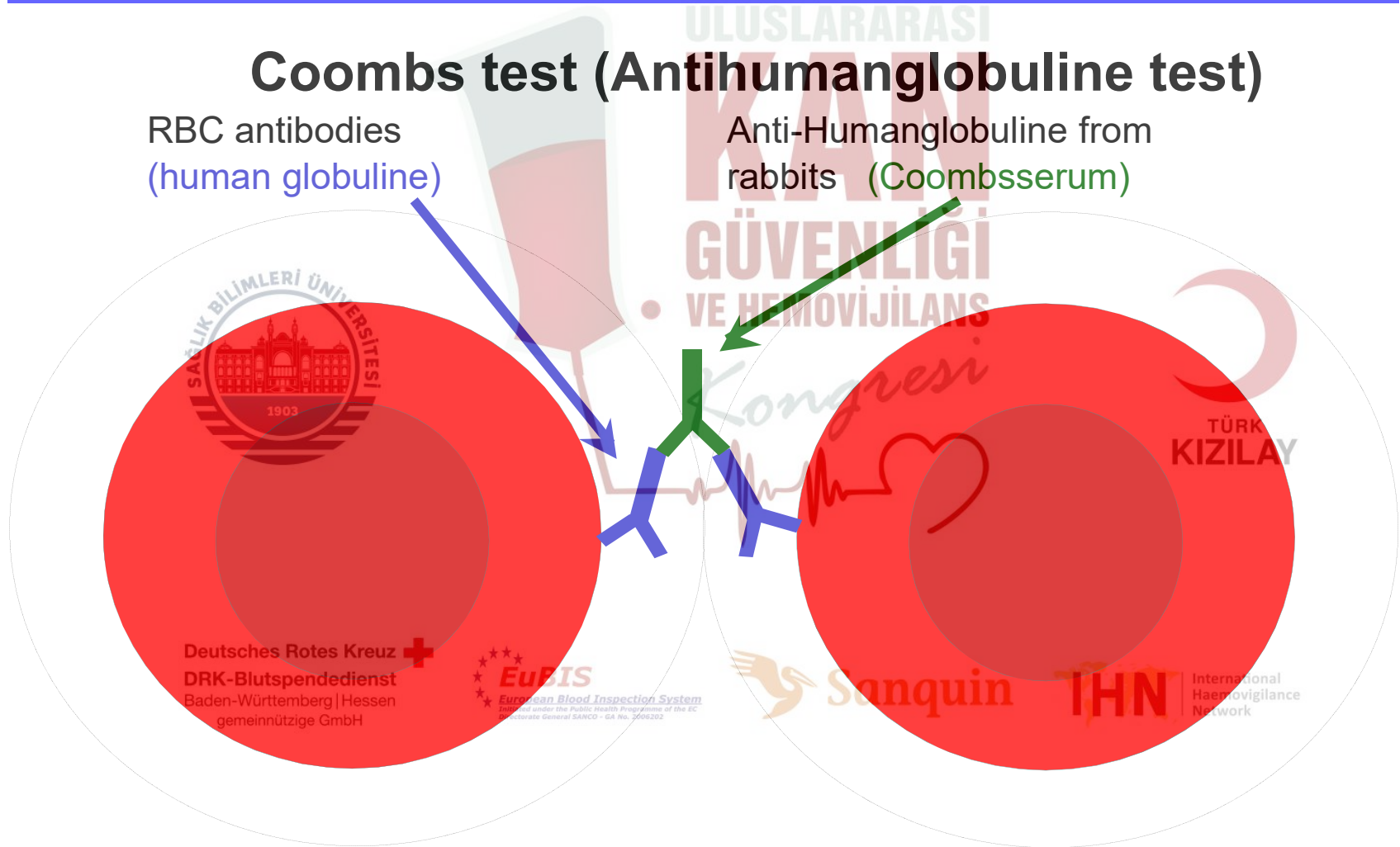
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International Haemovigilance Network

# Antihumanglobuline test

## Coombs test (Antihumanglobuline test)

RBC antibodies  
(human globuline)

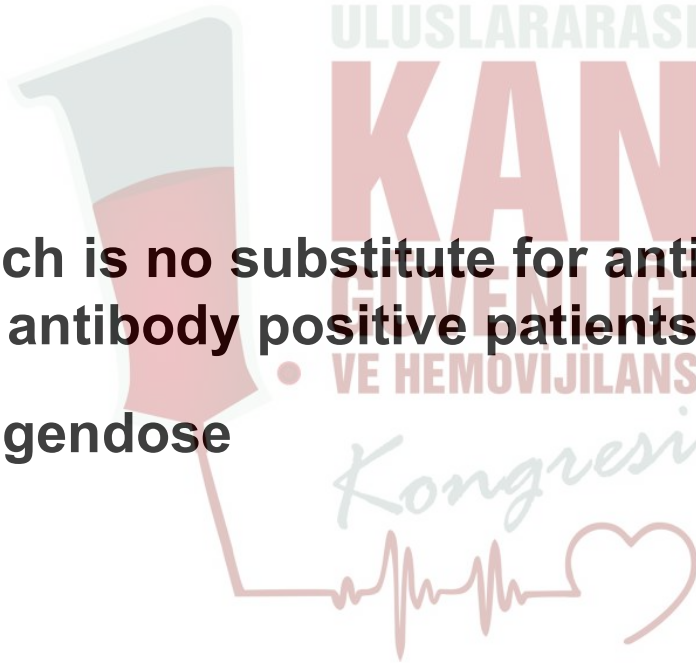
Anti-Humanglobuline from  
rabbits (Coombsserum)



# crossmatch

**Crossmatch is no substitute for antigen testing in antibody positive patients**

**Why: Antigendose**



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# Dose effect of blood group antigens

- Expression depends on genotype in various blood group systems

	Genotype	Antigens/RBC	Antigens/RBC
<b>Kidd (Jk)</b> 	(a+b-)	Jk (a) 10.000	Jk (b)
	(a-b+)	10.000	10.000
	(a+b+)	5.000	5.000
<b>Duffy (Fy)</b> 	(a+b-)	Fy (a) 14.000	Fy (b)
	(a-b+)	14.000	14.000
	(a+b+)	7.000	7.000
<b>Ss</b> 	SS <small>European Blood Inspection System Initiated under the Public Health Programme of the EC Directorate General SANCO - GA No. 2006202</small>	S 250.000	s
	ss	170.000	170.000
	Ss	125.000	85.000

# Compatibility testing

## Crossmatch / antibody screening (3 stage – tube test)

1.  
NaCl 22° C  
IgM

2.  
Suppl. 37° C  
IgM, IgG

3.  
Coombsphase  
IgG/Komplement

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# Compatibility testing

Relevance of antibodies according to reaction pattern

1. Stage  
NaCl; 22° C

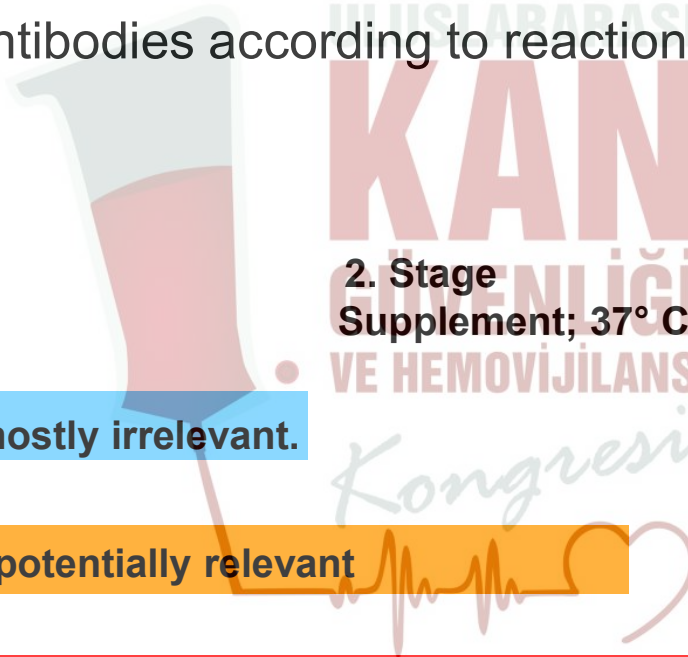
Cold reactive = mostly irrelevant.

Warm reactive = potentially relevant

Warm reactive = relevant

2. Stage  
Supplement; 37° C

3. Stage  
Coombsphase



# Irregular RBC antibodies

Clinically relevant:

Anti- ... —

C, c, D, E, e, C<sup>w</sup>

K, k, Kp<sup>a</sup>, Kp<sup>b</sup>, Js<sup>a</sup>, Js<sup>b</sup>

JK<sup>a</sup>, JK<sup>b</sup>

Fy<sup>a</sup>, Fy<sup>b</sup>

S, s, U

Lu<sup>b</sup>, Yt<sup>a</sup>

Vel, P, Tj<sup>a3</sup>

Co<sup>a</sup>, Di<sup>a</sup>, Di<sup>b</sup>, Do<sup>a</sup>, Do<sup>b</sup>,

und viele weitere

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Potentially relevant:

Anti- ...

A1, H, HI

M, N

Le<sup>a</sup>, Le<sup>b</sup>

P(1)

Xg<sup>a</sup>

Co<sup>b</sup>

Yt<sup>b</sup>

Lan

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Mostly irrelevant:

Anti- ...

Kälte-Ak (bei 37°C  
nicht wirksam)

N

P(1)

Sd<sup>a</sup>

Bg<sup>a</sup>, Bg<sup>b</sup>, Bg<sup>c</sup> (HLA)

HTLA: Chido, Rogers,



# Characteristic of irregular RBC antibodies

Antikörper	Herkunft		Antikörperaktivität in vitro			Verbunden mit		Kompatible Blute	
	natürlich	immun	NaCl	LISS/ Coombs	Enzym 37°	MHN	HTR	%*	Kompatibler Phänotyp
<b>Kell</b>									
Anti-K		ja	selten	meist	meist	ja	ja	91	kk
Anti-k		ja		meist	meist	ja	ja	0,2	KK
Anti-Kp <sup>a</sup>		ja		meist	einige	ja	ja	98	Kp(a-b+)
Anti-Kp <sup>b</sup>		ja		meist	meist	selten	ja	<0,1	Kp(a+b-)
Anti-Js <sup>a</sup>		ja		meist	einige	ja	ja	>99	Js(a-b+)
Anti-Js <sup>b</sup>		ja		meist	einige	ja	ja	<0,1	Js(a+b-)
<b>KIDD</b>									
Anti-Jk <sup>a</sup>		ja	selten	meist	meist	möglich	ja	23	Jk(a-b+)
Anti-Jk <sup>b</sup>		ja	selten	meist	meist	selten	ja	27	Jk(a+b-)
Anti-Jk <sup>3</sup>		ja	selten	meist	meist	ja	möglich	<0,1	Jk(a-b-)
<b>MNS</b>									
Anti-S	einige	ja	einige	meist	sehr selten	ja	ja	45	ss
Anti-s		ja	selten	meist	einige	ja	ja	11	SS
Anti-M	ja	einige	meist	einige	sehr selten	selten	einige	22	NN
Anti-N	ja	selten	meist	einige	sehr selten	nein	selten	28	MM
Anti-U	Deutsches Rotes Kreuz DRK-Blutspendedienst Baden-Württemberg   Hessen gemeinnützige GmbH	ja	selten	meist	meist	ja	ja	<1	S-s-U
<b>P</b>									
Anti-P, Anti-P <sup>k</sup>	ja	einige	meist	selten	meist	nein	selten	21	P, neg P <sup>k</sup>
Anti-Tj <sup>a3</sup> (P+P,+P <sup>k</sup> )	ja		meist	einige	einige	ja	ja	<0,1	p



# Compatibility testing

today:

- crossmatch often performed in column centrifugation test (equals 3. stage of tube testing)
- (difficult differentiation of warm-/cold reactive antibodies)

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TÜRK  
KIZILAY

# Antibody identification

Patient:	Case 1										Sample taken:											Method:	T = Tube								
Bloodgr.:	A1 Rh neg (ccddee), Kell neg.										Techn.:											Invest.-Date:					M = Micro column (Diamed)				
																						Sample:	S = Serum, E = Eluate								
																							22° 37° NaCl Sup. CT								
Test-																							St. 1	St. 2	St. 3	Co.-Co.					
Zellen	Rhesus							MNSs				P	Lewis		Lutheran		Kell				Duffy		Kidd		Xg						
	D	C	E	c	e	Cw	V	M	N	S	s	P1	Lea	Leb	Lua	Lub	K	k	Kpa	Jsa	Fya	Fyb	Jka	Jkb							
I	+	+	o	+	+	+	NT	+	+	o	+	+	o	+	o	+	o	+	o	o	+	o	o	+	o	+	NT	I	o	2	3
II	+	o	+	+	o	o	o	o	+	o	+	0	+	o	o	+	o	+	o	o	o	+	+	o	+	NT	II	o	2	3	
III	o	o	o	+	+	o	o	+	o	+	o	+s	o	+	+	+	+	+	o	o	o	+	+	o	+	NT	III	o	o	o	
1	o	o	o	+	+	o	o	o	+	+	+	+W	+	o	+	+	+	+	o	o	+	o	+	+	+	1	o	o	o		
2	o	o	o	+	+	o	o	+	+	o	+	+	o	o	+	+	+	+	o	o	+	+	o	+	o	2	o	o	o		
3	o	+	o	+	+	o	o	+	o	+	o	+s	o	+	+	+	+	+	o	o	+	+	o	+	+	3	o	o	o		
4	o	o	+	+	+	o	o	+	o	o	+	+	o	o	+	+	+	+	o	+	o	o	+	+	+	4	o	o	o		
5	o	o	o	+	+	o	o	+	o	+	o	+	o	+	+	+	+	+	o	o	+	+	+	+	+	5	o	o	o		
6	+	o	o	+	+	o	o	+	o	+	+	+s	o	o	+	+	+	+	o	o	o	o	+	o	+	6	o	2	3		
7	+	+	+	o	+	o	o	+	+	+	+	+W	+	o	+	+	+	+	o	o	o	+	+	o	o	7	o	2	3		
8	+	+	o	o	+	o	o	o	+	o	+	o	o	+	+	+	+	+	o	o	o	+	+	o	+	8	o	2	3		
9	+	+	o	o	+	+	o	+	o	o	+	+	o	+	+	+	+	+	o	o	+	o	+	+	o	9	o	2	3		
10	+	o	+	+	o	o	o	+	o	o	+	+	o	+	+	+	+	+	o	o	+	+	o	+	+	10	o	2	3		
11	+	+	o	+	+	o	o	+	o	o	+	+	o	+	+	+	+	+	o	o	+	+	o	+	+	11	o	2	3		
autolog																							AC	o	o	o					
																							A1	o	2	3					
																							A2	o	o	o	+				
																							B	4	3	3					

# Antibody identification

## -Checklist-

- Auto- or Allo-ab?
  1. Autolog. controll (AC) neg -> Allo-ab
  2. Patient is antigen negative
- Cold or warm reactive? (Stage 1, 2 oder 3)  
weak in 2., strong in 3. Stage -> warmreactice ab
- complete or incomplete reacting? (Stage 1 or 3)  
only in 3. Stage -> „incomplete“ ab

Conclusion:

Warm reactive, incomplete Allo-ab of the specificity XY

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				Invest.-Date:																															
					Sample: S = Serum, E = Eluate																														
					22° 37° NaCl Sup. CT																														
					Stufe 1 Stufe 2 Stufe 3 Co.-Co.																														
Test- Zellen	Rhesus															MNSs				P	Lewis		Lutheran		Kell				Duffy		Kidd				
	D	C	E	c	e	Cw	V	M	N	S	s	P1	Lea	Leb	Lua	Lub	K	k	Kpa	Jsa	Fya	Fyb	Jka	Jkb	Xg										
I	+	+	o	+	+	+	NT	+	+	o	+	+	o	+	o	+	o	+	o	o	+	o	o	+	NT	I	o	2	3						
II	+	o	+	+	o	o	o	o	+	o	+	o	+	o	o	+	o	+	o	o	o	+	+	o	NT	II	o	2	3						
III	o	o	o	+	+	o	o	+	o	+	o	+s	o	+	+	+	+	+	o	o	o	+	+	o	NT	III	o	o	o						
1	o	o	o	+	+	o	o	o	+	+	+	+W	+	o	+	+	+	+	o	o	+	o	o	+	+	1	o	o	o						
2	o	o	o	+	+	o	o	+	+	o	+	+	o	o	o	+	+	+	o	o	o	+	+	o	o	2	o	o	o						
3	o	+	o	+	+	o	o	+	o	+	o	+s	o	+	o	+	o	+	o	o	+	+	o	+	+	3	o	o	o						
4	o	o	+	+	+	o	o	+	o	o	+	+	o	o	o	+	+	+	o	+	o	o	+	+	+	4	o	o	o						
5	o	o	o	+	+	o	o	+	o	+	o	+	o	+	o	+	o	+	+	o	o	+	+	+	+	5	o	o	o						
6	+	o	o	+	+	o	o	+	o	+	+	+s	o	o	o	+	o	+	o	o	o	o	+	o	+	6	o	2	3						
7	+	+	+	o	+	o	o	+	+	+	+	+W	+	o	o	+	o	+	o	o	o	+	+	o	o	7	o	2	3						
8	+	+	o	o	+	o	o	o	+	o	+	o	o	+	o	+	+	+	o	o	o	+	+	o	+	8	o	2	3						
9	+	+	o	o	+	+	o	+	o	o	+	+	o	+	o	+	+	+	o	o	+	o	+	+	o	9	o	2	3						
10	+	o	+	+	o	o	o	+	o	o	+	+	o	+	o	+	+	+	o	o	+	+	o	+	+	10	o	2	3						
11	+	+	+	+	+	o	o	+	o	+	+	+	o	+	o	+	+	+	o	o	+	+	o	+	+	11	o	2	3						
autolog	gemeinnützige GmbH																																		
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	D	C	E	c	e	Cw	V	M	N	S	s	P1	Lea	Leb	Lua	Lub	K	k	Kpa	Jsa	Fya	Fyb	Jka	Jkb	Xg					
I	+	+	o	+	+	+	NT	+	+	o	+	+	o	+	o	+	o	+	o	o	+	o	o	+	+	NT	I	o	2	3
II	+	o	+	+	o	o	o	o	+	o	+	0	+	o	o	+	o	+	o	o	o	+	+	o	o	NT	II	o	2	3
III	o	o	o	+	+	o	o	+	o	+	o	+S	o	+	+	+	+	+	o	o	o	+	+	o	o	NT	III	o	o	o
1	o	o	o	+	+	o	o	o	+	+	+	+W	+	o	+	+	+	+	o	o	+	o	+	+	+	+	1	o	o	o
2	o	o	o	+	+	o	o	+	+	o	+	+	o	o	o	+	+	+	+	o	o	+	+	o	o	o	2	o	o	o
3	o	+	o	+	+	o	o	+	o	+	o	+S	o	+	o	+	o	+	o	o	+	+	o	+	+	+	3	o	o	o
4	o	o	+	+	+	o	o	+	o	o	+	+	o	o	+	+	+	+	o	+	o	o	+	+	+	+	4	o	o	o
5	o	o	o	+	+	o	o	+	o	+	o	+	o	+	o	+	o	+	+	o	o	+	+	+	+	+	5	o	o	o
6	+	o	o	+	+	o	o	+	o	+	+	+S	o	o	o	+	o	+	o	o	o	o	+	o	+	6	o	2	3	
7	+	+	+	o	+	o	o	+	+	+	+	+W	+	o	o	+	o	+	o	o	o	+	+	o	o	7	o	2	3	
8	+	+	o	o	+	o	o	o	+	o	+	o	o	+	o	+	+	+	o	o	o	+	+	o	+	8	o	2	3	
9	+	+	o	o	+	+	o	+	o	o	+	+	o	+	o	+	+	+	o	o	+	o	+	+	+	9	o	2	3	
10	+	o	+	+	o	o	o	+	o	o	+	+	o	+	o	+	+	+	o	o	+	+	+	+	+	10	o	2	3	
11	+	+	+	+	+	o	o	+	o	o	+	+	o	+	o	+	+	+	o	o	+	+	+	+	+	11	o	2	3	
autolog																										EP	o	o	o	
																											A1	o	2	3
																											A2	o	o	o
																											B	4	3	3

# Antibody identification

## -checklist-

- Auto- or Allo-ab? (AC, DAT? Antigenstatus of the Pt.?) ✓
- cold or warm reactive? (Stage 1, 2 or 3) ✓
- complete or incomplete reacting? (Stage 1 or 3) ✓
- identification of the specificity of the antibody ✓
- exclusion of antibodies against other antigens ✓

# DRK-BSD Identification RedCells

IVD CE 0344

LOT DRK-BSD IRC-0024 DRK-BSD IRC-0024P

2017-01-09



System			Rh - Hr					Kell				Duffy		Kidd		Lewis		P	MNS				Xg	Lutheran			Dombrock		Auberger		Results						
Rh - Hr	Component Lot		D	C	c	E	e	C <sup>w</sup>	K	k	Kp <sup>a</sup>	Kp <sup>b</sup>	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Le <sup>a</sup>	Le <sup>b</sup>	P <sub>1</sub>	M	N	S	s	Xg <sup>a</sup>	Lu <sup>a</sup>	Lu <sup>b</sup>	Do <sup>a</sup> *	Do <sup>b</sup> *	Au <sup>a</sup> *	Au <sup>b</sup> *	Spezial-Antigene Special types	IAT	Enzyme				
1	C*CD.ee R1* <sup>w</sup> R1	70116366386	+	+	0	0	+	+	0	+	0	+	+	0	+	0	+	0	0	0	+	0	+	+	0	+	+	+	+	+	+	+	+	+	Yk(a+ <sup>w</sup> ) <sup>\$</sup>	1	
2	CCD.ee R1R1	70116445550	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	+	+	+	0	+	+	+	0	+	+	+	+	0				Bg+ <sup>\$</sup> , Lu14+ <sup>*</sup>	2		
3	ccD.EE R2R2	70216289296	+	0	+	+	0	0	0	+	0	+	+	0	+	0	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	+	+		3		
4	ccD.ee Ror	70116489708	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+	+	+	+	+	+	+	+		4		
5	Ccddee r'r	70116347378	0	+	+	0	+	0	0	+	0	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	0	+	+	+	+	+		5			
6	ccddEe r'r	70216225861	0	0	+	+	+	0	+	+	0	+	+	+	+	0	0	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+		6			
7	ccddee rr	70416183562	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+	+	0	+	+	+	+	+	+	+	+	+	+	+		7			
8	ccddee rr	70116366349	0	0	+	0	+	0	0	+	+	+	+	0	+	+	0	+	0	0	+	+	0	+	0	+	+	+	+	0	0					8	
9	ccddee rr	70416383522	0	0	+	0	+	0	0	+	0	+	0	+	+	+	0	+	0	+	0	+	+	+	+	0	+	+	+	+	+	+		9			
10	ccddee rr	70116469213	0	0	+	0	+	0	0	+	0	+	+	0	+	+	0	+	0	0	+	+	+	+	0	+	0	+	+	0				Di(a+ <sup>s</sup> ), Lu14+ <sup>*</sup>	10		
11	ccddee rr	70116407009	0	0	+	0	+	0	0	+	+	+	0	+	0	+	0	0	0	+	0	+	0	+	0	+	+	+	+	+	+	+		11			
Patientenzellen Patient's cells																																			Patientenzellen Patient's cells		

\* / \$ Siehe Anmerkungen auf der Rückseite / Refer to remarks on the reverse

Name:

Deutscher Rotes Kreuz  
DRK-Blutspendedienst  
Baden-Württemberg | Hessen  
gemeinnützige GmbH

Blutgruppe:  
Blood group:

Interpretation:

**EuBIS**  
European Blood Inspection System  
Initiated under the Public Health Programme of the EC  
Directorate General SANCO - GA No. 2006202

**Sanquin**

Datum / Unterschrift:  
Date / Signature:

International  
Haemovigilance  
Network

# Antibody identification: further methods

- prewarming technique (“**strict warmth**“):  
IAT with prewarmed (37 °C) pt. Plasma and test cells  
(sometimes also gel cards):

Principle: prewarming avoids reacting of cold reacting antibodies with increased temperature altitude


Differentiation between “normal” warm reacting allo-ab (IgG) und “cold reacting ab (IgM) with increased temperature amplitude

Deutsches Rotes Kreuz   
DRK-Blutspendedienst  
Baden-Württemberg | Hessen  
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 **Sanquin**

 **IHN** International  
Haemovigilance  
Network

  
TÜRK  
KIZILAY

## Methods: Enzyme – treatment of testcells:

Specificity	Ficin/Papain	Trypsin	$\alpha$ -Chymo- trypsin	0.2 M DTT/AET*
Lu	pos	neg	neg	Weak/neg
Yt	neg	pos	neg	Weak/neg
M,N Ge2, 3, 4	neg (Ge3 +)	neg	pos	pos
S, s, Fy(a), Fy(b)	neg	pos	neg	pos
Kel / LW	pos	pos	pos	neg
Kn/Yk	?	neg	neg	neg
Ch/Rg	neg	neg	neg	pos
JMH	neg	neg/±	neg	neg
Cs(a)	pos	pos	pos	pos
Jr(a)	mf	pos	pos	pos
Do	pos	neg	weak	neg
ABO, Rh, U, P, U, Di	pos	pos	pos	pos
Fy3, P, Vel, Di	pos	pos	pos	pos

\* Aminoethylisothiuronium bromide. Immunohematology 1992 vol.8 nr.3 / Factsbook M. Reid

# Antibody identification: further methods

- „**Super-Coombs**“: IAT with enzym treated Cells

Suspicion of weal allo-ab, e.g. Kidd-ab

**Advantage:** very high sensitivity

**Disadvantage:** unspecific positivity due to cold reacting autoantibodies

# Antibody identification: further methods

- **REST** (rabbit erythrocyte stroma)-**Absorption**

In high titer cold reacting auto antibodies

principle: Incubation of Plasma with REST,  
cold reacting auto ab are adsorbed  
warm reacting ab remain

centrifugation: use of supernatant for further ab  
identification

# Antibody identification: further methods

- **HPC** (human platelet concentrate)-**Absorption**

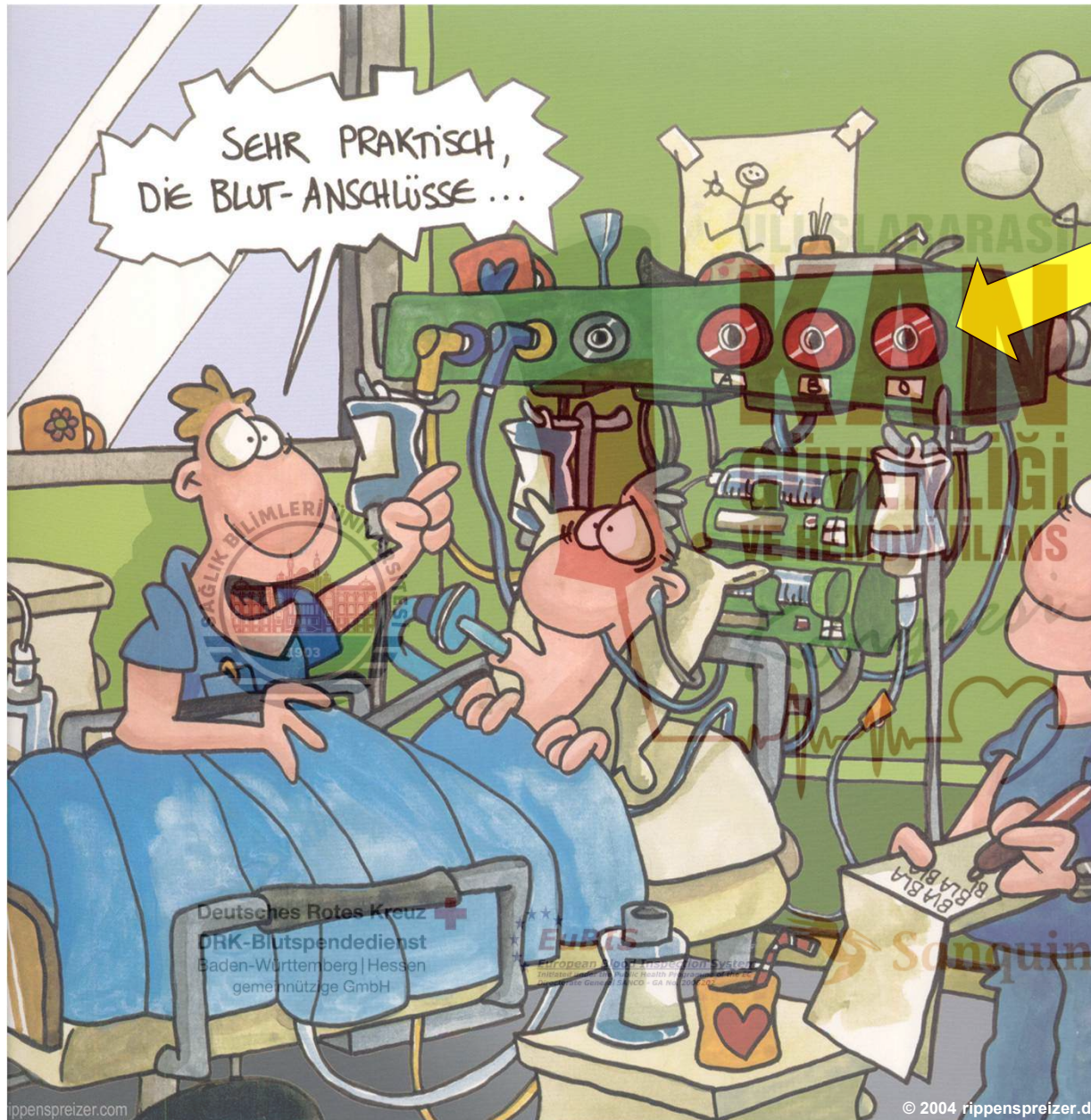
In accompanying HLA-ab (Bennet – Goodspeed ab)

principle: Incubation v. Plasma with HPC,  
HLA-ab are absorbed, anti-RBC-ab remain  
centrifugation: use of supernatant for further ab  
identification

# Antibody identification: further methods

- **AB-Serum-Inhibition**

In suspicion of HTLA-AK (Rogers, Chido) with characteristic reaction pattern (high titer, low avidity)  
principle: Mixing of Pt.-Plasma with AB-Serum, HTLA-ab will be inhibited (bound to soluble antigen)  
(Controll: Mixing with NaCl)



**Bluttransfusion  
2020 ??**



**Thank you  
For your  
attention!**

International  
Haemovigilance  
Network