

Case Studies – Resolving complex serological problems

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Case Studies

- Polyagglutination
- Antibody to a high-prevalence antigen
- Antibody to a low-prevalence antigen

Patient case 2011

- Male child; d.o.b. 2009-10-08
- Sample sent for ABO/Rh & antibody screen and DAT

Anti-A	Anti-A	Anti-B	Anti-B	Anti-D	Anti-D	Rh ctrl
0	0	0	0	4+	4+	1+

S I	S II	S III	DAT
0	0	0	2+

Group O, Rh NT
 DAT positive, Ab screen negative
 Sample sent to Regional laboratory

Repeat ABO type/screen & DAT



Anti-A	Anti-B	Anti-D	Anti-D	A1 RBCs	B RBCs
0	0	3+	4+	4+	2+

S I	S II	S III
0	0	0

Anti-IgG	Anti-C3d	Ctrl
0	4+	0

**Group O, RhD-positive
DAT positive, C3d on RBCs
Antibody screen negative**

Patient case – new sample

Hemolytic-
uremic
syndrome

Blood group m.m.

Personnummer: Male child; DOB
 (12 tocken) 20091008
 Namn: (efternamn, förnamn)

Kund-kod: S I V

Diagnos: HUS?

Provtagning: 24/3-11
 ID-kontroll utförd enligt gällande föreskrifter (Se remissens bilagda)

Önskad undersökning:

<input type="checkbox"/> Blodgr = ABO/RhD/antik. screen	<input type="checkbox"/> Antikropp-identifiering	<input type="checkbox"/> DAT	<input type="checkbox"/> Mono-DAT	<input checked="" type="checkbox"/> Annet: T-antigen på erythrocyter
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Tidigare transfusion? Datum: 110521

ABO och RhD erythrocyter	ABO plasm/serum					Tolk.	begr.					
	AB	Anti-A	Anti-B	Anti-A	Anti-B			A1	A2	B	O my	ABO

Tidigare transfusion? Datum: 110521

18003 05 11 - Lånegr. Skåne 011471 01 - 01

T antigen on the erythrocytes?

Pneumococcus infection

How can T-activation be detected in the laboratory?

Lectin	RBCs										
	T	Tk	Th	Tx	Tn	Cad	Nor	VA	HEMPAS	HbM _{Hlyde} Pak	Acquired-B
<i>Griffonia simplicifolia I</i>	0	0	0	0	+	0	0	0	0	0	+
<i>Griffonia simplicifolia II</i>	0	+	0	0	0	0	0	0	0	+	0
<i>Dolichos biflorus</i>	0	0	0	0	+	+	0	0	0	0	w/0
<i>Helix pomatia</i>	+	0	/	/	+	+	0	+	+	+	+
<i>Phaseolus limensis</i>	0	0	0	0	0	0	0	0	0	/	+
<i>Leonorus cardiaca</i>	0	0	0	0	0	+	0	0	0	0	0
<i>Arachis hypogaea</i>	+	+	+	+	0	0	0	0	0	w	(+)
<i>Glycine max</i>	+	0	0	0	+	0	0	0	0	+	0
<i>Salvia horminum</i>	0	0	0	0	+	+	0	0	0	w	0
<i>Salvia sclarea</i>	0	0	0	0	+	0	0	0	0	0	0
<i>Ulex europaeus</i> [†]	>	≤	=	=	≥	≤	=	≤	≤	>	=
<i>Vicia cretica</i>	+	0	+	0	0	0	0	0	0	w	0

How can T-activation be detected in the laboratory?



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	T	Tk	Th	Tx	Tn	Cad	Nor	VA	HEMPAS	HbM _{H₂O} Pak	Acquired-B
<i>Griffonia simplicifolia I</i>	0	0	0	0	+	0	0	0	0	0	+
<i>Griffonia simplicifolia II</i>	0	+	0	0	0	0	0	0	0	+	0
<i>Dolichos biflorus</i>	0	0	0	0	+	+	0	0	0	0	w/0
<i>Helix pomatia</i>	+	0	/	/	+	+	0	+	+	+	+
<i>Phaseolus limensis</i>	0	0	0	0	0	0	0	0	0	/	+
<i>Leonorus cardiaca</i>	0	0	0	0	0	+	0	0	0	0	0
<i>Arachis hypogaea</i>	+	+	+	+	0	0	0	0	0	w	(+)
<i>Glycine max</i>	+	0	0	0	+	0	0	0	0	+	0
<i>Salvia horminum</i>	0	0	0	0	+	+	0	0	0	w	0
<i>Salvia sclarea</i>	0	0	0	0	+	0	0	0	0	0	0
<i>Ulex europaeus</i> [†]	>	≤	=	=	≠	≤	=	≤	≤	>	=
<i>Vicia cretica</i>	+	0	+	0	0	0	0	0	0	w	0

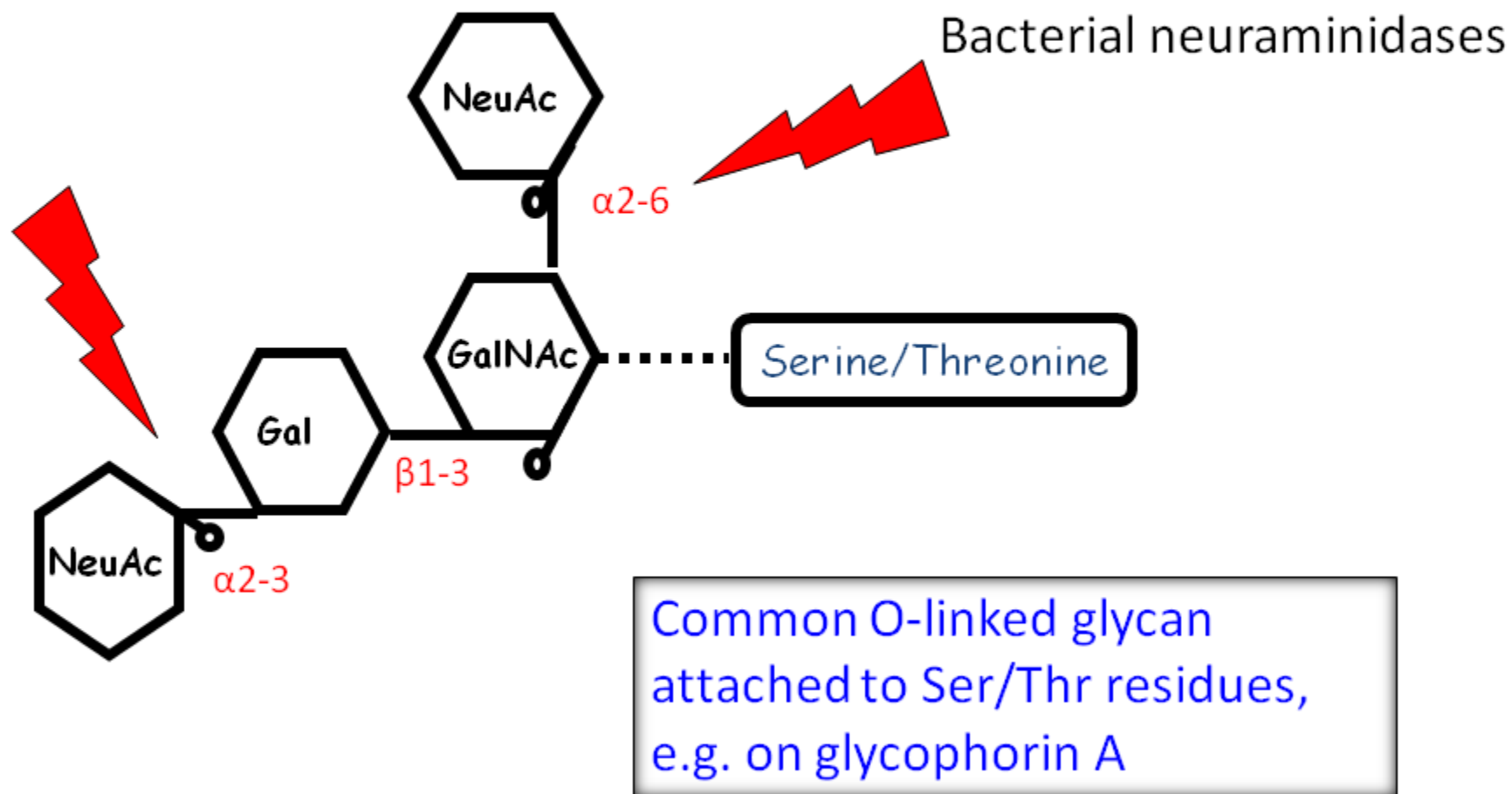
Patient's cells

++++
 ++++
 0
 0

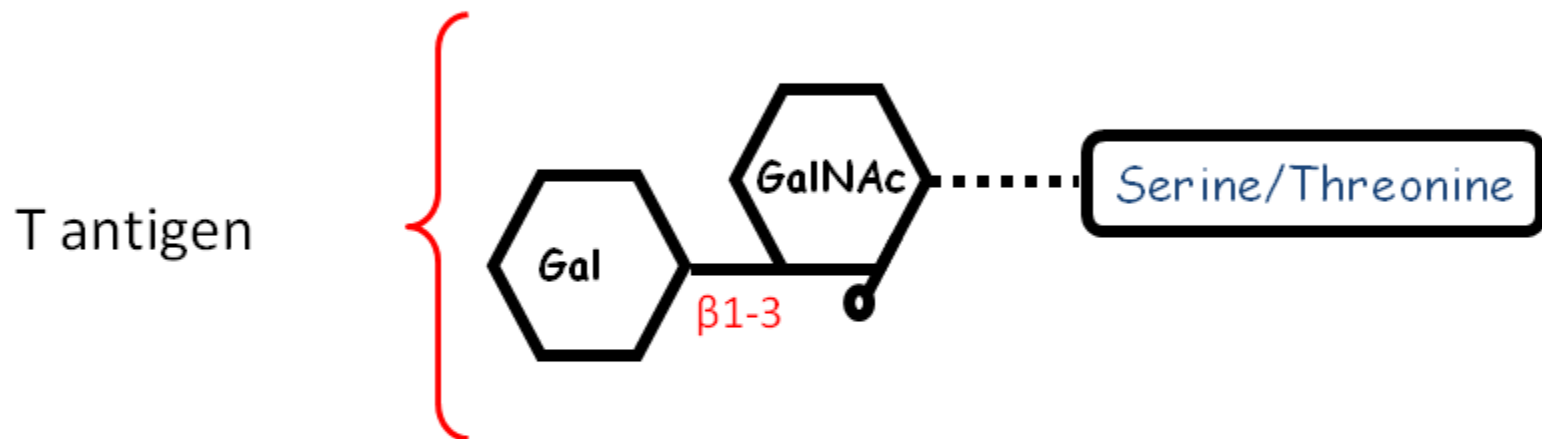
Conclusions

- Patient is O RhD+, DAT+: IgG 0 C3d 4+
- Current Recommendations:
 - Washed RBCs and platelets
 - Avoid plasma (naturally occurring anti-T)

What is T-activation?



What is T-activation?



All human sera contain anti-T

How common is T-activation in HUS?



RARE!

Waters AM, Kerecuk L, Luk D, et al. Hemolytic uremic syndrome associated with invasive pneumococcal disease: the United kingdom experience. J Pediatr. 2007;151(2):140-4.

- ◆ 43 cases of pneumococcal HUS 1998-2005
- ◆ Median age 13 months (range 5-39 months)
- ◆ Pneumococcal infection identified in 34/43
- ◆ T-activation in 36 cases
- ◆ **Mortality** 11%

Blood component therapy – current practice/dogma

- Washed RBCs
- No plasma
- Washed platelets
- What the patient received:
 - 3 units O RhD– RBCs 20/3
 - 2 units AB RhD– plasma 20/3
 - 1 unit O RhD– platelets 21/3
 - 2 units O RhD+ washed RBCs 22/3

Polyagglutination

Acquired

acquired-B

T

Tk

Th

Tx

VA

Tr

Genetic

Cad

Hb M^{Hyde Park}

HEMPAS

Nor

Tn

Clinical Findings

microbial-induced polyagglutination

- septicemia
- carcinoma of the colon
- other lesions of the bowel, GI tract
- wound infections
- neonatal necrotizing enterocolitis

ORIGINAL ARTICLE

Thomsen-Friedenreich activation in infants with necrotizing enterocolitis in Taiwan

Lin-Yen Wang, Yung-Shu Chan, Feng-Chuan Chang, Chang-Ling Wang, and Marie Lin

**43 infants with necrotizing enterocolitis
4/43 T-activation**

Infant with NEC	With T activation	Without T activation	Total	p value
Number of patients	4	39	43	
Gestational age at birth (weeks)	28 (26-32)* _u	29 (24-39)* _u	29 (24-39)* _u	0.519
Birth weight (g)	1160 (856-1800)* _u	1256 (520-3500)* _u	1170 (520-3500)* _u	0.494
Male:female ratio (n)	1:3	27:12	28:15	0.08
Surgery	1	29	30	0.312
Age at onset of NEC (days)	22.5 (20-26)* _u	14 (2-52)* _u	15 (2-52)* _u	0.514
NEC Bell Stage II	3	13	16	0.105
NEC Bell Stage III	1	26	27	0.105
Antibiotics given before the diagnosed NEC (days)	7 (5-7)* _u	7 (0-30)* _u	7 (0-30)* _u	0.564
Sepsis	2	8	10	0.192
Mortality	0	7	7	0.366
RBCs	4 _u †	31	35	0.326
FFP	0	17	17	0.093
PLTs	0	19	19	0.064

Polyagglutination - Summary

An atypical finding in which RBCs are agglutinated by allogeneic blood group-compatible adult human sera, but not by sera from newborns

- bacterial enzymes
- incomplete RBC membrane biosynthesis
- genetic factors

Case 2 – Lab referral

- 26 year-old woman, Serbian origin
- Second pregnancy
- Antibody reacted 2+ with all panel RBCs
 - Titre 32
 - 3+ with PEG
 - Autologous RBCs negative
- Did not react with papain-treated RBCs
- Reactive with A1 & A2 RBCs, and cord RBCs
- DAT-negative

Alloantibody

Not anti-H

developed at birth



Patient/Donor Information		Test Ordering Information	
Full Name:		Ordered by:	
Patient or donor #:		Institution:	
Sample Information		Test Performed	
Sample ID#: [REDACTED]		Test Type: IDCore+; PGKWS v2.0	
Sample Type: DNA		Developed by Progenika Biopharma, S.A. www.progenika.com	
Accession date: 23/8/2013		Lot#: IDCP01010022	



Blood Group	Genotype	Predicted Phenotype		Recommendations
RHCE	Cc	C	+	We recommend that the test should always be interpreted within the clinical context of the donor/patient as well as serology data.
	r's No variant	c	+	
	Ee	E	+	
		e	+	
	Cx No variant	Cx	0	
	Cw No variant	Cw	0	
KELL	VS No variant	VS	0	Notes +: Normal antigen expression 0: undetectable antigen expression +w: Weak antigen expression NC: No call, result inconclusive NV: Not valid, test should be repeated
	kk	K	0	
		k	+	
	KPB/KPB	Kpa	0	
		Kpb	+	
	Kmod No variant			
KIDD	JsB/JsB	Jsa	0	Comments
	JKA/JKB	Jsb	+	
	Jk null NO JKnull	Jka	+	
DUFFY	FYA/FYB	Jkb	+	<div style="border: 2px solid red; padding: 5px; text-align: center; color: white; font-weight: bold;"> Group A RhD+ K-, N-, s- </div>
	NO FYX	Fya	+	
	NO FYGATA	Fyb	+	
MNS	MM	M	+	Review & Approval
		N	0	
	SS	S	+	
		s	0	
DIEGO	U No variant	U	+	Date:
	GPMur No variant	GPMur	0	
DOMBROCK	DIB/DIB	Dia	0	Technician:
		Dib	+	
	DOA/DOB	Doa	+	
		Dob	+	
COLTON	Hy No variant	Hy	+	Laboratory Responsible:
	Joa No variant	Joa	+	
	COA/COA	Coa	+	
CARTWRIGHT		Cob	0	
		Yta	+	
		Ytb	+	

Namn: [REDACTED]		Beställare: GÖBLSA
Provtdatum: 2018	Analysdatum: 29/8	Labtext: 3P 24/a-13

Testery	IAT gel	Pap gel	IAT/ PEG	IAT rör	NaCl rör	Testery	IAT gel	Gel Station	Pap. gel	IAT/ PEG	IAT rör	NaCl rör
Egna	-	-				S 1		+				
AK 1	+	+				S 2		+				
AK 2	+	+				S 3		+				
AK 3	+	+				B 1						
AK 4	+	+				B 2						
AK 5	+	+				B 3						
AK 6	+	+				B 4						
AK 7	+	+				Kontr.						
AK 8	+	+				Datum						
AK 9	+	+				Satt/Läst						
AK 10	+	+				Fenotypning						
AK 11	+	+				Antigen	Kn b					
Kontr.		+				Pos	+					
Datum	28/8	28/8				Neg						
Satt/Läst	PWC	PWC				Susp 1	+					
DAT	Rör	Gel	IgG	C3d	ctl gel	Susp 2						
Datum						Datum	29/8					
Satt/Läst						Satt	ap					
						Läst	14/9					
NOTERING						UTLÅTANDE						

Antibody to a high frequency antigen?

Kn b
+
+

Guesswork – Knops system antigens not enhanced by papain; common “nuisance” (clinically insignificant) antibody

Me	Testery								
	MH051243	+							Ku ^a -
	RN ID W00435920	+							Ku ^a -
	SP 90312	+							Ku ^a -
	HS201010717	+							Ge - 2,3
	From KAPed	+							JMH -
	MME	+							Yt ^a -

Guesswork – referring lab found it non-reactive with papain-treated RBCs

Papain	Possible specificity
0	MNS, Ge, Fy, Xg ^a , Ch/Rg, In, JMH
+/0	Yt
+	Cromer, Knops, Lu, Do, AnWj, Raph, Kell, LW, Sc ABO, P1, Rh, Le, Jk, Fy3, Di, Co, Ge3, Ok ^a , li, P, At ^a , Cs ^a , Emm, Er ^a , Jr ^a , Lan, Sd ^a , PEL, MAM, ABTI, Vel, Kx

Enzymes and DTT

Metod Testery	IG DTT	IG Tryp.	IG dit	IG Pronase			Kontr DTT & K	
AK 2	+	+	+	+			-	
AK 5	+	+	+	+			+sv	
AK 8	+	+	+	+			+sv	
Untreated RBCs								
Kontroll	-k	-M	-F _γ ^a	-F _γ ^a	-M	-F _γ ^a	-k	
AK 2	-	-	-	-	###	#	-	
AK 5	+sv	-	-	-	###	-	###	
AK 8	-	-	-	-	-	###	###	

Not affected by DTT, trypsin or pronase
 Weaker with α -chymotrypsin

Antigen sensitivity

Papain	Trypsin	α -ct	Pronase	DTT	Möjligt specificitet
0	0	0	0	+	Ch/Rg, Xg ^a
0	0	0	0	0	In, JMH
0	0	+	0	+	MN, En ^a TS, Ge2, Ge4
0/+	+	0	0	+	'N', Ss, Fy ^a /Fy ^b , Fy6
0/+	+	0	0	0	Yt ^a
0	+	+	0	+	En ^a FS
+	0	0	0	+ ^w	Lu, MER2
+	0	0	+	+ ^w	Knops
+	+	+	+	0	Kell
+	+	+	+ ^w /0	0	Sc
+	0	+ ^w	0	0	Do, Ge3
+	+	0	0	0	Cromer,
+	+	+ ^w	0	0	LW
+	+	+	+	+	Jk3, Fy3, Di ^b , Co ^a , Ge3; Ok ^a , P, LKE, At ^a , Cs ^a , Emm, Er ^a , Jr ^a , Lan, PEL
+	+	+	+	++	Kx
+	+	+	+	+/0	Vel

Metod Testery	IG DTT	I _h Tryp.	I _g αct	I _h Pronase			Kontroll DTT αct
AK2	+	+	+	+			-
AK5	+	+	+	+			+sv
AK8	+	+	+	+			+sv
Oberhandlade							
Kontroller	-k	-M	-Fy ^a	-Fy ^a	-M	-Fy ^a	-k
AK2	-	-	-	-	###	##	-
AK5	+sv	-	-	-	###	-	###
AK8	-	-	-	-	-	###	###

Papain	Trypsin	α-ct	Pronase	DTT	Possible specificity
+	+	+	+	+	Jk ^a , Fy ^b , D ^a , Co ^a , Ge ³ ; O ^a , R, L ^x E, A ^x , Cs ^a , Emm, Er ^a , Jr ^a , L ^x n, PEL

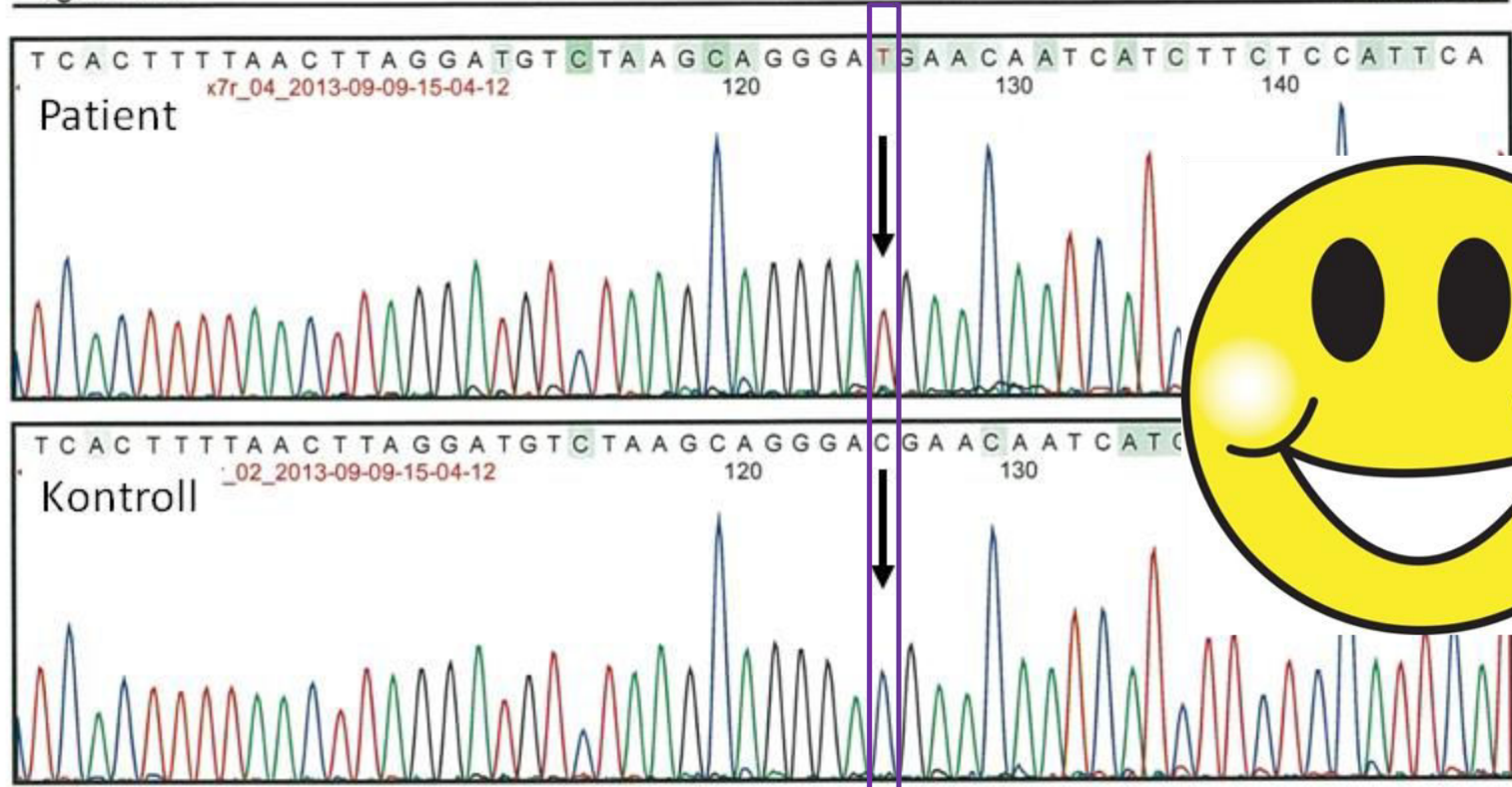
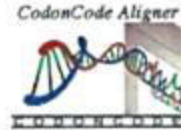
	IG	IG						
SR092001		+						Cs(a-)
3-6002		+						D ₁ (b-)
1048282		-						Jr(a-)
841296781		+						
AK8	+							
MC32	-							Jr(a-)
60115395	-							Jr(a-)

No anti-Jr^a available for phenotyping

Reference allele <i>ABCG2*01</i> encodes Jr1 (Jr ^a)				
Phenotype	Allele Name	Nucleotide change	Intron/Exon	Amino acid change
Jr(a+)	<i>ABCG2*01</i>			
Null phenotypes				
Jr(a-)	<i>ABCG2*01N.01</i>	376C>T	Exon 4	Gln126X
Jr(a-)	<i>ABCG2*01N.02.01</i>	706C>T	Exon 7	Arg236X
Jr(a-)	<i>ABCG2*01N.02.02</i>	34G>A 706C>T	Exon 2 Exon 7	Val12Met Arg236X
Jr(a-)	<i>ABCG2*01N.03</i>	736C>T	Exon 7	Arg246X
Jr(a-)	<i>ABCG2*01N.04</i>	337C>T	Exon 4	Arg113X
Jr(a-)	<i>ABCG2*01N.05</i>	784G>T	Exon 7	Gly262X
Jr(a-)	<i>ABCG2*01N.06</i>	34G>A 1591C>T	Exon 2 Exon 13	Val12Met Gln531X
Jr(a-)	<i>ABCG2*01N.07</i>	187_197delATATTATCGAA	Exon 2	Ile63TyrfsX
Jr(a-)	<i>ABCG2*01N.08</i>	542_543insA	Exon 6	Phe182ValfsX
Jr(a-)	<i>ABCG2*01N.09</i>	730C>T	Exon 7	Gln244X
Jr(a-)	<i>ABCG2*01N.10</i>	791_792delITT	Exon 7	Leu264HisfsX
Jr(a-)	<i>ABCG2*01N.11</i>	875_878dupACTT	Exon 8	Phe293Leuf sX
Jr(a-)	<i>ABCG2*01N.12</i>	1111_1112delAC	Exon 9	Thr371Leuf sX
Jr(a-)	<i>ABCG2*01N.13</i>	34G>A 244_245insC	Exon 2 Exon 3	Val12Met Thr82HisfsX
Jr(a-) ^	<i>ABCG2*01N.14</i>	1017_1019delCTC	Exon 9	Ser340del
Altered phenotypes				
Jr(a ^W)	<i>ABCG2*01W.01</i>	421C>A	Exon 5	Gln141Lys
Jr(a ^W)	<i>ABCG2*01W.02</i>	1858G>A	Exon 16	Asp620Asn

ABCG2 exon 7 sequencing

Contig1: Traces for 2 samples
den 10 september 2013 15:44:45 CEST
Page 1 of 2



706 C>T => Arg236Ter

Clinical relevance of anti-Jr^a

- According to Reid, Lomas Francis & Olsson: The blood group antigen factsbook 3rd ed. 2012 Academic Press
 - Possible reduced cell survival
 - Baby most likely DAT+
 - One report of a fatal case of HDFN
 - Otherwise, not much information

What did the referring lab do?



- Followed the baby's progress
 - With ultrasound, Doppler MCA
 - Antibody titre
- Purchased 4 units O RhD- Jr(a-) blood from Spain
- Patient delivered v. 39
 - Baby was DAT+
 - Healthy, no blood required

Why use enzymes?

- Taught that enzymes "reduce the zeta potential" by removing sialic acid
 - But modification is very specific
 - Can be used selectively
- Sensitivity depends on "access"
 - Proteins on intact RBCs less accessible than solubilized membranes
- Can be used as a **fingerprint**
 - Antibodies to high incidence antigens
 - Antibodies to low incidence antigens
 - Can differentiate mixtures of antibodies



Common "treating" agents

Proteases:

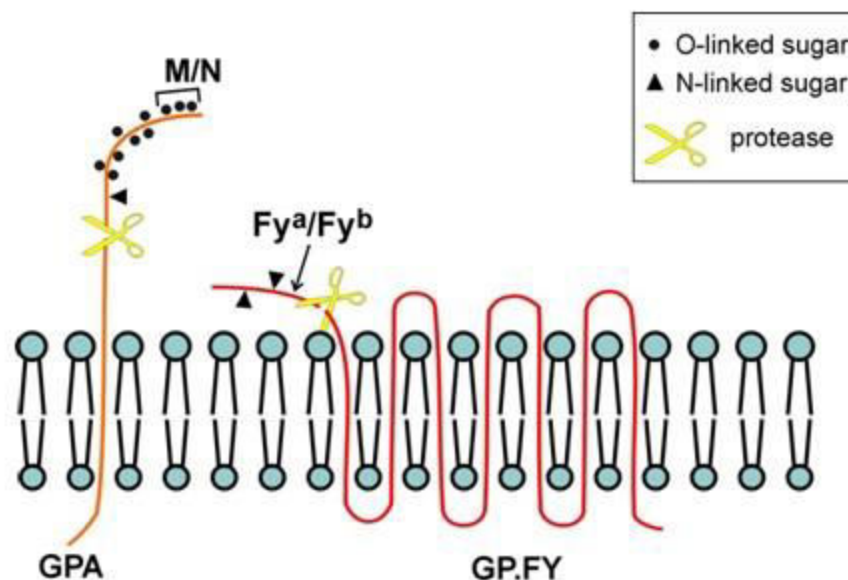
- Papain, Ficin, Trypsin, α -chymotrypsin, Pronase

Glycosidases

- Sialidase

Reducing agents

- DTT, AET



Case 3

- 🔴 2-day old baby
- 🔴 Hyperbilirubinemia
- 🔴 Sample sent for group, screen and DAT

Anti-A	Anti-B	Anti-D	Anti-D	Rh ctrl
0	0	4+	4+	0

S I	S II	S III
0	0	0

Anti-IgG	Anti-C3d	Ctrl
4+	0	0

Group O, RhD-positive
 DAT positive, IgG on RBCs
 Antibody screen negative

What next?

- The baby's RBCs were strongly DAT and had an elevated => suggests hemolysis despite the negative antibody screen
- Mother and baby were both O RhD+
 - Mother had no detectable antibodies at last screen
 - Antibody to a low frequency antigen?
- What can be done?
 - Elute the antibody from the baby's RBCs
 - Test with different RBCs carrying low frequency antigen
 - Test the mother's plasma and baby's eluate with the father's RBCs

Elution

- Removes IgG (and IgM) from RBCs
 - Acid (low pH)
 - Heat (56°C)
 - Freeze-thaw
 - Ether
- We eluted the antibody using Elu-Kit II
 - Low pH solution (0,3 M Glycine-HCl, pH 3.0)
 - Rebuffer after elution to pH ~7.0
 - 500 uL eluate
 - What RBCs should be tested?

Testing the eluate

RBCs	Eluate	Last wash
S I	0	0
S II	0	0
S III	0	0

- ❖ Antibody screening RBCs were nonreactive
- ❖ Tested rare RBCs thawed for a previous case
 - ❖ Based on patient's background
- ❖ Di^b has a low frequency partner Di^a, so Di(b-) RBCs are Di(a+)

RBCs	Eluate	Last wash
Di(a+b-)	4+	0
Di(a+b-)	4+	0
Di(a+b-)	4+	0

Tests with the mother's plasma

Metod										Antigen
Testery	16									
S1	-									
S2	-									
S3	-									
J756	##									Di(atb-)
B4115	#									Di(atb-)
GMM	##									Di(atb-)

Titrerad antikropp: Diego				Testerytrocyt id: 216033 266-614				Metod: 14794			
Spädning	1	2	4	8	16	32	64	128	256	512	Titer
Resultat	##	##	##	##	##	##	##	##	+	-	256
										Datum	10/9
										Satt	kol
										Läst	kol 14

1:256 = High titre antibody

Notes on Di^a

- Di^a and Di^b are antigens in the Diego blood group system on Band 3
 - Anti-Di^a first discovered as the cause of fatal HDFN
- Di^a is a very low incidence antigen across Europe but varies greatly in other populations:
 - South America >25%
 - Native Canadians (Chippewa) 11%
 - Native Americans 4%
 - Chinese 4 – 10%
 - Japanese ~13%
 - Hispanics 1%
 - Polish 0.5%
- Not included in our antibody screening RBCs

Clinical progress of the baby

- Treated originally with phototherapy
- Received 1 unit of "baby" blood one week post-partum
- No further transfusions since then
 - Control sample requested in 6 months
- Sample received from the father
 - RBCs type (Di(a+b+))

Thank you!

Questions??