

### An Unexpected Needle in the Haystack!

The case of a "surprise" antibody to high prevalence antigen.



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HAABB Winter Meeting  
1/11/2023



1

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### Objectives

- Review the case of a patient with a complicated serology history requiring chronic transfusion therapy.
- Briefly discuss options for selecting red cells for transfusion for patients that have antibodies of variable clinical significance.



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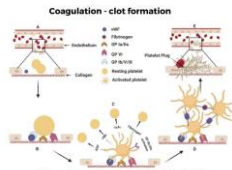
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### Patient History



- Hispanic female
- Born 1971
- Chief complaint: Von Willebrand Disease, recurrent GI Bleed, anemia

Von Willebrand disease is an inherited or acquired bleeding disorder caused by the qualitative or quantitative deficiency of the pro-von Willebrand factor. Affected people may complain of excessive bruising, prolonged bleeding from mucosal surfaces, and prolonged bleeding after minor trauma.



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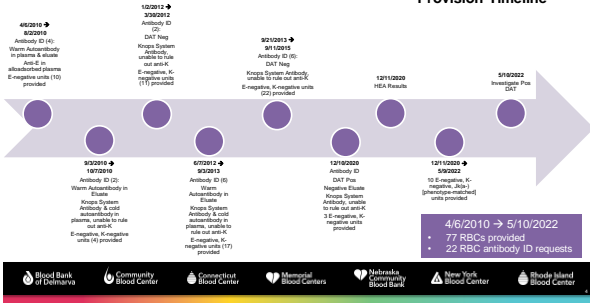
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### Patient Serology & Unit Provision Timeline



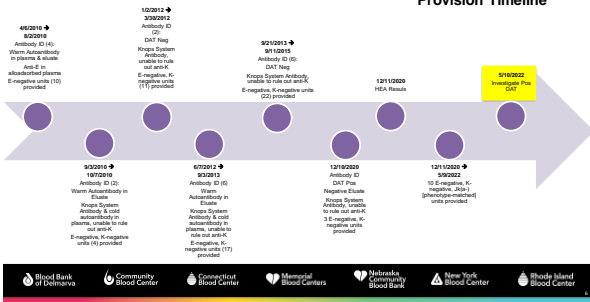
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Previous Serologic Findings Summary		
Source	Antibody	Clinical Significance
Plasma & Eluate	Evanescent warm autoantibody	Transfusion of patients with warm autoantibodies carries a greater than normal risk.
Plasma	Anti-E	Significant
Plasma	Knops system antibody	Insignificant
Plasma	Cold autoantibody	Not usually clinically significant
<b>Transfusion Recommendation</b>	E-negative, K-negative Due to high incidence of Knops antigens on donor and reagent cells, most cells selected will appear reactive with the patient's plasma by IAT. Repeat antibody identification may be necessary for each transfusion episode.	

- Notes on Knops system antibodies:**
- Antigens present on most red cells (95%)
  - Antibody demonstrates "high-titer, low-avidity" (HTLA) reactivity
  - Frequently found in multiphasic sera
  - Variable results in tests on different samples from the same patient have been described
  - Limited availability of well-characterized rare cells
  - Antibodies not known to cause Transfusion reaction or Hemolytic Disease of the Fetus/Newborn (HDFN)

5

### Patient Serology & Unit Provision Timeline



6

### 5/10/2022 Request to Investigate Positive DAT

- Description of reactivity:
  - DAT 2+ Gel IgG
  - All panel cells 1+ reactive with plasma in Gel
- Comments:
  - DAT and autocontrol have been negative for > 6 months
  - 10 phenotype-matched "least-incompatible" RBCs have been transfused since April
  - Laboratory evidence of hemolysis

RBC Units Transfused			5/6/2022	5/9/2022	5/10/2022	5/11/2022	5/12/2022
4/21/2022	1 unit	Hgb g/dL	4.3 L	NA	NA	7.3 L	8.3
5/6/2022	2 units	Bili Total mg/dL	1.1	NA	3.9 H	NA	NA
5/7/2022	2 units	Retic %	NA	8.5 H	NA	NA	NA
5/8/2022	3 units	LDH IU/L	NA	231	NA	NA	NA
5/9/2022	2 units	Haptoglobin mg/dL	NA	< 10 L	NA	NA	NA



7

### 5/10/2022 Workup: ABO/Rh & DAT

	ABO Group				Rh Type	
	Anti-A	Anti-B	A <sub>1</sub> Cells	B Cells	Anti-D	Control
IS	0	0	3+	3+	3+	0

Group O, Rh Pos

Direct Antiglobulin Test			
Poly	IgG	C'	Saline
(+)	(+)	(0) ✓	(0)

DAT Positive IgG



8

### 5/10/2022 Workup: Plasma panel #1

	Rh			Kell					Duffy			Kidd			MNSs			Lutheran		Plasma Results			
	D	C	E	c	e	K	k <sub>1</sub>	Ko	Jk <sup>a</sup>	Jk <sup>b</sup>	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	M	N	S	s	Lup	Lup	S	REG IAT	
1 rr	0	0	0	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	0	(+)
2 rr	0	0	0	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	0	(+)
3 rr	0	0	0	+	+	+	+	+	0	+	+	+	+	0	+	+	+	+	+	+	+	0	(0) ✓
4 R <sub>1</sub> R <sub>2</sub>	+	+	0	+	+	+	+	+	0	+	+	+	+	0	+	+	+	+	+	+	+	0	(+)
5 R <sub>1</sub> R <sub>2</sub>	+	+	0	+	+	+	+	+	0	+	+	+	+	0	+	+	+	+	+	+	+	0	(+)
6 Fy	+	+	0	+	+	+	+	+	0	+	+	+	+	0	+	+	+	+	+	+	+	0	(+)
7 R <sub>1</sub> R <sub>2</sub>	+	+	0	+	+	+	+	+	0	+	+	+	+	0	+	+	+	+	+	+	+	0	(+)
8 R <sub>1</sub> R <sub>2</sub>	+	+	0	+	+	+	+	+	0	+	+	+	+	0	+	+	+	+	+	+	+	0	(+)
9 Patient Cell																						0	(+)



9

5/10/2022 Workup: Eluate panel #1

	Rh	Kell														Duffy	Kidd	MNSs	Lutheran	Acid Eluate Results					
		D	C	E	c	e	K	k	Kp <sup>a</sup>	Kp <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>					Jk <sup>b</sup>	M	N	S	s	Lp <sup>a</sup>
1 r <sup>r</sup>	0	0	0	+	+	+	+	+	0	+	0	+	+	+	+	+	+	0	+	+	+	0	+	+	1+
2 r <sup>r</sup>	0	0	0	+	+	+	+	+	0	+	0	+	+	+	+	+	+	0	+	+	+	0	+	+	1+
3 r <sup>r</sup>	0	0	0	+	+	+	+	+	0	+	0	+	+	+	+	+	+	0	+	+	+	0	+	+	(0) ✓
4 R <sub>1</sub> R <sub>2</sub>	+	+	0	0	+	+	+	+	0	+	0	+	+	+	+	+	+	0	0	+	0	+	+	1+	
5 R <sub>1</sub> R <sub>2</sub>	+	0	+	+	0	0	+	+	0	+	0	+	0	+	0	+	+	0	+	+	+	0	+	1+	
6 r <sup>r</sup>	0	0	0	+	+	+	+	+	0	+	0	+	+	+	+	+	+	0	+	+	+	0	+	1+	
7 R <sub>1</sub> R <sub>2</sub>	+	+	+	+	0	0	+	+	0	+	0	+	+	+	+	+	+	0	0	+	0	+	+	1+	
8 R <sub>1</sub> R <sub>2</sub>	+	+	0	0	+	+	+	+	0	+	0	+	+	+	+	+	+	0	0	+	0	+	+	1+	
9 Patient Cell																								NT Recarity Transfused	



10

5/10/2022 Workup: Panel #1 Insights

Plasma & eluate nonreactive with Yt(a-) cell

	Rh	Kell														Duffy	Kidd	MNSs	Lutheran	Plasma Results		Acid Eluate Results				
		D	C	E	c	e	K	k	Kp <sup>a</sup>	Kp <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>					Jk <sup>b</sup>	M		N	S	s	Lp <sup>a</sup>
1 r <sup>r</sup>	0	0	0	+	+	+	+	+	0	+	0	+	+	+	+	+	+	0	+	+	+	0	+	+	(+)	1+
2 r <sup>r</sup>	0	0	0	+	+	+	+	+	0	+	0	+	+	+	+	+	+	0	+	+	+	0	+	+	(+)	1+
3 r <sup>r</sup> Yt(a-)	0	0	0	+	+	+	+	+	0	+	0	+	+	+	+	+	+	0	+	+	+	0	+	+	(0) ✓	(0) ✓
4 R <sub>1</sub> R <sub>2</sub>	+	+	0	0	+	+	+	+	0	+	0	+	+	+	+	+	+	0	0	+	0	+	+	(+)	1+	
5 R <sub>1</sub> R <sub>2</sub>	+	0	+	+	0	0	+	+	0	+	0	+	0	+	0	+	+	0	+	+	+	0	+	(+)	1+	
6 r <sup>r</sup>	0	0	0	+	+	+	+	+	0	+	0	+	+	+	+	+	+	0	+	+	+	0	+	(+)	1+	
7 R <sub>1</sub> R <sub>2</sub>	+	+	+	+	0	0	+	+	0	+	0	+	+	+	+	+	+	0	0	+	0	+	+	(+)	1+	
8 R <sub>1</sub> R <sub>2</sub>	+	+	0	0	+	+	+	+	0	+	0	+	+	+	+	+	+	0	0	+	0	+	+	(+)	1+	
9 Patient Cell																		0						(+)	NT Recarity Transfused	



11

5/10/2022 Workup: Rare cell panel

Plasma & eluate nonreactive with Yt(a-) cells

	Rh	Kell														Duffy	Kidd	MNSs	Lutheran	Plasma Results		Acid Eluate Results			
		D	C	E	c	e	K	k	Kp <sup>a</sup>	Kp <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>					Jk <sup>b</sup>	M		N	S	s
1 Yt(a-)	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	+	NT	NT	+	+	0	NT	(0) ✓	(0) ✓	
2 Yt(a-)	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	+	+	0	0	+	+	0	+	(0) ✓	(0) ✓
3 Yt(a-)	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	+	+	0	0	+	+	0	NT	(0) ✓	(0) ✓
4 Yt(a-)	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	+	+	0	0	+	+	0	NT	(0) ✓	(0) ✓
5 Yt(a-)	0	0	0	+	+	0	+	0	+	0	+	0	+	+	+	+	+	0	0	0	NT	(0) ✓	(0) ✓	(0) ✓	
6 Kn(a-)	0	0	0	+	+	0	+	0	+	0	+	0	+	+	+	+	+	0	+	+	0	+	1+	1+	
7 Kn(a-)	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	+	+	0	0	+	0	+	(+)	1+	

Plasma & eluate were reactive with 2 examples of Kn(a-) cells the patient's plasma was previously nonreactive with when Knops antibody originally identified.



12

### Yt<sup>a</sup> Antigen

Terminology	History	Occurrence	In vitro characteristics of alloanti-Yt <sup>a</sup>	Clinical significance of alloanti-Yt <sup>a</sup>
<ul style="list-style-type: none"> <li>• ISBT # Y11</li> <li>• Obsolete name: Cartwright</li> </ul>	<ul style="list-style-type: none"> <li>• Named "Cartwright" after 1<sup>st</sup> antibody producer in 1956</li> <li>• Most letters in "Cartwright" had been taken by other antigens</li> <li>• The authors then thought "why not T", and eventually "why T" or "Yt"</li> <li>• Yt<sup>a</sup> achieved system status in 1964</li> </ul>	<ul style="list-style-type: none"> <li>• Most populations &gt;99.8%</li> <li>• Israeli Jews 99.6%</li> <li>• Israeli Arabs 97.6%</li> <li>• Israeli Druse 97.4%</li> </ul>	<ul style="list-style-type: none"> <li>• IgG</li> <li>• IAT optimal technique</li> <li>• Capable of complement binding</li> </ul>	<ul style="list-style-type: none"> <li>• Transfusion reaction: No to moderate (rare)/delayed</li> <li>• HDFN: No</li> </ul>



13

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### Yt<sup>a</sup> Antigen

Effects of enzymes and chemicals on Yt <sup>a</sup> antigen on intact RBCs	
Ficin/Papain	Sensitive (variable)
Trypsin	Resistant
α-Chymotrypsin	Sensitive
DTT	Sensitive/weakened
Acid	Resistant



14

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5/10/2022 Workup: DTT-treatment of cells from Panel #1

	Target antigen is sensitive to DTT-Treatment																									
	Rh				Kell							Duffy		Kidd				MNSs				Lutheran		Plasma Results		Acid Eluate Results
	D	C	E	c	K	k	K <sup>o</sup>	Kp <sup>a</sup>	Jk <sup>a</sup>	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>b</sup>	M	N	S	s	L <sup>a</sup>	L <sup>b</sup>	PEG IAT	DTT-Tx Cells PEG IAT	DTT-Tx Cells PEG IAT	DTT-Tx Cells PEG IAT				
1 <sup>II</sup>	0	0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	(+)	(0) ✓	1+	(0) ✓				
2 <sup>II</sup>	0	0	0	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	(+)	(0) ✓	1+	(0) ✓				
4 R,R <sub>b</sub>	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	(+)	(0) ✓	1+	(0) ✓				
5 R,R <sub>b</sub>	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	(+)	(0) ✓	1+	(0) ✓				
6 <sup>rr</sup>	0	+	0	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	(+)	(0) ✓	1+	(0) ✓				
7 R,R <sub>b</sub>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	(+)	(0) ✓	1+	(0) ✓				
8 R,R <sub>b</sub>	+	+	0	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	(+)	(0) ✓	1+	(0) ✓				



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5/10/2022 Workup: Ficin & Trypsin-Treatment of cells

Target antigen is sensitive to Ficin-treatment, resistant to Trypsin-treatment

	Kell												Lutheran		Plasma Results				Acid Eluate Results						
	D	C	E	c	K	N	Kp1	Kp2	20P	Fy1	Fy2	20k	20k	M	N	S	s	L <sup>u</sup>	L <sup>v</sup>	PEE IAT	Ficin-Tx Cells PEE IAT	Trypsin- Tx Cells PEE IAT	PEE IAT	Ficin-Tx Cells PEE IAT	
rr	0	0	0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
R <sub>1</sub> R <sub>2</sub>	+	+	0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
R <sub>1</sub> R <sub>2</sub>	+	+	0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
R <sub>1</sub> R <sub>2</sub>	+	+	0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
R <sub>1</sub> R <sub>2</sub>	+	+	0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+



16

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5/10/2022 Workup Conclusions

<b>ABO/Rh</b>	Group O, Rh Positive
<b>DAT</b>	Positive IgG
<b>Plasma &amp; Eluate</b>	Anti-Yt*
<b>Additional Comments</b>	<ul style="list-style-type: none"> <li>The previously reported anti-E, Knops system antibody, and warm and cold autoantibodies were not detected in the current sample.</li> </ul>
<b>Transfusion Recommendation</b>	<ul style="list-style-type: none"> <li>E-negative</li> <li>The clinical significance of anti-Yt* ranges from none to moderate (rare)/delayed. Since anti-Yt* is present in the patient's plasma and eluate, Yt(a-) donor units are recommended at this time.</li> </ul>
<b>Additional units provided</b>	<ul style="list-style-type: none"> <li>None after the identification of anti-Yt*</li> <li>Requesting facility reports patient is being supported without transfusion therapy.</li> </ul>



17

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Yt and Kn Genotyping

New York Blood Center  
 Laboratory of Immunohematology and Genetics  
 610 West 64th Street, New York, NY 10023  
 718-224-4771 • fax 718-224-4767

Date Reported: 05/24/22

**SEQUENCE RESULTS:**  
 Kp: c.4223C/T, c.4619A/A, c.4681G/G, c.4768A/G, c.4801A/G, c.4828T/T, c.4843A/G  
 Jt: c.1057A/A (p.353Asn)

**Predicted Knops phenotype:** Yt(a+), Kn(a+b-), McC(a+b-), Sl(a+), Vii-, Sll+ (Sll 1, 2, 3), KCAM+,  
**Predicted Yt phenotype:** Yt(a-b+)

**COMMENTS:** The RBCs are not predicted to lack a high prevalence antigen in the Knops system but are predicted to be Yt(a-b+).

Although the DNA analysis indicates that the patient is not at risk to make an antibody to the Knops antigens that were tested for, there is always the possibility that the antibody may be against Knops antigens not yet defined or not detected by the current assays. Knops system antibodies are clinically insignificant.



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### Knops vs. Yt<sup>a</sup>

	Knops (Kn <sup>a</sup> )	Yt <sup>a</sup>
Antigen occurrence	>95%	>99.8%
Optimal technique	IAT	IAT
Effects of enzymes & chemicals on antigen:	<ul style="list-style-type: none"> <li>• Weakened</li> <li>• Sensitive</li> <li>• Sensitive</li> <li>• Resistant</li> </ul>	<ul style="list-style-type: none"> <li>• Sensitive (variable)</li> <li>• Sensitive</li> <li>• Sensitive</li> <li>• Resistant</li> </ul>
Clinical Significance of alloantibody	Not clinically significant in transfusion medicine.	Experts agree that anti-Yt <sup>a</sup> are often benign and antigen-negative blood may not need to be transfused.



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### Objectives

- Review the case of a patient with a complicated serology history requiring chronic transfusion therapy.
- Briefly discuss options for selecting red cells for transfusion for patients that have antibodies of variable clinical significance.



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### When Uncommon or Rare Blood is Needed

-  IRL fresh and/or frozen inventory
-  NYBCE Partners
-  Autologous (if clinical situation allows)
-  Family Members
-  American Rare Donor Program



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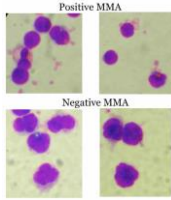
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Options for selecting red cells for transfusion for patients that have antibodies of variable clinical significance:  
**MMA**

**Monocyte Monolayer Assay (MMA)**

- Used to predict the risk of a clinically significant reaction if a patient is transfused antigen-positive RBCs.
- Steps:
  - Monocytes isolated from donors and placed in single layer on a plate.
  - Antigen-positive RBCs are mixed with patient antibody and added to monocytes.
  - Monocytes with one or more RBCs adhered/engulfed are counted under a microscope.
  - If the monocyte index is low (cutoff is lab-dependent), indicates RBCs won't be rapidly destroyed during transfusion.
- Rare assay, not many labs in the country are able to perform.
- Should not be used in emergent situations.



BloodBankGuy.com  
 BBO Guy Essentials 093  
 The Mighty MMA with Sandy Neuner  
 Released October 13, 2021

Highest number of evaluations is for anti-YtH

https://www.bbguy.org/2021/10/13/093/

Logos for Blood Bank of Delaware, Community Blood Center, Connecticut Blood Center, Memorial Blood Centers, Nebraska Community Blood Bank, New York Blood Center, Rhode Island Blood Center.

22

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Options for selecting red cells for transfusion for patients that have antibodies of variable clinical significance:  
**Transfusing Antigen-Positive Units**

**TRANSFUSION**

**YtGT: A new high-prevalence antigen in the Yt blood group system in two unrelated Native Americans and transfusion management**

Authors: Nancy L. Van Boren, Barbara Gillet, Christine Lemaire-Franco, James England, Shantel Frank, Maria Rivera, Alan Landwehr, Felix Engel, Alexander Papadopoulos, Eric Haber, Michelle Lederman, Jennifer Hogg, Charles McManis

**TRANSFUSION**

**TRANSFUSION PRACTICE**

Transfusions of least-incompatible blood with intravenous immunoglobulin plus steroids cover in two patients with rare antibody

Authors: Anusha Nand, Anuj Thakral, Robert Robinson, Cheryl Chang

**Guidelines**

- Select heterozygous antigen-positive units if available.
- Baseline hemolysis markers should be evaluated (CBC, retic, Bilirubin, LDH, Haptoglobin, urine dipstick for Hgb).
- Patient should be informed and provide consent.
- Steroids and IVIG given prior to or within 24 hours of "incompatible" transfusion (if clinically appropriate).
- Transfusion should be given at slowest rate and monitor patient carefully.
- Stop transfusion immediately if reaction develops.

Logos for Blood Bank of Delaware, Community Blood Center, Connecticut Blood Center, Memorial Blood Centers, Nebraska Community Blood Bank, New York Blood Center, Rhode Island Blood Center.

23

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### References

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