Community Blood Center

It REALLY Was A UNICORN!

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New York Blood Center Enterprises

Objectives

- Evaluate requests for antigen negative units.
- Develop an approach to complex antibody identification.
- Utilize genomics to explain or confirm serologic results.
- Explore transfusion options in extremely rare cases.
- Describe the importance of educating the community of rare donors through donor letters.

Initial Background

Received request

- 1 group O Rh negative RBC
- E-, c-
- Per registry, patient has anti-c

Initial Internal thoughts

- Group O, Rh negative, c- prevalence **<0.01%**
- r'r'

- ✓ Per hospital, patient RBCs type c-
- ✓ 1 frozen unit thawed and delivered
- ✓ We recommend genotyping to confirm c status

What makes r'r' SO rare?

Phenotype (alternative)	Caucasians	Blacks	Asians
D-positive			
R_1R_1 (R_1r')	18.5	2.0	51.8
R_2R_2 (R_2r'')	2.3	0.2	4.4
R_1r (R_1R_0; R_0r')	34.9	21.0	8.5
R_2r (R_2R_0; R_0r'')	11.8	18.6	2.5
R_0r (R_0R_0)	2.1	45.8	0.3
R_zR_z (R_zr^y)	0.01	Rare	Rare
R_1R_z (R_zr'; R_1r^y)	0.2	Rare	1.4
R_2R_z (R_zr''; R_2r^y)	0.1	Rare	0.4
R_1R_2 (R_1r''; R_2r'; R_zr; R_0R_z; R_0r^y)	13.3	4.0	30.0
D-negative			
$r'r$	0.8	Rare	0.1
$r'r'$	Rare	Rare	0.1
$r''r$	0.9	Rare	Rare
$r''r''$	Rare	Rare	Rare
rr	15.1	6.8	0.1
$r'r''$ (r^yr)	0.05	Rare	Rare
$r'r^y$; $r''r^y$; r^yr^y	Rare	Rare	Rare
r'^Sr	0	1–2	0

Taken from AABB Technical Manual, 21st ed.

Patient Genotype Results Received

Serology:

- Confirmed D-

Genotyping:

- Confirmed c-

Full phenotype:

D-E-c-; **K-**; **Fy(a-**b+); Jk(a+b+); **M-**N+S+s+

Blood Group	Antigen	Result	Comments
Rh	c	0	
	C	+	
	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	+	
Kidd	Jk^a	+	
	Jk^b	+	
MNS	M	0	
	N	+	
	S	+	
	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	

Guess what happened? The patient returned!

- This time a workup is requested
  - we finally get to see this anti-c in action!

- We also learned more history on the patient.
  - 28 yrs old, female
  - Presents for surgery
  - Requesting 2 units of RBCs

IRL Approach – Initial Testing

	Rh					Kell		Duffy		Kidd		MNS			
	D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s
1	0	0	0	+	+	0	+	+	+	+	+	+	+	+	+
2	0	0	0	+	+	+	+	0	+	0	+	0	+	0	+
3	+	+	0	0	+	0	+	+	0	+	+	+	0	+	+
4	+	+	0	0	+	0	+	0	0	+	0	+	+	0	+
5	+	+	0	0	+	0	+	+	0	+	0	+	+	+	0
6	+	+	0	0	+	0	+	0	+	+	+	0	+	0	+
7	+	+	0	0	+	+	+	0	+	+	0	+	0	+	+
8	+	+	0	0	+	0	+	+	+	0	+	0	+	+	+
9	+	+	+	0	+	0	+	+	+	0	+	+	0	0	+
10	+	+	+	0	+	0	+	+	0	+	+	+	+	+	0
11	+	+	0	0	+	+	+	0	0	+	+	0	+	+	+
Auto															

IRL Approach – Initial Testing

Anti-c or something else?

	Rh					Kell		Duffy		Kidd		MNS				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	0	0	0	+	+	0	+	+	+	+	+	+	+	+	+	1+	2+
2	0	0	0	+	+	+	+	0	+	0	+	0	+	0	+	0	2+
3	+	+	0	0	+	0	+	+	0	+	+	+	0	+	+	1+	1+
4	+	+	0	0	+	0	+	0	0	+	0	+	+	0	+	1+	2+
5	+	+	0	0	+	0	+	+	0	+	0	+	+	+	0	1+	1+
6	+	+	0	0	+	0	+	0	+	+	+	0	+	0	+	0	2+
7	+	+	0	0	+	+	+	0	+	+	0	+	0	+	+	1+	2+
8	+	+	0	0	+	0	+	+	+	0	+	0	+	+	+	0	1+
9	+	+	+	0	+	0	+	+	+	0	+	+	0	0	+	1+	1+
10	+	+	+	0	+	0	+	+	0	+	+	+	+	+	0	1+	1+
11	+	+	0	0	+	+	+	0	0	+	+	0	+	+	+	0	1+
Auto																0	0v

IRL Approach – Initial Testing

	Rh					Kell		Duffy		Kidd		MNS				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	0	0	0	+	+	0	+	+	+	+	+	+	+	+	+	1+	2+
2	0	0	0	+	+	+	+	0	+	0	+	0	+	0	+	0	2+
3	+	+	0	0	+	0	+	+	0	+	+	+	0	+	+	1+	1+
4	+	+	0	0	+	0	+	+	0	+	0	+	+	0	+	1+	2+
5	+	+	0	0	+	0	+	+	0	+	0	+	+	+	0	1+	1+
6	+	+	0	0	+	0	+	0	+	+	+	0	+	0	+	0	2+
7	+	+	0	0	+	+	+	0	+	+	0	+	0	+	+	1+	2+
8	+	+	0	0	+	0	+	+	+	0	+	0	+	+	+	0	1+
9	+	+	+	0	+	0	+	+	+	0	+	+	0	0	+	1+	1+
10	+	+	+	0	+	0	+	+	0	+	+	+	+	+	0	1+	1+
11	+	+	0	0	+	+	+	0	0	+	+	0	+	+	+	0	1+
Auto																0	0v

Anti-M at RT

IRL Approach – Initial Testing

	Rh					Kell		Duffy		Kidd		MNS				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	0	0	0	+	+	0	+	+	+	+	+	+	+	+	+	1+	2+
2	0	0	0	+	+	+	+	0	+	0	+	0	+	0	+	0	2+
3	+	+	0	0	+	0	+	+	0	+	+	+	0	+	+	1+	1+
4	+	+	0	0	+	0	+	0	0	+	0	+	+	0	+	1+	2+
5	+	+	0	0	+	0	+	+	0	+	0	+	+	+	0	1+	1+
6	+	+	0	0	+	0	+	0	+	+	+	0	+	0	+	0	2+
7	+	+	0	0	+	+	+	0	+	+	0	+	0	+	+	1+	2+
8	+	+	0	0	+	0						0	+	+	+	0	1+
9	+	+	+	0	+	0						+	0	0	+	1+	1+
10	+	+	+	0	+	0	+	+	0	+	+	+	+	+	0	1+	1+
11	+	+	0	0	+	+	+	0	0	+	+	0	+	+	+	0	1+
Auto																0	0v

All M-, c- RBCs reactive at IAT

IRL Approach – Initial Testing

	Rh					Kell		Duffy		Kidd		MNS				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	0	0	0	+	+	0	+	+	+	+	+	+	+	+	+	1+	2+
2	0	0	0	+	+	+	+	0	+	0	+	0	+	0	+	0	2+
3	+	+	0	0	+	0	+	+	0	+	+	+	0	+	+	1+	1+
4	+	+	0	0	+	0	+	0	0	+	0	+	+	0	+	1+	2+
5	+	+	0	0	+	0	+	+	0	+	0	+	+	+	0	1+	1+
6	+	+	0	0	+	0	+	0	+	+	+	0	+	0	+	0	2+
7	+	+	0	0	+	+	+	0	+	+	0	+	0	+	+	1+	2+
8	+	+	0	0	+	0	+	+	+	0	+	0	+	+	+	0	1+
9	+	+	+	0	+	0	+	+	+	0	+	+	0	0	+	1+	1+
10	+	+	+	0	+	0	+	+	0	+	+					1+	1+
11	+	+	0	0	+	+	+	0	0	+	+					0	1+
Auto																0	0v

Autocontrol negative

Time to chase that party!

- Plasma reactive with all cells
- All c-,M- RBCs reactive micro to 2+ at IAT
- Negative auto control

Testing Phenotype-Matched RBC

Phenotype-matched = RBC negative for all common antigens the patient's RBCs lack

D-E-c-; **K-**; **Fy**(**a-**b+); Jk(a+b+); **M-**N+S+s+

Reactivity with phenotype-matched RBC	Interpretation
Reactive	Antibody to high prevalence antigen
Non-reactive	Multiple antibodies to common antigens

Testing Phenotype-Matched RBC

	Rh					Kell		Duffy		Kidd		MNS				Results	
	D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	5’ RT	PEG IAT
r’r’	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	1+

Interpretation: **Antibody to a high prevalence antigen (anti-c + anti-M + …??)**

Cell Treatments

Common treatments	• **Ficin/Papain** • **DTT** • **Trypsin**
How do treatments work?	• Test treated reagent RBCs against patient plasma • Treatments destroy some antigens; enhance some antibodies
What do you do with the results?	• Pattern of reactivity narrows down specificity of antibody to high prevalence antigen • May provide negative reactions for ruleouts
Lab logistics 	• Each treatment takes time • QC to ensure appropriate antigens destroyed

Results: Treatments

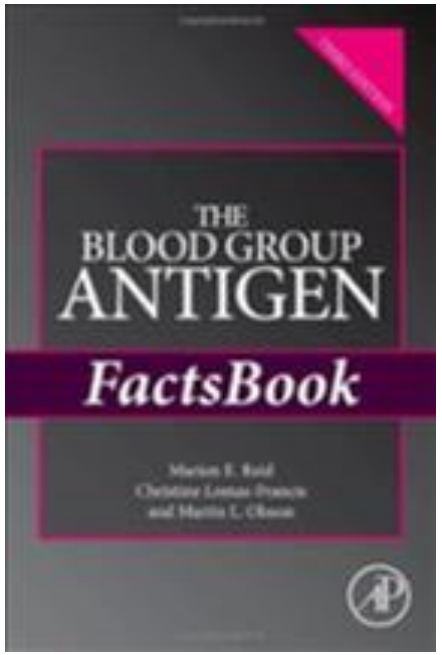
All cells reactive at PEG IAT

Rh					Kell		Duffy		Kidd		MNS				Results		
D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	**PEG IAT**	**Ficin IAT**	**Trypsin IAT**
+	+	0	0	+	0	+	+	+	+	+	0	+	+	+	1+	0v	0v
+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	(+)	0v	0v
+	+	0	0	+	0	+	+	0	+	+	0	0	+	+	2+	0v	0v
0	0	0	+	+	0	+	0	+	+	0	0	0	+	+	2+	2+	2+
0	0	0	+	+	0	+	+	+	0	+	0	+	+	+	2+	2+	2+
+	+	0	0	+	+	+	0	+	+	+	+	0	+	+	1+	0v	0v
+	+	0	0	+	0	+	+	+	+	0	+	+	+	+	1+	0v	0v

- Ficin/Trypsin circumvent reactivity (anti-c identified)
- Antibodies to common RBC antigens ruled out (not anti-M)

Next Steps

Ficin/Papain	Trypsin	α-chymotrypsin	DTT	Possible Specificity
Negative	Negative	Negative	Positive	Bp^a; Ch/Rg; XG
Negative	Negative	Negative	Negative	IN; JMH
Negative	Negative	Positive	Positive	M, N, En^aTS; Ge2, Ge4
Negative	Positive	Negative	Positive	'N'; Fy^a, Fy^b
Variable	Positive	Negative	Positive	S, s
Variable	Positive	Negative	Weak or negative	YT
Negative	Positive	Positive	Positive	En^aFS
Positive	Negative	Negative	Weak or negative	LU, MER2
Positive-Papain Weak or negative – ficin	Negative	Negative	Negative	KN
Positive	Negative	Weak	Negative	DO
Positive	Positive	Negative	Weak	CROM
Positive	Positive	Negative	Positive	Some DI
Positive	Positive	Positive/weak	Negative	LW
Positive	Positive/weak	Positive/weak	Positive	SC
Positive	Positive	Positive	Negative	KEL
Positive	Positive	Positive	Positive	ABO; En^aFR, U; P1PK; RH; LE; FY3; JK; most DI; CO; H; Ge3; OK; I/I; P, FORS; JR; LAN, Cs^a; ER; LKE; PX2; Vel, ABTI; At^a; Emm; AnWj; Sd^a; PEL; MAM
Positive	Positive	Positive	Enhanced	Kx

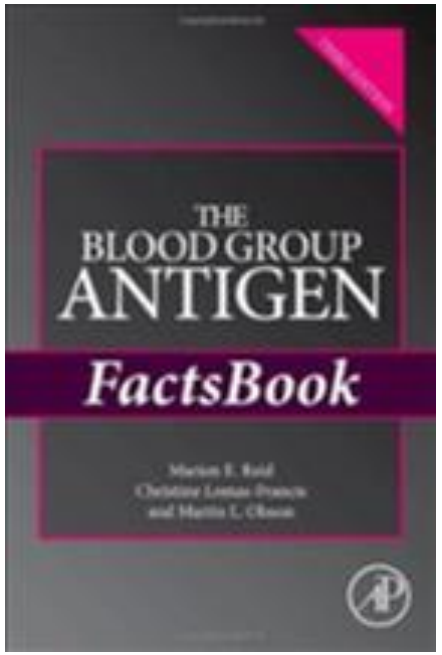


Blood Group Antigen FactsBook

Next Steps

Ficin/Papain	Trypsin	α-chymotrypsin	DTT	Possible Specificity
Negative	Negative	Negative	Positive	Bp^a; Ch/Rg; XG
Negative	Negative	Negative	Negative	IN; JMH
Negative	Negative	Positive	Positive	M, N, En^{a}TS; Ge2, Ge4
Negative	Positive	Negative	Positive	'N'; Fy^a, Fy^b
Variable	Positive	Negative	Positive	S, s
Variable	Positive	Negative	Weak or negative	YT
Negative	Positive	Positive	Positive	En^{a}FS
Positive	Negative	Negative	Weak or negative	LU, MER2
Positive-Papain Weak or negative – ficin	Negative	Negative	Negative	KN
Positive	Negative	Weak	Negative	DO
Positive	Positive	Negative	Weak	CROM
Positive	Positive	Negative	Positive	Some DI
Positive	Positive	Positive/weak	Negative	LW
Positive	Positive/weak	Positive/weak	Positive	SC
Positive	Positive	Positive	Negative	KEL
Positive	Positive	Positive	Positive	ABO; En^{a}FR, U; P1PK; RH; LE; FY3; JK; most DI; CO; H; Ge3; OK; I/I; P, FORS; JR; LAN, Cs^a; ER; LKE; PX2; Vel, ABTI; At^a; Emm; AnWj; Sd^a; PEL; MAM
Positive	Positive	Positive	Enhanced	Kx

Blood Group Antigen FactsBook



Testing Rare RBCs

- Test cells with rare phenotype
  - Also have to be c-, M-
- Kn(a-), Ch-, Rg-, Yk(a-), McC(a-), En(a-), Yt(a-)

All cells reactive micro to 2+ at IAT

Neutralization

- Specifically investigating CH/RG antibody
- Soluble CH/RG substance in inert plasma/serum

Rh					Kell		Duffy		Kidd		MNS				Results		
D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	PEG IAT	Neutralized PEG IAT	Control PEG IAT
+	+	0	0	+	0	+	+	+	+	+	0	+	+	+	1+	1+	1+
+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	1+	1+	1+
+	+	+	0	+	0	+	+	0	+	+	0	0	+	+	(+)	(+)	(+)
+	+	0	0	+	0	+	0	0	+	0	0	+	0	+	2+	2+	2+
+	+	0	0	+	0	+	+	0	+	0	0	+	0	0	1+	1+	1+

Results: antibody not neutralizable; not CH/RG

Titers

- Determine if reactivity could be considered HTLA
  - High Titer, Low Affinity
- Can help further narrow down specificity
- Test c-, M- RBC; saline 60’ 37C, IAT

	Neat	2	4	8	16	32	64	128
Saline 60’37C; IAT M-, c-	(+)	(+)	$(+)^{w}$	0√	0√	0√	0√	0√

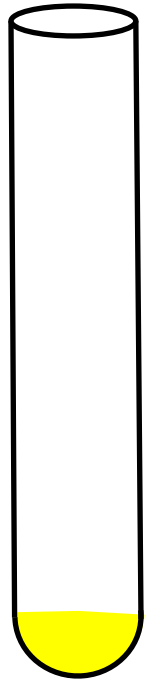
Results: antibody not HTLA

Adsorption?

- Separate out known antibodies (anti-c, anti-M) from unidentified antibody
- Unsuccessful

Sample Exhausted….

(and so were we)



Conclusion: First Sample

- No additional sample submitted
- **Antibodies reported:** anti-c, anti-M, **other**
- **Clinical significance of unidentified antibody:** unknown
- **Transfusion recommendation:** phenotype matched
  - **D-, E-, c-; K-; Fy(a-); M-**

Rare r'r' phenotype
<1% of donors
(without matching for unidentified antibody)

Patient update:
- Not available to provide more sample (outpatient)
- Not transfused at this time

2nd Sample:

New York Blood Center

- Anti-M, anti-c not detectable in this sample
- ***Had use of all rare cells***

Plasma reactive with all RBCs except Lu(a-b-) and LU:-13

Anti-Lu13?

Sample submitted for *LU* genotyping

Fun Facts:

Lu^a and Lu^b in LU system on licensed genotyping platform

Lu13
- High prevalence antigen
- Not included in common genotyping

Lutheran Sequencing Results

- Reviewed genotype
  - Lu(a-b+)
  - Lu13 not included on genotyping platform
- *LU* sequencing.
  - Patient phenotype: Lu13-
  - Lu13 is a high prevalence antigen.

Unidentified antibody =

Anti-Lu13

Blood Group	Antigen	Result	Comments
Rh	c	0	
	C	+	
	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	+	
Kidd	Jk^a	+	
	Jk^b	+	
MNS	M	0	
	N	+	
	S	+	
	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	

Transfusion Options

Searching Rare Inventory

- liquid units
- frozen units
- NYBCe partners
- call in donors, if available

American Rare Donor Program (ARDP)

- Purpose= to assist in locating rare units
- Headquartered in PA
- Submitting requests
- Filling requests

Autologous Donation

- Patient can donate autologous or allogeneic unit ahead of time
  - Preferably allogeneic
- Patient needs to meet criteria for donation
- Can be encouraged to donate when recovered from present illness

Family Studies

- Contact family members for testing
  - Preferably siblings
    - ABO/Rh compatibility
    - Crossmatch with the patient
    - Confirm with serology / genotyping as needed

Transfusion Recommendations

- Only transfusing when absolutely necessary
- Transfusing slowly and monitor for reactions
- Full phenotype matched units (for common antigens), if possible

Communication

- Donor letters
  - We send out letters to donors if we discover that they have a rare phenotype/genotype.
    - They have the option to be submitted to ARDP
- Patient letters
  - Patients can become donors
  - Reference lab sends the patient a letter via the hospital blood bank

Case Conclusion

- After antibodies have been identified
  - No transfusions have been requested
  - Patient had hysterectomy: no concern for HDFN
  - Next time the workup will be easier, finding units will continue to be nearly impossible

Questions?

References

Cohn, Claudia S., et al. *Technical Manual*. 21st ed., AABB, 2023.

Reid, Marion E., et al. *The Blood Group Antigen FactsBook*. 3rd ed., Elsevier Science, 2012.