Immunohematology Case Studies 2016 - 5

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Clinical History

A 78 yrs old patient had coronary artery bypass surgery.

In the perioperative period, she received 14 red cell units (A Rh pos) within 1 week.

Antibody screen remained negative throughout.

Three weeks later, she had severe pneumonia and was on artificial ventilation.
An antibody screen was requested when the patient had hemoglobin 8 g/dl.

This antibody screen was positive.

Serologic History

Initial presentation:

Blood type A

Rh: D+ C+c+E-e+ (CcDee, R1r)

Kell: K-

Antibody screen (3 cells): negative (IAT + neutral)

All transfused units were A CcDee K-

Current Sample Presentation Data

ABO/Rh: A Rh pos

DAT: negative

Antibody Screen Method: IAT (BioRad ID gel)

Antibody Screen Results: positive (0.5 to 1+)

Antibody Identification Method: IAT (BioRad ID gel)

Antibody Identification Preliminary Results:

Almost all cells weak positive, autocontrol negative

Challenge with the Current Presentation

Based on the initial panel this case is of category “autocontrol negative / almost all cells positive”

This constellation suggests that transfused red blood cells might not be compatible and requires further, often laborious work-up.

Panel

			Rh-Hr									Kell						Duffy		Kidd		Lewis		P	MNS			Luther.		Xg		
	Rh-Hr	ID	C	D	E	c	e	C^w	f	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P1	M	N	S	s	Lu^a	Lu^b	Xg^a		
1	$R_1^wR_1$	170744	+	+	0	0	+	+	/	/	0	+	0	+	/	+	0	+	0	0	0	0	0	+	+	+	+	0	+	+	Co(b+)	+
2	R_1R_1	331530	+	+	0	0	+	0	/	/	+	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	0	0	+	+	Bg(a+) Do(a+b+)	+
3	R_2R_2	384848	0	+	+	+	0	0	/	/	+	+	0	+	0	+	+	+	0	+	+	0	+	0	+	+	+	0	w	0	Bg(a+)	+
4	R_0	074858	0	+	0	+	+	0	/	/	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	+	0	+	+	Co(b+)	+
5	r'r'	1783510	+	0	0	0	+	0	/	/	0	+	0	+	/	+	0	+	+	+	0	+	+	0	+	+	+	0	+	+		+
6	r'r''	732530	0	0	+	+	0	0	/	/	0	+	0	+	/	+	0	+	+	+	0	+	+	+	0	+	0	0	+	+		+
7	rr	329238	0	0	0	+	+	0	/	/	0	+	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	+	0	+
8	rr	303099	0	0	0	+	+	0	/	/	0	+	+	+	0	+	+	0	0	+	0	+	+	+	+	0	+	0	+	+	0	+
9	rr	1703902	0	0	0	+	+	0	/	/	+	0	0	+	/	+	+	0	+	+	0	+	0	+	+	+	0	+	+	+		+
10	rr	331559	0	0	0	+	+	0	/	/	0	+	0	+	0	+	+	+	+	0	0	+	+	0	+	0	0	0	+	0	Bg(a+)	+
11	$R_2R_1^w$	356770	+	+	+	0	+	+	/	/	0	+	0	+	0	+	0	+	+	+	+	0	+	+	+	0	+	0	+	0		+
12	R_2R_2	202024	w	+	+	+	0	0	/	/	+	+	0	+	0	+	+	0	+	+	+	0	+	0	+	0	+	0	+	+		+
13	rr	143225	0	0	0	+	+	0	/	/	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	+	+	+	Bg(a+)	+
14	r'^wr	1779780	+	0	0	+	+	+	/	/	+	+	0	+	/	+	+	0	+	0	0	+	0	0	+	0	+	0	+	+		+
15	R_2r	1718113	0	+	+	+	+	0	/	/	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	+	+	0	+		+
16	rr	382612	0	0	0	+	+	0	/	/	+	+	0	+	0	+	+	+	+	0	0	+	0	+	+	0	+	0	+	+	Wr(a+) Bg(a+)	+

All cells positive independent of the antigen constellation!

Challenge with the Current Presentation

The most frequent causes for the “autocontrol negative / almost all cells positive” pattern are:

- An antibody to an antigen of high prevalence
- A combination of antibodies to “the usual” antigens
- An antibody interfering with the commercial cell preparation
- An autoantibody with a suppressed autoantigen

→ The laboratory did some additional work

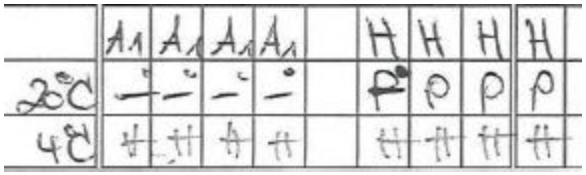
Panel

No. Nr. N° Szám Br.	Rh	Donor Spender Donneur Donatore Donante Davatelj Doador Giver	Ag = antigen Other Andere / Autres Altri / Otros Andet / Egyéb Ostalo / Outros Andre	Rhesus								MNS				P	Lewis		Lutheran		Kell				Duffy		Kidd		Sex linked	No. Nr. N° Szám Br.
				D	C	E	c	e	f	C^w	V	M	N	S	s	P_1	Le^a	Le^b	Lu^a	Lu^b	K	k	Kp^a	Js^a	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	
SC I*																													SC I*
SC II*																													SC II*
SC III*																													SC III*
1	rr	M3284TR		0	0	0	+	+	+	0	NT	0	+	0	+	0	+	0	0	+	0	+	0	0	0	+	+	+	+	1
2	rr	M3133CC		0	0	0	+	+	+	0	0	+	0	+	+	+*	0	+	0	+	+	+	0	0	0	+	+	+	0	2
3	r'r	M3266CC		0	+	0	+	+	+	0	NT	+	+	+	0	+	0	+	0	+	0	+	0	0	+	+	0	+	+	3
4	r"r	M3440HM		0	0	+	+	+	+	0	NT	+	+	+	+	+	0	+	0	+	0	+	0	0	+	+	0	+	+	4
5	rr	M3035HM	Kp(a+)	0	0	0	+	+	+	0	0	+	+	+	+	+	0	+	0	+	0	+	+	0	+	+	+	0	+	5
6	R_oR	M3233TR	Co(b+)	+	0	0	+	+	+	0	NT	+	0	+	+	0	+	0	0	+	0	+	0	0	+	0	+	+	+	6
7	R_1R_1	M3199CC		+	+	0	0	+	NT	0	NT	+	+	+	+	+	+	0	+	+	0	+	0	0	+	+	+	0	+	7
8	R_1R_1	M2145HM		+	+	0	0	+	NT	0	NT	+	0	+	+	+	0	+	0	+	+	+	0	0	+	+	0	+	0	8
9	R_1R_1"	M596CC		+	+	0	0	+	NT	+	NT	0	+	0	+	0	0	+	0	+	+	+	0	0	+	0	0	+	+	9
10	R_1R_2	M3358HE		+	0	+	+	0	NT	0	NT	+	+	+	+	+	0	+	0	+	0	+	0	0	+	+	0	+	+	10
11	r'r	M3344CC		0	+*	0	+	+	+	0	NT	+	+	+	+	+	0	0	0	+	0	+	0	0	0	+	+	+	+	11
Auto																													Auto

In another panel, there were some weak to negative cells
There was no obvious correlation with the antigen pattern

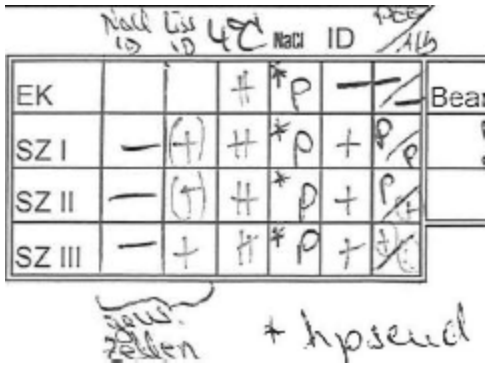
note: antigen typing was considered confounded by recent massive transfusion

Influence of ABO type and test conditions



Agglutination at 20° C and 4° C
with 4 test cells type A1 and 4 test cells type O (“H“)

There was no correlation with ABO type



Autocontrol („EK“) and reactivity with 3 cell antibody screen in the following techniques:
Neutral (NaCl), Liss, 4° C saline, standard ID, PEG and Albumine

In the cold, there was non-specific reactivity.
Albumin-IAT was positive

Panel

	SYSTEM	Rh - Hr							Kell						Duffy		Kidd		Lewis		P	MN				Lutheran		Xg	Special Antigen Type
	Donor	D	C	c	E	e	V	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*}	
1	R1wR1 B4749	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	0	
2	R1R1 B9193	+	+	0	0	+	0	0	+	+	0	+	0	+	+	0	+	+	+	0	+	+	0	+	0	0	+	0	
3	R1R1 B7357	+	+	0	0	+	0	0	0	+	0	+	0	+	0	+	+	0	0	+	0	+	0	+	0	0	+	+	Sc:2
4	R1R1 B8224	+	+	0	0	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	0	0	+	+	+	0	+	+	
5	RzR1 A3909	+	+	0	+	+	0	0	0	+	0	+	0	+	+	+	+	+	+	0	+	0	+	+	+	0	+	+	
6	RzR2 A4210	+	W	+	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	+	+	+	0	+	+	0	+	0	
7	R2R2 C1461	+	0	+	+	0	0	0	0	+	0	+	0	+	+	+	0	+	0	+	+	0	+	0	+	0	+	+	Yt(b+)
8	R2R2 C6003	+	0	+	+	0	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+	+	+	0	+	+	
9	R2R2 C6000	+	0	+	+	0	0	0	0	+	0	+	0	+	+	+	+	+	+	0	+	+	0	0	+	0	+	+	
10	R1wR1 B7306	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	0	+	+	0	0	+	+	+	0	0	+	+	
		D	C	c	E	e	V	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*}	
11	r'r E974	0	+	+	0	+	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	0	+	0	+	0	+	+	
12	r''r F660	0	0	+	+	+	0	0	+	0	0	+	0	+	+	0	0	+	0	+	+	+	+	0	+	0	+	+	
13	rr H1352	0	0	+	0	+	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	0	+	+	+	0	+	+	
14	r'r E621	0	+	+	0	+	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	+	0	+	0	0	+	+	
15	rr N4189	0	0	+	0	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	
16	rr H1720	0	0	+	0	+	0	0	0	+	0	+	0	+	+	0	+	0	0	0	+	+	+	0	+	+	+	+	
17	rr G1327	0	0	+	0	+	0	0	+	0	0	+	0	+	+	+	+	+	0	+	+	+	+	0	+	0	+	0	
18	rr H1721	0	0	+	0	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	0	0	+	0	+	0	+	+	
19	rr N4010	0	0	+	0	+	0	0	0	+	0	+	0	+	0	+	+	+	0	+	0	0	+	+	0	0	+	+	
20	Ror D1196	+	0	+	0	+	+	0	0	+	0	+	0	+	0	0	+	+	+	0	+	+	+	0	+	0	+	+	

A third panel was done but did not give much additional information

Challenge with the Current Presentation

Based on the three panels, the following explanations are unlikely:

- An antibody to an antigen of “really” high prevalence, like Vel, Lu^b, Kp^b, Yt^a
  - For these antigens, all cells would be expected to be positive
  - In addition, there was no correlation Lu^a and Kp^a status
- A combination of antibodies to “the usual” antigens
  - In that case, a pattern would be expected

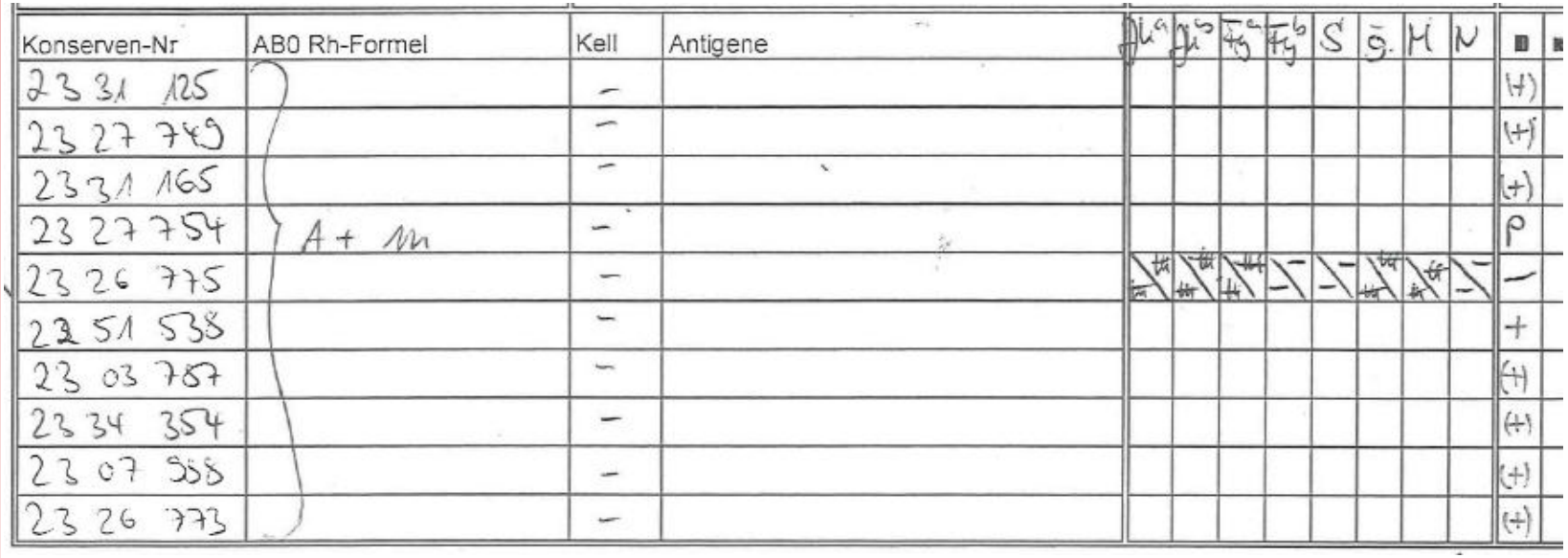
Crossmatch

Konserven-Nr	ABO Rh-Formel	Kell	Antigene	Jk^a	Jk^b	Fy^a	Fy^b	S	s	M	N	
23 31 125	A+ M	–										(+)
23 27 749		–										(+)
23 31 165		–										(+)
23 27 754		–										P
23 26 775		–		tr	tr	tr	–	–	tr	tr	–	–
23 51 538		–										+
23 03 787		–										(+)
23 34 354		–										(+)
23 07 558		–										(+)
23 26 773		–										(+)

Crossmatches with ABO / RH compatible units (all A R1r) were mostly positive:

Only 2 of 40 units were negative

Crossmatch



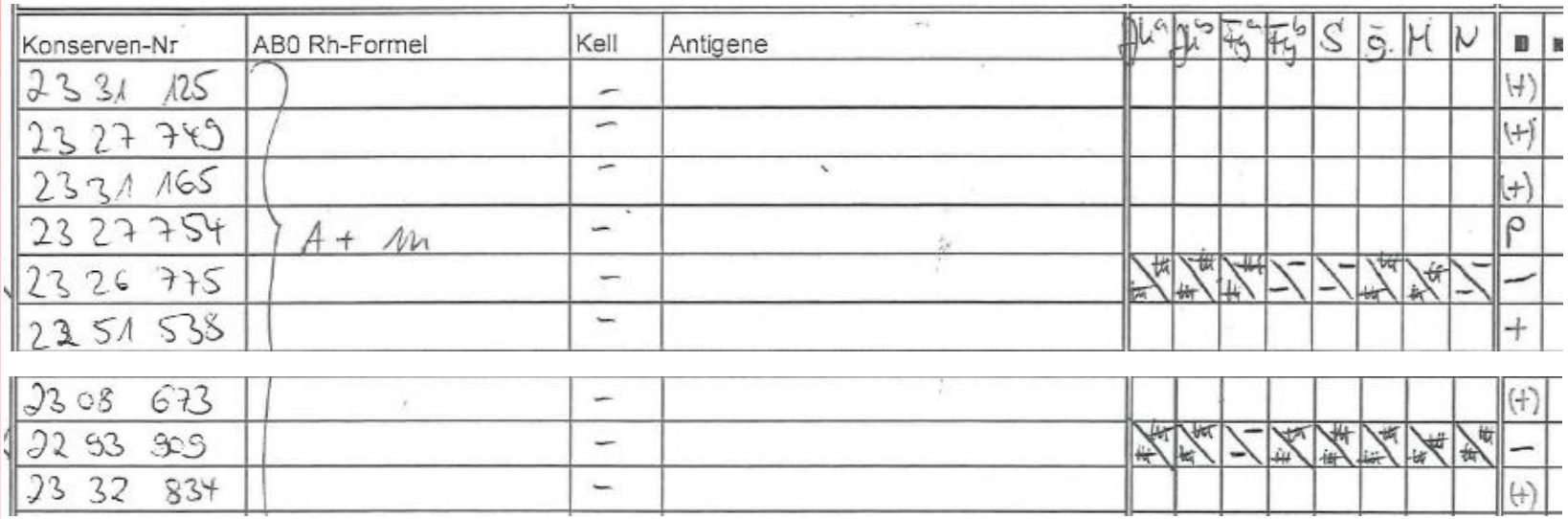
Konserven-Nr	ABO Rh-Formel	Kell	Antigene	Jk^a	Jk^b	Fy^a	Fy^b	S	s	M	N	
23 31 125		–										(+)
23 27 749		–										(+)
23 31 165		–										(+)
23 27 754	A+ M	–										P
23 26 775		–		th	th	th	–	–	th	th	–	–
23 51 538		–										+
23 03 757		–										(+)
23 34 354		–										(+)
23 07 558		–										(+)
23 26 773		–										(+)

Crossmatches with ABO / RH compatible units (all A R1r) were mostly positive:

Only 2 of 40 units were negative

The positive crossmatch with ABO-identical cells is an argument against
- antibodies to commercial test cells
- antibodies to blood O cells (e.g. anti-IH)

Crossmatch



Konserven-Nr	AB0 Rh-Formel	Kell	Antigene	Jk^a	Jk^b	Fy^a	Fy^b	S	s	M	N	
23 31 125		–										(+)
23 27 749		–										(+)
23 31 165		–										(+)
23 27 754	A + M	–										P
23 26 775		–		+	+	+	–	–	+	+	–	–
23 51 538		–										+
23 08 673		–										(+)
22 93 909		–		+	+	–	+	+	+	+	+	–
23 32 834		–										(+)

The two compatible units differed in their antigen pattern

- Another argument against a combination of antibodies to “conventional“ antigens

Interim Antibody Identification
Possible Answers and Next Steps

The laboratory suspected an antibody to an antigen of higher to high frequency.

Therefore, two additional tests were done:

(1) Cross-match with rare cells

(2) Neutralization with plasma

Crossmatch with rare cells

Konserven-Nr	AB0 Rh-Formel		Kell	Antigene									
Yt^a-ø-Zelle	BG: O	Nr.	160										+
Yt^a-ø-Zelle	BG: O	"	474										+
Yk^a-ø-Zelle	BG: O	"	1										+
Yk^a-ø-Zelle	BG: A	"	22										+
Kn^a-ø-Zelle	BG: A	"	493										+
Kn^a-ø-Zelle	BG O	"	525										+
Kp^b-ø-Zelle	BG: O	"	491										+
Kp^b-ø-Zelle	BG: O	"	529										+
Lan-ø-Zelle	BG: O	"	323										(+)
Lan-ø-Zelle	BG: O	"	542										+

All available rare cells showed a positive cross-match

Crossmatch with rare cells

Konserven-Nr	AB0 Rh-Formel		Kell	Antigene									D
Yt^a-ø-Zelle	BG: O	Nr.	160										+
Yt^a-ø-Zelle	BG: O	"	474										+
Yk^a-ø-Zelle	BG: O	"	1										+
Yk^a-ø-Zelle	BG: A	"	22										+
Kn^a-ø-Zelle	BG: A	"	493										+
Kn^a-ø-Zelle	BG O	"	525										+
Kp^b-ø-Zelle	BG: O	"	491										+
Kp^b-ø-Zelle	BG: O	"	529										+
Lan-ø-Zelle	BG: O	"	323										(+)
Lan-ø-Zelle	BG: O	"	542										+

All available rare cells showed a positive cross-match

The ability to identify an antibody to an antigen of higher frequency heavily depends on the "rare" cells available in a laboratory.

In the case shown, only Yt^a, Yk^a, Kn^a, Kp^b and Lan were tested.
Yk^a and Kn^a have a frequency of 98% resp. 92%
and would have fitted the "all positive with some negative" pattern.

Neutralization with plasma

Makropanel 16-P LOT 8000227400 CE 0344
Cellbind ID16 LOT 8000227409 CE 0344
Cellbind ID16-P LOT n.a. CE 0344
Column panel 16 LOT 8000227405 CE 0344

IVD 2016-05-13

Sanquin Reagents
Plesmanlaan 125
1066 CX Amsterdam
The Netherlands

	Rh-Hr	ID	Rh-Hr: C	D	E	c	e	C^w	C^x	V	Kell: K	k	Kp^a	Kp^b	Js^a	Js^b	Duffy: Fy^a	Fy^b	Kidd: Jk^a	Jk^b	Lewis: Le^a	Le^b	P	MNS: M	N	S	s	Luther.: Lu^a	Lu^b	Xg^a				
1	$R_1^wR_1$	170744	+	+	0	0	+	+	/	/	0	+	0	+	/	+		0	+	0	0	0	0				+	0	+	+	Co(b+)			(+)	(+)
2	R_1R_1	331530	+	+	0	0	+	0	/	/	+	+	0	+	0	+		+	0	+	0	+	+				0	0	+	+	Bg(a+)	Do(a+b+)		+	+
3	R_2R_2	384848	0	+	+	+	0	0	/	/	+	+	0	+	0	+		+	0	+	+	0	+				+	0	w	0	Bg(a+)			+	+
4	R_0	074858	0	+	0	+	+	0	/	/	0	+	0	+	0	+		0	0	+	0	+	0				+	0	+	+	Co(b+)			+	+
5	r'r'	1783510	+	0	0	0	+	0	/	/	0	+	0	+	/	+		0	+	+	0	+	+				+	0	+	+				+	+
6	r'r''	732530	0	0	+	+	0	0	/	/	0	+	0	+	/	+		0	+	+	0	+	+		0		+	0	+	+				+	+
7	rr	329238	0	0	0	+	+	0	/	/	0	+	0	+	0	+		0	+	+	0	+	+		0		+	0	+	0				+	+
8	rr	303099	0	0	0	+	+	0	/	/	0	+	+	+	0	+		0	0	+	0	+	+		0		+	0	+	0				+	+
9	rr	1703902	0	0	0	+	+	0	/	/	+	0	0	+	/	+		0	+	+	0	+	0		0		+	+	+	+				+	+
10	rr	331559	0	0	0	+	+	0	/	/	0	+	0	+	0	+		+	+	0	0	+	+		0		+	0	+	0	Bg(a+)			+	+
11	$R_zR_1^w$	356770	+	+	+	0	+	+	/	/	0	+	0	+	0	+		0	+	+	+	+	0	+			0	+	0	+	0				(+)	(+)
12	R_zR_2	202024	w	+	+	+	0	0	/	/	+	+	0	+	0	+		0	+	+	+	+	0	+	0		0	+	0	+	+				+	+
13	rr	143225	0	0	0	+	+	0	/	/	0	+	0	+	0	+		0	+	0	+	0	+	+	0		0	+	+	+	+	Bg(a+)			+	+
14	r'^wr	1779780	+	0	0	+	+	+	/	/	+	+	0	+	/	+		0	+	0	0	+	0		0		0	+	0	+	+				+	+
15	R_2r	1718113	0	+	+	+	+	0	/	/	0	+	0	+	0	+		+	+	+	0	+	0		+		+	+	0	+	+				+	+
16	rr	382612	0	0	0	+	+	0	/	/	+	+	0	+	0	+		+	+	0	0	+	0		+		0	+	0	+	+	Wr(a+)	Bg(a+)		+	+

AB-Plasma 1:4 Neat 1:4

Neutralization with plasma

Some blood group antibodies are directed to proteins that are abundantly present in plasma.

The by far most important example is the CH/RG blood group system:

Ch antigens are on complement C4B,

Rg antigens are on complement C4A

These antibodies are inhibited by plasma

Although both specifities would fit the pattern, no inhibition was observed

Further Work

The case was referred to our laboratory

Further Work

The case was referred to our laboratory

Which tests should be repeated?

Is there any test lacking that we would have done?

Did the colleagues miss some special condition?

Further Work

Which tests should be repeated?

Decision 1: Let`s do our basal diagnostics, because we know how to interpret these tests

1: ABD Conf Angefordert: Dorner / 12.05.2016 08:06:01 Getestet: Innova J/02-3422/12.05.2016 08:55:05	10..	002996 1 Anti-A ++++	002996 2 Anti-B 0	002996 3 Anti-D ++++			
1: 8BVSF Poly Angefordert: Dorner / 12.05.2016 08:06:01 Getestet: Kroll/12.05.2016 09:11:48	22..	032061 2 AKS 1 0	032061 3 AKS 2 (+)	032061 4 AKS 3 0			
1: Auto Poly Angefordert: Dorner / 12.05.2016 08:06:01 Getestet: Kroll/12.05.2016 09:11:53	22..	032061 1 Auto Ctrl (+)					
1: 8Fic ABScr Angefordert: Dorner / 12.05.2016 08:07:02 Getestet: Kroll/12.05.2016 09:30:40	55..	018069 1 AKS 1 (+)	018069 2 AKS 2 (+)	018069 3 AKS 3 (+)	018069 4 AKS E1 0	018069 5 AKS E2 0	018069 6 AKS E3 0

Tests were done in BioVue technique using AutoVue Innova:

[ABD Conf - ABD confirmation; 8BVSF Poly - 3 cell antibody screen in IAT; Auto Poly - autocontrol in IAT; FicABScr - 3 cell antibody screen in IAT (left) and with ficin-treated cells on neutral column (right)]

Further Work

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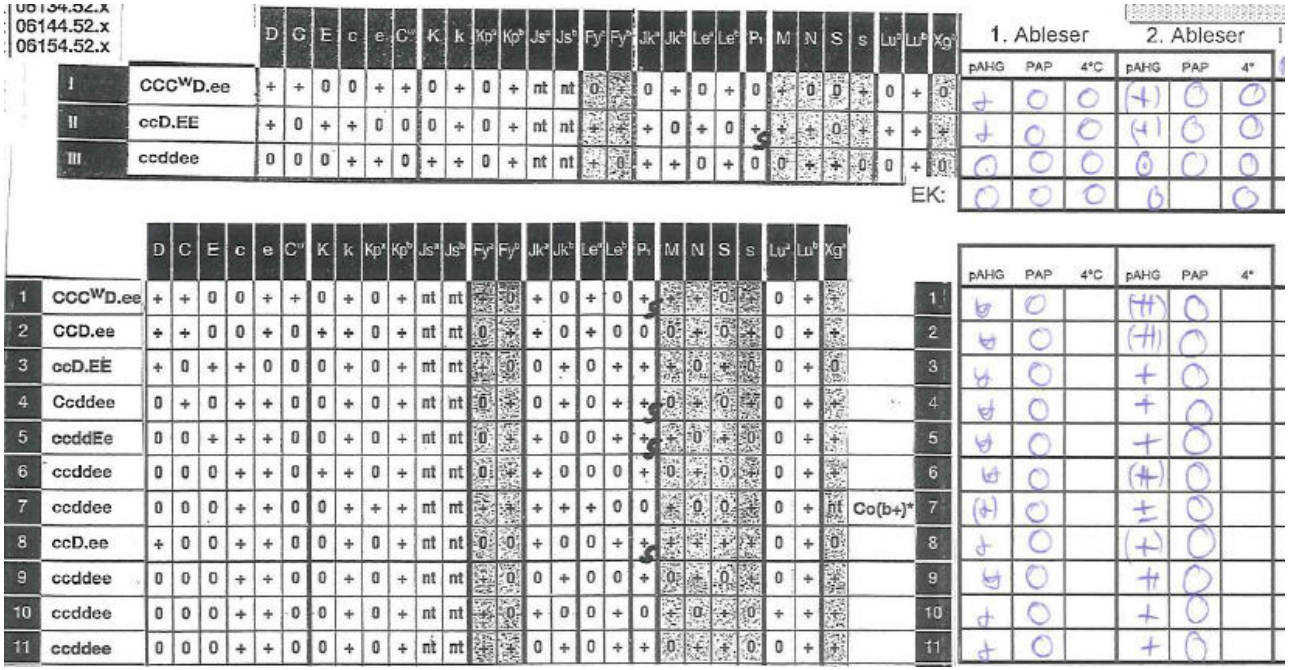


Legend: Test were done in IAT (pAHG), papain-IAT (PAP) and Neutral 4° C; results were independently scored by two persons (“1. Ableser“ and “2. Ableser“)

Further Work

Decision 1: Let`s do our basal diagnostics, because we know how to interpret these tests

→ Confirmed:
The antibody reacts with almost all cells

→ New:
The antibody does not react in direct agglutination in the cold

→ New:
The antibody did not react
- with papain-treated cells in IAT
- with ficin-treated cells in direct test

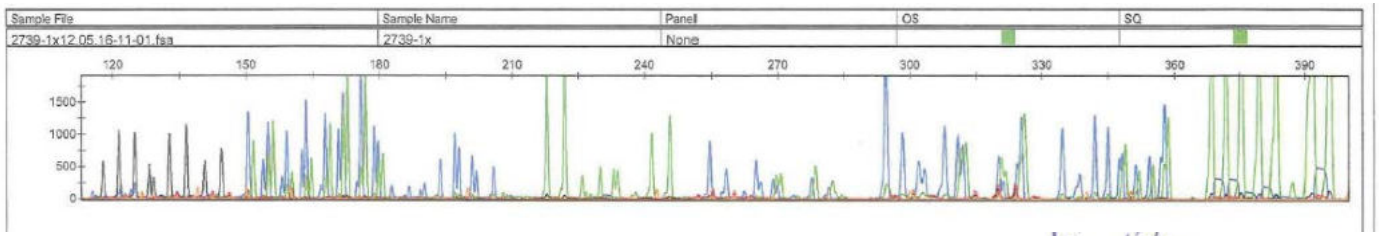
Further Work

Is there any test lacking that we would have done?

Our scheme for antibodies to high frequency antigens includes the following additional tests:

- Typing by molecular and serologic methods for "usual" antigens
- Neutralization with recombinant proteins
- Use of a commercial "special cell" panel

Genotyping Results



Molekulare Typisierung
Extended Typing

12.05.2

Datum	Name	Jk(a)	Jk(b)	Fy(a)	Fy(b)	M	N	S	s	Vel	Lu(a)	Lu(b)	Yt(a)	Yt(b)	Kp(a)	Kp(b)	Lauf
		POS	NEG	POS	NEG	POS	POS	NEG	POS	POS	NEG	NEG	POS	POS	NEG	NEG	POS	2739-1-1-4

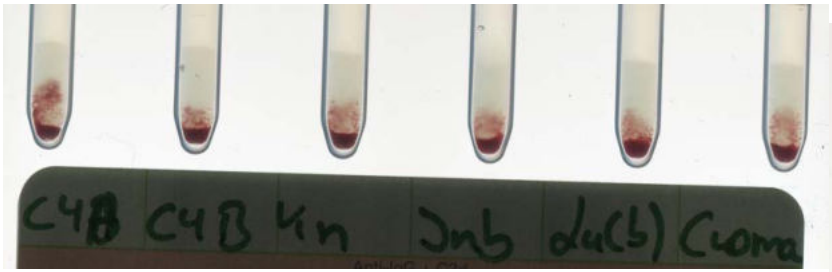
Jk (a) mono		+
Jk(b) mono		(+)
S mono		(+)
s mono		+
M mono		+
N mono	+	+
Cw mono		0
Fy(a) poly		+
Fy(b) poly		(+)

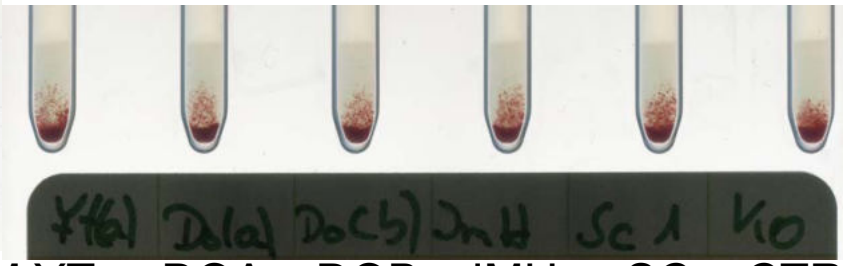
For molecular typing, we used a „exploratory“ in-house single SNP method. This method suggested that the patient could form an anti-Jk^b and anti-S. The serologic typing was in concordance, showing only weak reactivity with anti-Jk^b and anti-S (a remnant of the massive transfusion)

In addition, molecular typing suggested that the patient was likely positive for Vel, Lu^b, Yt^a and Kp^b

As the pattern was not suggestive of a mixture of antibodies,
no further genotyping was instituted

Neutralization with recombinant proteins





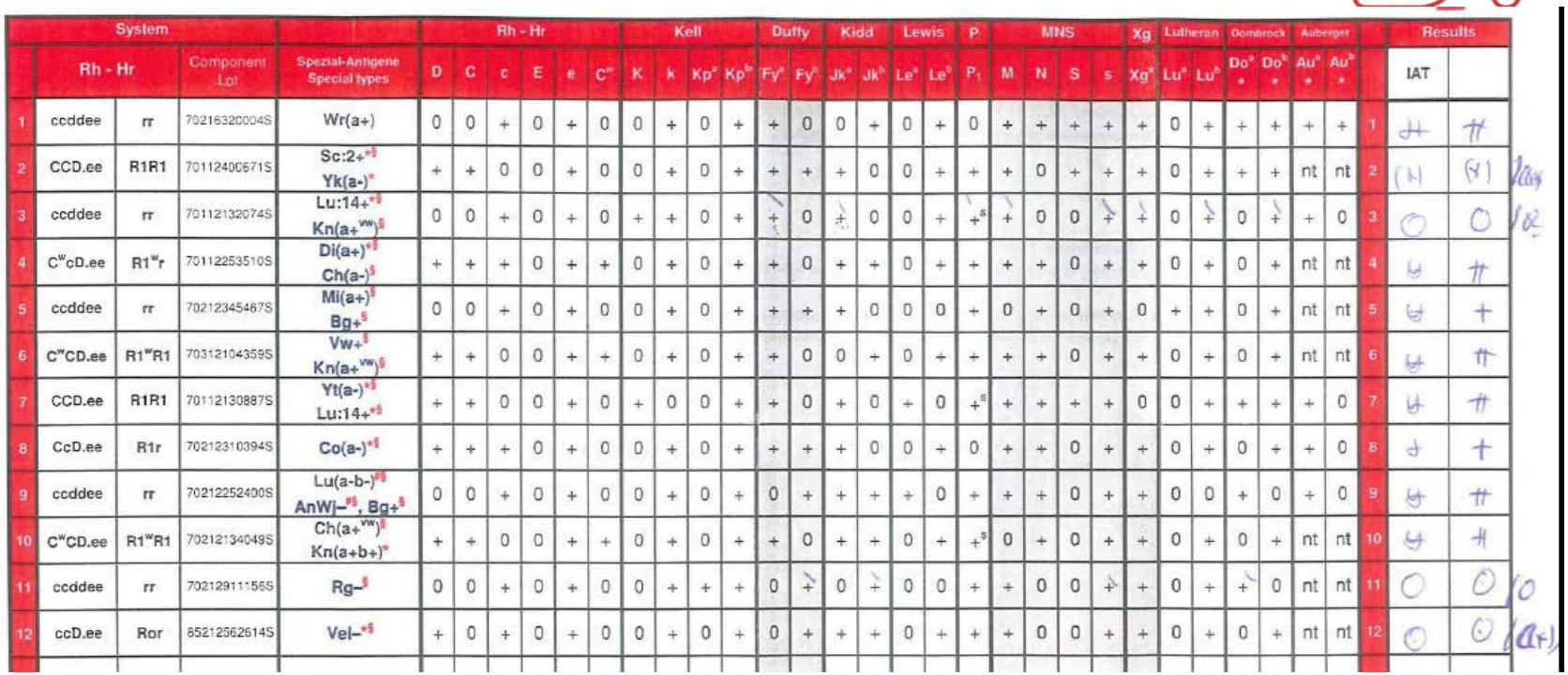
C4A C4B KN IN LU CROM YT DOA DOB JMH SC CTRL

C4A	C4B	Kn	Inb	Lu(b)	Croma	Yt(a)	Do(a)	Do(b)
+	+	+	+	+	+	+	+	+

Jmh	Scianna 1	Ko
+	+	+

We have some recombinant proteins available for neutralization.
The set-up is similar to neutralization with plasma:
Patient plasma is first incubated with recombinant protein (or saline control), the mixture is than used for a normal IAT.
For the test, we use cells compatible for DCcEe, K, Jk^a/Jk^b, Fy^a/Fy^b, Ss in order to prevent masking by these antibodies.
All tests remained reactive, hence no antibody specificity was identified.

“Special cell” panel



	System				Rh - Hr						Kell				Duffy		Kidd		Lewis		P	MNS				Xg	Lutheran		Dombrock		Auberger			Results	
	Rh - Hr		Component Lot	Spezial-Antigene Special types	D	C	c	E	e	C^{w}	K	k	Kp^{a}	Kp^{b}	Fy^{a}	Fy^{b}	Jk^{a}	Jk^{b}	Le^{a}	Le^{b}	P$_{1}$	M	N	S	s	Xg^{a}	Lu^{a}	Lu^{b}	Do^{a} *	Do^{b} *	Au^{a} *	Au^{b} *		IAT	
1	ccddee	rr	70216320004S	Wr(a+)	0	0	+	0	+	0	0	+	0	+	+	0	0	+	0	+	0	+	+	+	+	+	0	+	+	+	+	+	1	H	H
2	CCD.ee	R1R1	70112400671S	Sc:2+*§ Yk(a-)*	+	+	0	0	+	0	0	+	0	+	+	+	+	0	0	+	+	+	0	+	+	+	0	+	+	+	nt	nt	2	(H)	(H)
3	ccddee	rr	70112132074S	Lu:14+*§ Kn(a+^{vw})§	0	0	+	0	+	0	+	+	0	+	+	0	+	0	0	+	+^{s}	+	0	0	+	+	0	+	0	+	+	0	3	O	O
4	C^{w}cD.ee	R1^{w}r	70112253510S	Di(a+)*§ Ch(a-)§	+	+	+	0	+	+	0	+	0	+	+	0	+	+	0	+	+	+	+	0	+	+	0	+	0	+	nt	nt	4	H	H
5	ccddee	rr	70212345467S	Mi(a+)§ Bg+§	0	0	+	0	+	0	0	+	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	nt	nt	5	H	+
6	C^{w}CD.ee	R1^{w}R1	70312104359S	Vw+§ Kn(a+^{vw})§	+	+	0	0	+	+	0	+	0	+	+	0	0	+	0	+	+	+	+	0	+	+	0	+	0	+	nt	nt	6	H	H
7	CCD.ee	R1R1	70112130887S	Yt(a-)*§ Lu:14+*§	+	+	0	0	+	0	+	0	0	+	+	0	+	0	+	0	+^{s}	+	+	+	+	0	0	0	+	+	+	+	0	7	H	H
8	CcD.ee	R1r	70212310394S	Co(a-)*§	+	+	+	0	+	0	0	+	0	+	+	+	+	0	0	+	0	+	+	0	+	+	0	+	0	+	+	+	0	8	H	+
9	ccddee	rr	70212252400S	Lu(a-b-)*§ AnWj–*§, Bg+§	0	0	+	0	+	0	0	+	0	+	0	+	+	+	+	0	+	+	+	0	+	+	0	0	+	0	+	0	9	H	H
10	C^{w}CD.ee	R1^{w}R1	70212134049S	Ch(a+^{vw})§ Kn(a+b+)*	+	+	0	0	+	+	0	+	0	+	+	0	+	+	0	+	+^{s}	0	+	0	+	+	0	+	0	+	nt	nt	10	H	H
11	ccddee	rr	70212911156S	Rg–§	0	0	+	0	+	0	0	+	+	+	0	+	0	+	0	0	+	+	0	0	+	+	0	+	+	0	nt	nt	11	O	O
12	ccD.ee	Ror	85212562614S	Vel–*§	+	0	+	0	+	0	0	+	0	+	0	+	+	+	0	+	+	+	0	0	+	+	0	+	0	+	nt	nt	12	O	O

As part of our work-up, we use a special cell panel that includes many cells with rare phenotypes. Two cells were negative:

- one cell marked as Lu:14+ Kn(a+^{vw})
  - → Anti-Lu8??? (should have been inhibited by LU-protein)
  - → Anti-Kn^{a}??? (should have been inhibited by KN-protein)
- one cell marked as Rg-
  - → Anti-Rg??? (should have been inhibited by C4A and by plasma)

Further Testing Results and Interpretations

What did we miss?

Further Testing Results and Interpretations

What did we miss?

→ The “many positive, some negative pattern” is extremely suspicious for an antibody in the CH/RG or KN blood group system

→ Anti-Kn^{a} (and anti-Lu8) are expected to be reactive in papain-IAT

→ Anti-Rg would fit the pattern perfectly, but should be neutralized by plasma or C4A

Further Testing Results and Interpretations

What did we miss?

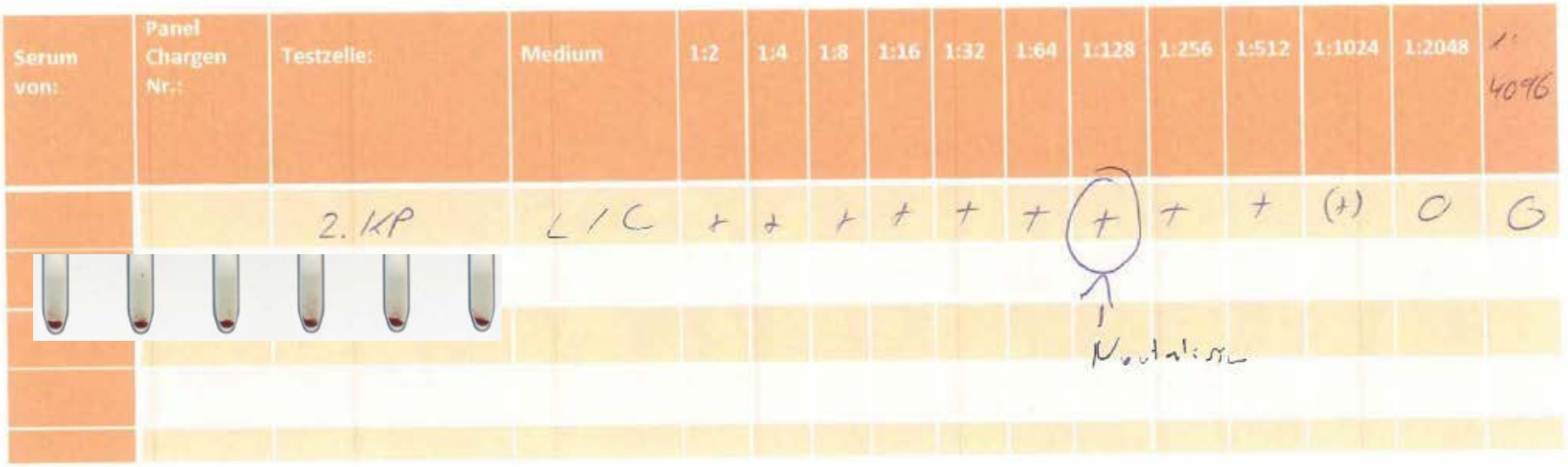
→ Anti-Rg would fit the pattern perfectly, but should be neutralized by plasma or C4A

→ Anti-Rg often shows a HTLA pattern ("high titer low avidity")

→ Apparently, a titer was lacking in the work-up.

Further Testing Results and Interpretations

What did we miss?



Serum von:	Panel Chargen Nr.:	Testzelle:	Medium	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1: 4096
		2. KP	L / C	+	+	+	+	+	+	+	+	+	(+)	0	0

Neutralisation

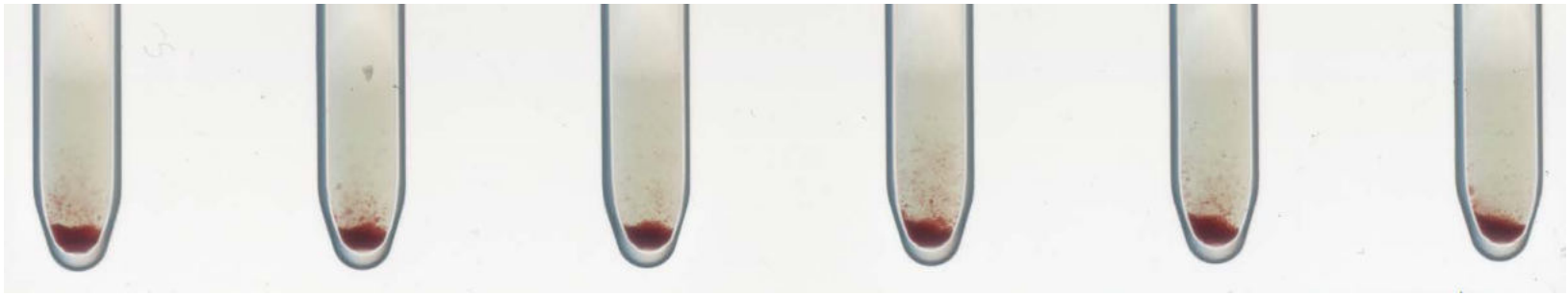
→ Antibody titer was 1:1024

→ Was it “too much antibody” for our neutralization reagents?

→ Neutralization was repeated with diluted plasma

Further Testing Results and Interpretations

What did we miss?



1:2 1:4 1:8 1:16 1:32 1:64

The typical “high titer low avidity” pattern is seen:

Reaction at 1:2 is only faint (even in gel technique).
The reactions remain unchanged up to a 1:64 dilution

Conclusions

What did we miss?

Plasma 1:128 verdünnt
Zelle 2 KP

C4A	C4B	Kn	Inb	Lu(b)	Croma	Yt(a)	Do(a)	Do(b)
0	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)

→ Neutralization was repeated with diluted plasma (1:128)

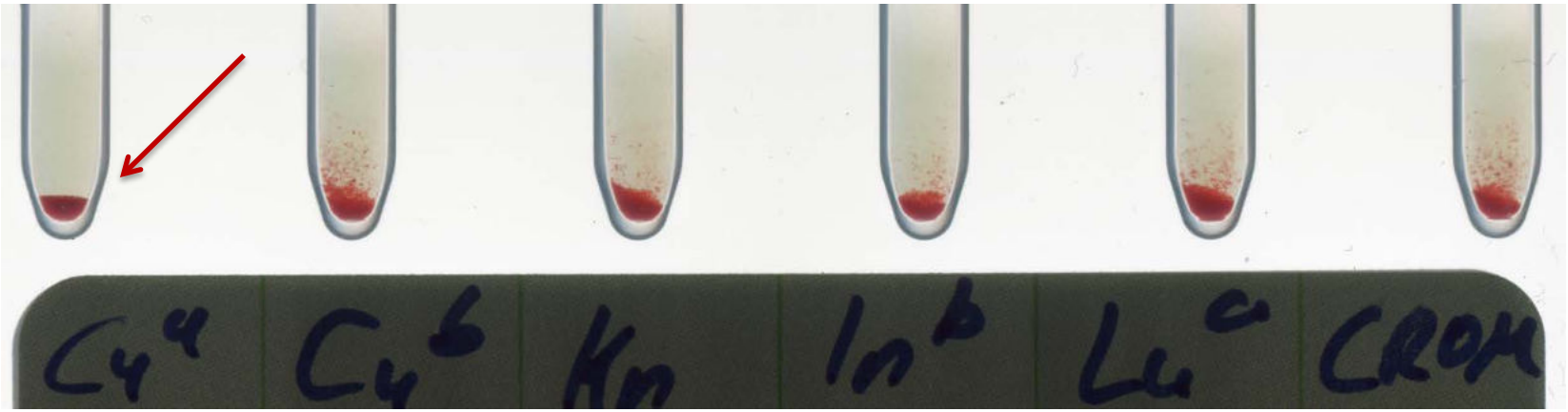
→ C4A specifically neutralized the diluted antibody

→ The anti-Rg hypothesis was correct!

Conclusions:

Neutralization of diluted plasma

What did we miss?



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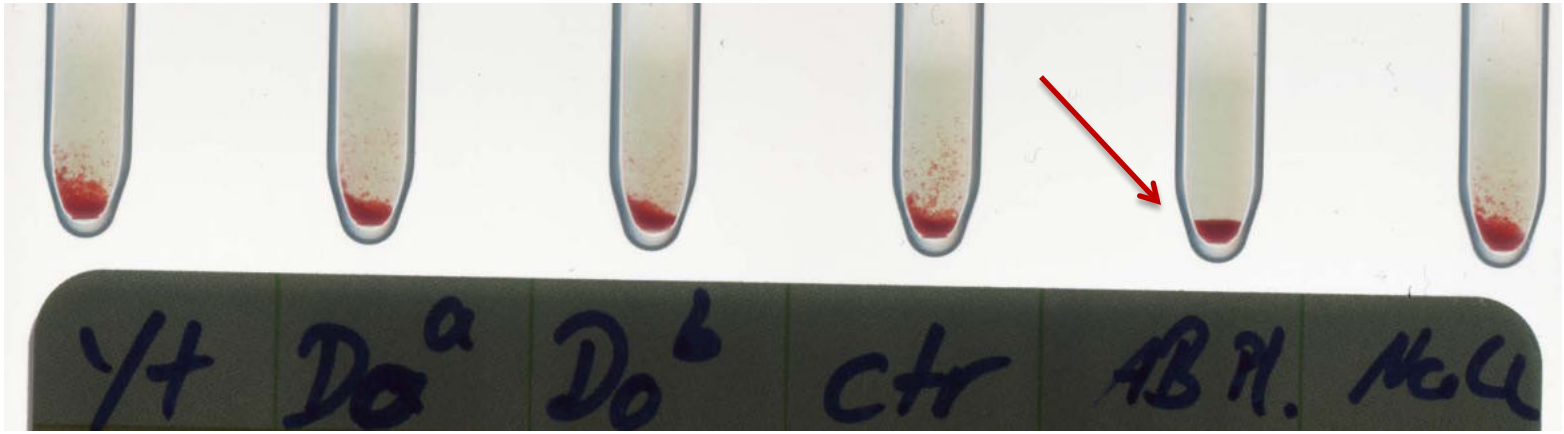

→ Plasma did also work with the diluted patient plasma

Anti-Rg

Anti-Rg is a “generic” name for antibodies to complement C4B

There are two specificities, Anti-Rg1 and Anti-Rg2:

Rg1 depends on Val at position 1188 and Leu at position 1191

Rg2 in addition needs Asn at position 1157

The discrimination of Anti-Rg1 and Anti-Rg2 is beyond the capacities of our laboratory. Almost all antisera contain both specificities.

Anti-Rg

Anti-Rg reacts with ~98% of all cells.

It does best react in IAT (in columns) and does not react with papain-treated cells. Typically, there are some cells in a panel that react slightly weaker.

The presence of “weak” or even negative cells in a panel with many positive cells may cause diagnostic confusion and long hunts for possible combinations of antibodies present in the serum.

Anti-Rg

Anti-Rg is considered to be clinically insignificant. There are reports of allergic reactions to plasma or plasma-containing platelet units.

We usually recommend transfusion with antigen positive units tested for compatibility after neutralization with plasma. In addition we try to match for clinically relevant antigens in order to avoid a missed weak yet clinically relevant antibody.

Summary of Case Challenges

This case included several challenges and pitfalls:

- The patient had a recent massive transfusion confounding serologic antigen typing
- The patient had rapidly developed an antibody of very high titer that overwhelmed the usual neutralization capacity of neutralization tests
- The case was referred after considerable prior testing. We had to decide which tests were to be repeated and which results were to be considered valid.

Lessons Learned by the Case

- Neutralization tests may fail, if the antibody titer is extremely high.
- The reaction pattern gives important information. In our case, we were almost certain that the antibody was an anti-Rg because the pattern fitted so well. Therefore, we were eager to repeat the neutralization test.
- Even a clinically irrelevant antibody may harm the patient, because he may not receive the blood units he needs in time
    - In our case, no transfusion was needed

References

Reid ME, Lomas-Francis C, The blood group antigen facts book

Seltsam A. et al. Recombinant blood group proteins facilitate the detection of alloantibodies to high-prevalence antigens and reveal underlying antibodies: results of an international study. Transfusion. 2014;54(7):1823-30.

Rare Cell Panel:
DRK-BSD Identification Red Cell – Special
(produced by Red Cross Blood Service BaWü-He)