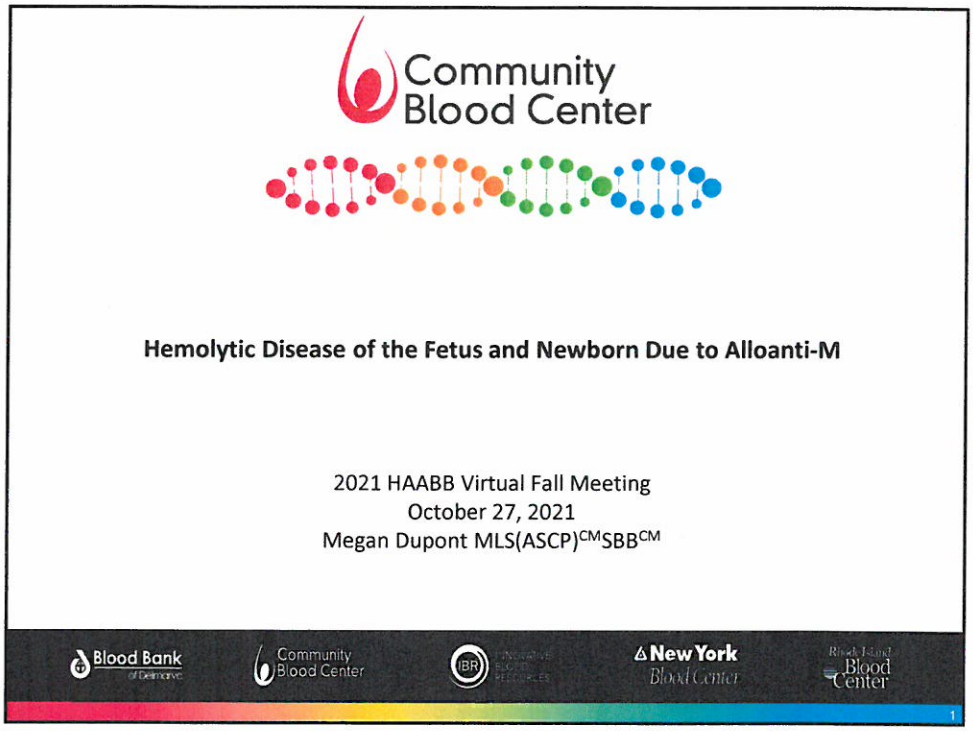
Community
Blood Center
Hemolytic Disease of the Fetus and Newborn Due to Alloanti-M
2021 HAABB Virtual Fall Meeting
October 27, 2021
Megan Dupont MLS(ASCP)CMSBBCM
Blood Bank of Delmarva
Community Blood Center
New York Blood Center
Rhode Island Blood Center
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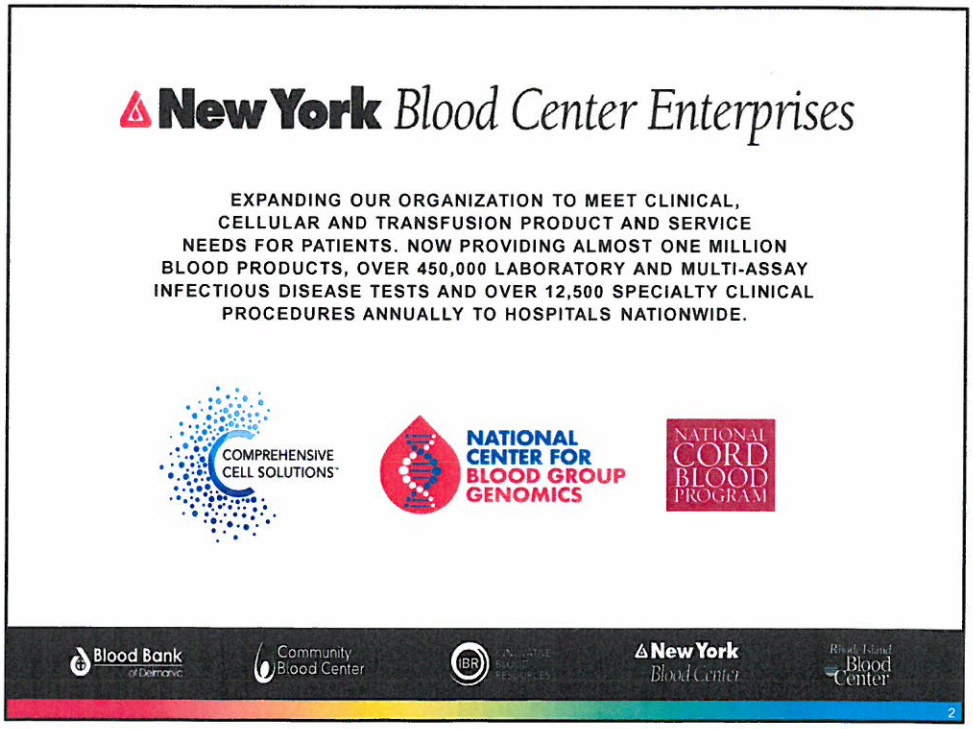
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Objectives

- **Briefly review Hemolytic Disease of the Fetus and Newborn**
- **Describe how dithiothreitol (DTT) is helpful in differentiating IgM from IgG antibodies**
- **Review of patient case**

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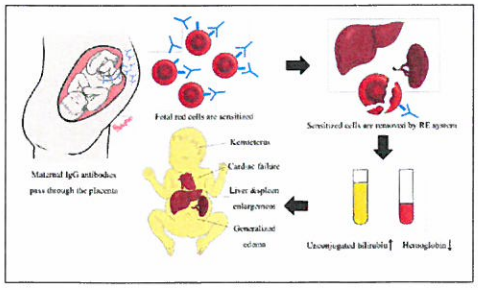
Hemolytic Disease of the Fetus and Newborn (HDFN)

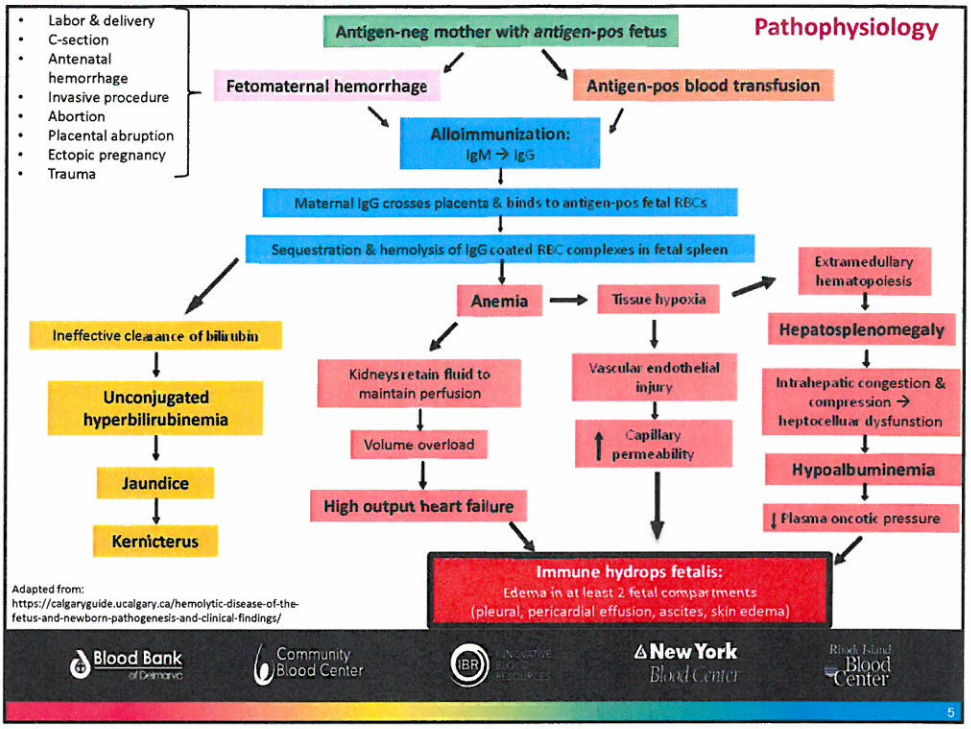
The destruction of fetal and newborn red cells by maternal red cell antibodies specific for paternally inherited antigens on fetal red cells or erythroid precursors.

Clinically unaffected newborn → **Severe anemia** → **Fetal demise**



https://dokumen.tips/documents/-hemolytic-disease-of-the-fetus.html

4



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Blood Group Antigens and HDFN in Populations of European and African Ancestry

Antigen	Antibody frequency (per 100 pregnant women)	Severity of HDFN in infants with antigen		
		None/Mild	Moderate	Severe
A, B, AB	Not relevant	Not HDFN, but antibody in 90%	<10%	<1%
D	2.6	51%	30%	19%
c,cE	0.9	70%	23%	7%
E	2.0	Almost all	NA	Rare
C, Ce, C^W,e	0.7	86%	14%	Rare
Le^a, Le^b	3.0	No HDFN		
Kell (K1)	3.2	30-50%	30-37%	13-38%
Fy^a	0.8	67-94%	16%	6-16%
Fy^b	Rare	Rare cause		
Kidd (Jk^a)	0.2			Rare
M	0.5			Rare
N	0.1			Rare
U	Rare			Rare
N	0.03	No HDFN		
P (P1)	0.03	No HDFN		

Adapted from Rossi's Principles off Transfusion Medicine, 5th ed.

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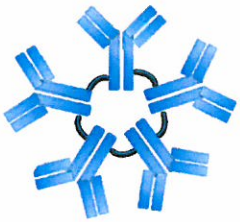
Anti-M Rarely Associated with HDFN

All evidence from Caucasian and black populations indicates severe HDFN cases due to Anti-M are very rare.



IgM
970 kDa

IgG
150 kDA

https://vivadifferences.com/understanding-igg-vs-igm/

Typical alloanti-M characteristics:
- IgM or mix of IgM & IgG
- Often naturally occurring

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Hemolytic disease of the fetus and newborn due to alloanti-M: three Chinese case reports and a review of the literature.
Transfusion 2019
Si Li, Chunyan Mo, Linhaun Haung, Xiaomei Shi, Guangping Lo, Yanli Ji, and Qun Fang

- In Chinese population, most common causes of HDFN:
  1. Anti-Rh (including anti-D, -E, -C, -c)
  2. Anti-M
- Literature review of HDFN cases due to alloanti-M from 1959 to 2017
  - 67 cases
    - 59 of 67 cases (88.06%) Asian ethnicity
  - Low retic levels in neonates & reduced erythroid precursors in fetal bone marrow indicate anti-M may inhibit growth of M-positive precursor cells (as seen with anti-K)
  - Low titer anti-M resulting in stillbirth and severe fetal anemia indicates high-affinity IgG antibody that binds to RBCs efficiently and leads to rapid destruction

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Diagnosis & Monitoring

1ST Prenatal Visit
- ABORh
- Antibody screen

Alloantibody Considerations
- D-negative women who have not made anti-D → RhIG
- Anti-I,-P1,-Le^a, -Le^b do not cause HDFN
- Anti-M: distinguish IgG from IgM

Clinical Significant Alloantibody
- Test father for corresponding antigen
- Genotype fetal DNA
- Monthly to biweekly maternal antibody titers
- Critical titer or "two tube" titer increase → Doppler fetal ultrasound to assess fetal anemia

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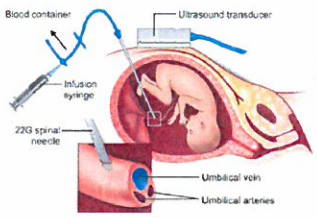
Treatment



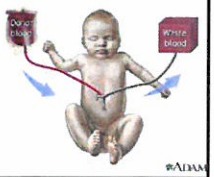
Fetal Transfusion
Maternal plasma exchange and/or IVIG

After Birth

Phototherapy

Simple Transfusion

Exchange Transfusion



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Images: https://www.[illegible]/chapter/ch26 https://medlineplus.gov/

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Objectives

- Briefly review Hemolytic Disease of the Fetus and Newborn
- **Describe how dithiothreitol (DTT) is helpful in differentiating IgM from IgG antibodies**
- Review of patient case

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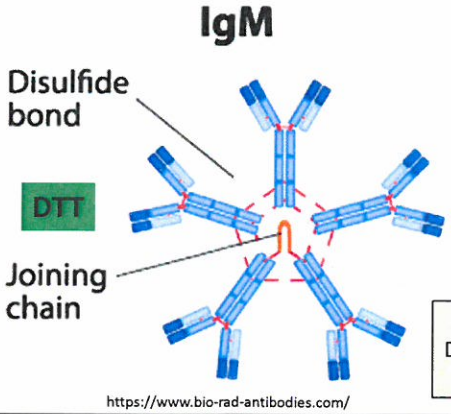
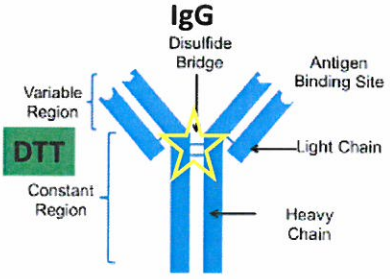
Differentiating IgM from IgG Antibodies Using Dithiothreitol (DTT)

IgM Molecules consist of 5 radially arranged subunits linked by inter-subunit disulphide bonds. Each subunit consists of two heavy chains and two light chains that are linked by interchain bonds. The intersub-unit S-S bonds are susceptible to cleavage by thiol reagents, whereas interchain S-S bonds of IgG and IgA are not readily cleaved by thiol reagents.





https://www.bio-rad-antibodies.com/

We can use the pattern of reactivity of the DTT-Treated plasma vs the neat plasma to help determine the class of antibody present.

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Objectives

- **Briefly review Hemolytic Disease of the Fetus and Newborn**
- **Describe how dithiothreitol (DTT) is helpful in differentiating IgM from IgG antibodies**
- **Review of patient case**

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Case Background

- 25 year old Asian female
- 1st pregnancy
- 33 weeks gestation
- A Pos
- History of anti-M from outside facility
  - Titer <2
  - No testing to determine IgM/IgG
- Now being seen at local Children's hospital
  - Hydrops fetalis
  - 4+ in 3-cell Gel screen
  - Submitting facility requesting antibody ID + 5 units RBCs for PUBS procedure scheduled for next morning

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ABORh

Group A, Rh Pos

	ABO Group				Rh Type	
	Anti-A	Anti-B	A_1 Cells	B Cells	Anti-D	Control
IS	4+	0	0	4+	3+	0

DAT Negative

Direct Antiglobulin Test

Poly	IgG	C′	Saline
(0) ✓	NT	NT	(0)

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Antibody Identification

Anti-M Rouleaux at RT

		Rh					Kell		Duffy		Kidd		MNS				Plasma Results		
		D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	5′ RT	Sal Rep	PEG IAT
1	R_2R_2	+	0	+	+	0	0	+	+	0	+	0	0	+	0	+	R	(0)	(0) ✓
2	r′r	0	+	0	+	+	0	+	0	+	+	0	+	0	0	+	$2+^R$	1+	$1+^S$
3	R_1R_1	+	+	0	0	+	0	+	0	+	+	0	0	+	0	+	R	(0)	(0) ✓
4	rr	0	0	0	+	+	0	+	0	+	+	+	+	0	+	0	$1+^R$	1+	$1+^W$
5	R_1R_1	+	+	0	0	+	0	+	+	0	+	+	0	+	0	+	R	(0)	(0) ✓
6	R_2R_2	+	0	+	+	0	0	+	0	+	+	+	0	+	0	+	R	(0)	(0) ✓
7	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	+	+	R	(0)	(0) ✓
8	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	+	0	R	(0)	(0) ✓
9	rr	0	0	0	+	+	0	+	0	+	0	+	0	+	+	0	R	(0)	(0) ✓
Pt. Cell																	R	(0)	(0) ✓

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Determining Clinical Significance of Anti-M

	Anti-M
Pos	2+^{s}
Neg	0
Patient Cells	0

Patient types M-negative

		Rh					Kell		Duffy		Kidd		MNS				Plasma Results	
		D	C	E	c	e	K	k	Fy^{a}	Fy^{b}	Jk^{a}	Jk^{b}	M	N	S	s	PEG IAT	Strict Prewarm IAT
1	r'r	0	+	0	+	+	0	+	0	+	+	0	+	0	0	+	1+^{s}	1+
2	rr	0	0	0	+	+	0	+	0	+	+	+	+	0	+	0	1+^{w}	(+)

Anti-M reactivity not circumvented by prewarmed testing is considered clinically significant

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Determining Clinical Significance of Anti-M

		MNS				Plasma Results						
		M	N	S	s	5' RT	Sal Rep	PEG IAT	DTT-Tx 5 'RT	Saline Ct 5' RT	DTT-Tx PEG IAT	Saline Ct PEG IAT
1	r'r	+	0	0	+	2+^{R}	1+	1+^{s}	(0)	1+	1+	1+
2	rr	+	0	+	0	1+^{R}	1+	1+^{w}	(0)	1+	1+	(+)

DTT treatment of the patient's plasma circumvented the anti-M reactivity at RT but the reactivity in PEG by the IAT was not circumvented, indicating the anti-M has **both IgM & IgG** components.

10' RT anti-M Titer	
Immunoglobulin Class	Titer Results
IgG/IgM (untreated)	2
IgG (DTT-Tx)	<2

60' 37C IAT anti-M Titer	
Immunoglobulin Class	Titer Results
IgG/IgM (untreated)	8
IgG (DTT-Tx)	8

Critical titers for anti-M have not been established.

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Additional Testing

We've identified anti-M,
however the submitting hospital reports all 3 screening cells reacting 4+ in Gel testing...

		Rh					Kell		Duffy		Kidd		MNS				Plasma Results	
		D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	30' RT	30' 4C
1	R_2R_2	+	0	+	+	0	0	+	+	0	+	0	0	+	0	+	1+	3+
2	R_1R_1	+	+	0	0	+	0	+	0	+	+	0	0	+	0	+	1+	3+
3	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	+	+	1+	3+
4	I-												0				r	2+
5	Tj(a-)												0				r	3+
6	Vel-												0				r	3+
7	Ge:-2												0				r	3+
Pt. Cell																	1+	3+

Cold Autoantibody

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Workup Conclusions

ABORh	A Pos
DAT	Negative
Plasma	• Anti-M Clinically significant Both IgM & IgG components IgG Titer 8 • Cold Autoantibody • Rouleaux
Transfusion Recommendation	M- Donor blood selected for transfusion should be nonreactive with the patient' plasma
Units Provided	4 A Pos M- 2 O Neg, M-, CMV- <7 days old

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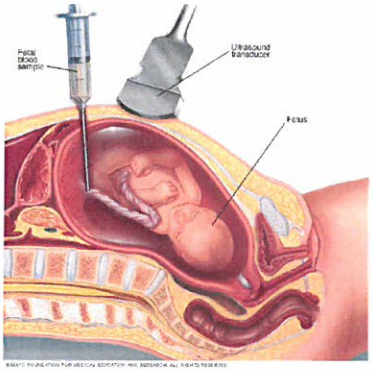
1st Percutaneous Umbilical Blood Sampling (PUBS)



https://www.mayoclinic.org/tests-procedures/percutaneous-umbilical-blood-sampling/about/pac-20393638

- Hemoglobin 2.0 g/dL (10.8-14.8 g/dL)
- Hematocrit 6.7% (36.5-45.3%)
- Reticulocyte not performed
- 107 mL RBCs transfused via IUT

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Additional Testing

Possible antibody to Low Prevalence Antigen?

Maternal plasma tested against M-negative cells positive for:

Di^a
Lu^a
Js^a
Kp^a
Co^b
V
VS
C^w
Yt^b
Wr^a
Go^a
He
DAK
Rh: 32

No reactivity detected in tube tests with PEG by the IAT.

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Paternal Testing

RBCs type group O, Rh Pos and M+N+

Red cells from the paternal sample were reactive when tested in PEG by IAT.

Ficin treatment of the paternal red cells circumvented the reactivity with the maternal plasma.

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Paternal HEA Results

Blood Group	Antigen	Result	Comments
Rh	c	+	
	C	0	
	e	+	
	E	0	
	V	0	
	VS	0	
Kell	K	0	
	k	+	
	Kp^a	0	
	Kp^b	+	
	Js^a	0	
	Js^b	+	
Duffy	Fy^a	0	
	Fy^b	(0)*	Not at risk for anti-Fy^a
Kidd	Jk^a	+	
	Jk^b	+	
MNS	M	+	
	N	+	
	S	0	
	s	+	
	U	+	
Lutheran	Lu^a	0	
	Lu^b	+	
Diego	Di^a	0	
	Di^b	+	
Colton	Co^a	+	
	Co^b	0	
Dombrock	Do^a	+	
	Do^b	+	
	Hy	+	
	Jo^a	+	
Landsteiner-Wiener	LW^a	+	
	LW^b	0	
Scianna	Sc1	+	
	Sc2	0	

Maternal Mismatch

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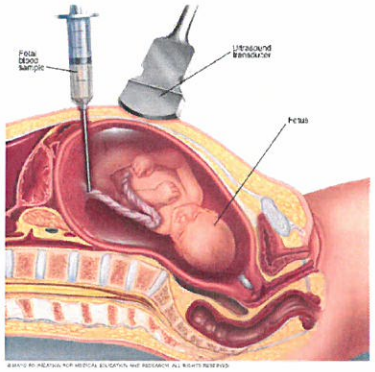
4 Days Later...
2nd Percutaneous Umbilical Blood Sampling (PUBS)



https://www.mayoclinic.org/tests-procedures/percutaneous-umbilical-blood-sampling/about/pac-20393638

- Hemoglobin 12.0 g.dL (10.8-14.8 g/dL)
- Hematocrit 36.4% (36.5-45.3%)
- Reticulocyte 1.2% (3-7%)
- 2nd IUT

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Cord Blood Sample Submitted to Investigate Positive DAT

ABORh

	ABO Group				Rh Type	
	Anti-A	Anti-B	A_1 Cells	B Cells	Anti-D	Control
IS	$1+^{mf}$	0	NT	NT	$1+^{mf}$	0

*Transfused 107mL O Neg; M-negative RBCs

Direct Antiglobulin Test

Poly	IgG	C'	Saline	Gel IgG
(0) ✓	(0) ✓	(0) ✓	(0)	$1+^{w}$

DAT Pos IgG (Gel only)

	Anti-M
Pos	4+
Neg	0
Patient Cells	$1+^{mf}$

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Anti-M in Eluate

		Rh					Kell		Duffy		Kidd		MNS				Eluate Results	
		D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	5' RT	PEG IAT
1	R_2R_2	+	0	+	+	0	0	+	+	0	+	0	0	+	0	+	0	(0)
2	r'r	0	+	0	+	+	0	+	0	+	+	0	+	0	0	+	0	(+)
3	R_1R_1	+	+	0	0	+	0	+	0	+	+	0	0	+	0	+	0	(0)
4	rr	0	0	0	+	+	0	+	0	+	+	+	+	0	+	0	0	(+)
5	R_1R_1	+	+	0	0	+	0	+	+	0	+	+	0	+	0	+	0	(0)
6	R_2R_2	+	0	+	+	0	0	+	0	+	+	+	0	+	0	+	0	(0)
7	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	+	+	0	(0)
8	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	+	0	0	(0)
9	rr	0	0	0	+	+	0	+	0	+	0	+	0	+	+	0	0	(0)
10	R_2R_2	+	0	+	+	0	+	+	+	0	+	0	+	0	+	0	0	(+)
11	rr	0	0	0	+	+	0	+	0	+	+	0	+	0	0	+	0	1+

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Workup Conclusions

ABORh	Mixed field with anti-A and anti-D; no reactivity with anti-B and Rh control *No testing was performed to verify sample submitted was solely fetal blood
DAT	Pos (Gel only)
M Antigen Typing	Mixed field reactivity
Eluate	Anti-M *Can not be determined if the anti-M detected was due to in vitro or in vivo binding of the antibody to cord RBCs
Transfusion Recommendation	M-negative Donor blood selected for transfusion should be nonreactive with the maternal plasma.

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Approximately 3 Weeks Later

- Baby boy born at 37 weeks gestation
- Hemoglobin 12.7 g/dL (14.0-24.0 g/dL)
- Hematocrit 39.8% (55-68%)
- Bilirubin not performed on day of birth, was 1.2 mg/dL (<5.2 mg/dL) on day 2
- Sample submitted to investigate pos DAT

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Cord Blood Sample Submitted to Investigate Positive DAT

ABORh

	ABO Group				Rh Type	
	Anti-A	Anti-B	A_1 Cells	B Cells	Anti-D	Control
IS	$2+^{mf}$	0	NT	NT	$2+^{mf}$	0

*Multiply transfused O Neg; M-negative RBCs

Direct Antiglobulin Test			
Poly	IgG	C'	Saline
$1+^{mf}$	$1+^{mf}$	(0)	(0)

DAT Pos IgG

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Anti-M in Plasma

		Rh					Kell		Duffy		Kidd		MNS				Plasma Results	
		D X	C X	E X	c X	e X	K X	k X	Fy^a X	Fy^b X	Jk^a X	Jk^b X	M	N X	S X	s X	5' RT	PEG IAT
1	R_1R_1	+	+	0	0	0	0	+	0	+	+	0	0	+	0	+	0	(0)
2	rr	0	0	0	+	+	0	+	+	+	+	0	0	+	0	+	0	(0)
3	rr	0	0	0	+	+	0	+	0	+	0	+	+	0	+	0	0	(+)
4	R_1R_1	+	+	0	0	+	+	+	+	+	+	0	0	+	0	+	0	(0)
5	rr	0	0	0	+	+	0	+	0	+	0	+	0	+	+	0	0	(0)
6	R_2R_2	+	0	+	+	0	0	+	0	+	0	+	0	+	0	+	0	(0)
7	R_2R_2	+	0	+	+	0	0	+	0	+	+	0	0	+	0	+	0	(0)
8	rr	0	0	0	+	+	0	+	+	+	+	0	0	+	+	0	0	(0)
9	R_0r	+	0	0	+	+	0	+	0	0	0	+	+	0	0	+	3+	3+
10	rr	0	0	0	+	+	+	+	+	0	+	0	0	+	+	+	0	(0)
11	R_1R_1	+	+	+	0	+	0	+	+	0	+	+	0	+	0	+	0	(0)
12	R_2R_2	+	0	+	+	0	+	+	+	0	+	0	+	0	+	+	1+	2+
13	rr	0	0	0	+	+	0	+	0	+	0	+	+	0	+	0	1+	2+

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Anti-M in Eluate

		Rh					Kell		Duffy		Kidd		MNS				Eluate Results	
		D X	C X	E X	c X	e X	K X	k X	Fy^a X	Fy^b X	Jk^a X	Jk^b X	M	N X	S X	s X	5' RT	PEG IAT
1	R_1R_1	+	+	0	0	0	0	+	0	+	+	0	0	+	0	+	0	(0)
2	rr	0	0	0	+	+	0	+	+	+	+	0	0	+	0	+	0	(0)
3	rr	0	0	0	+	+	0	+	0	+	0	+	+	0	+	0	0	(0)
4	R_1R_1	+	+	0	0	+	+	+	+	+	+	0	0	+	0	+	0	(0)
5	rr	0	0	0	+	+	0	+	0	+	0	+	0	+	+	0	0	(0)
6	R_2R_2	+	0	+	+	0	0	+	0	+	0	+	0	+	0	+	0	(0)
7	R_2R_2	+	0	+	+	0	0	+	0	+	+	0	0	+	0	+	0	(0)
8	rr	0	0	0	+	+	0	+	+	+	+	0	0	+	+	0	0	(0)
9	R_0r	+	0	0	+	+	0	+	0	0	0	+	+	0	0	+	0	2+
10	rr	0	0	0	+	+	+	+	+	0	+	0	0	+	+	+	0	(0)
11	R_1R_1	+	+	+	0	+	0	+	+	0	+	+	0	+	0	+	0	(0)
12	R_2R_2	+	0	+	+	0	+	+	+	0	+	0	+	0	+	+	0	(+)
13	rr	0	0	0	+	+	0	+	0	+	0	+	+	0	+	0	0	(+)

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Additional Testing

One M+ panel cell reacted strongly with the infant's plasma at RT and by PEG IAT; the reactivity was removed by ficin treatment.

Testing of additional reagent red cells positive for low prevalence antigens:

Mi^a
Mur
He
Hut
Dantu

No specificity was observed

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Infant HEA Results

Blood Group	Antigen	Result	Comments
Rh	c	+	
	C	0	
	e	+	
	E	0	
	V	0	
	VS	0	
Kell	K	0	
	k	+	
	Kp^a	0	
	Kp^b	+	
	Js^a	0	
	Js^b	+	
Duffy	Fy^a	0	
	Fy^b	(0)*	Not at risk for anti-Fy^b
Kidd	Jk^a	+	
	Jk^b	+	
MNS	M	+	
	N	+	
	S	0	
	s	+	
	U	+	
Lutheran	Lu^a	0	
	Lu^b	+	
Diego	Di^a	0	
	Di^b	+	
Colton	Co^a	+	
	Co^b	+	
Dombrock	Do^a	+	
	Do^b	+	
	Hy	+	
	Jo^a	+	
Landsteiner Wiener	LW^a	+	
	LW^b	0	
Sciana	Sc1	+	
	Sc2	0	

Maternal Mismatch

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Workup Conclusions

ABORh	A Pos
DAT	Pos IgG
Plasma & Eluate	Anti-M *Can not be determined if the anti-M detected was due to in vitro or in vivo binding of the antibody to cord RBCs
Transfusion Recommendation	While maternal antibody is detectable, donor blood selected for transfusion should be M-negative and nonreactive with the infant's plasma.

No additional transfusions given after birth or serological requests...
we can only hope this is good news!
Neonatal clinical characteristics and developmental problems are unknown.

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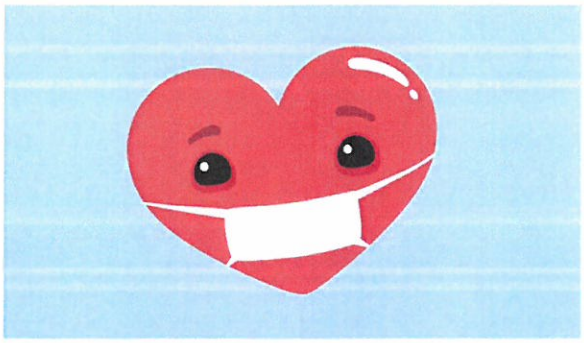
Thank you for your time and attention!



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