Workups Are Like A Box Of Chocolates... You Never Know What You Are Going To Get!

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Objectives

List some benefits of obtaining a patient's genotype during a serologic investigation.

Describe some possible causes of panreactivity in a patient's plasma.

Discuss selection of donor units for transfusion.

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Patient

- 83 year old Caucasian male
- 7 g Hgb
- Diagnosis: generalized weakness, potential GI bleed
- No prior tx hx at hospital, patient states no tx since 1960's
- Facility reports screening cells and all panels 3+ gel testing with positive auto controls
- Requesting 1 unit ASAP

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Reviewing Patient History

- Recent UTI, completed 7 day course cephalexin 2 days ago
- Patient has indwelling suprapubic catheter
  - Changed ever 3 weeks. Last changed 1 week ago
- Patient receives most of his care at local government facility

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CBC Workup

ABO/Rh

	ABO Group					Rh Type	
	Anti-A	Anti-B	Anti-A1	A_1 Cells	B Cells	Anti-D	Control
IS	4+	0	4+	0	4+	4+	2+
warm washed x 4						3+	0

Direct Antiglobulin Test

Poly	IgG	C'	Saline
$3+^{s}$	$3+^{s}$	3+	2+
3+ *	2+ *	3+ *	(0) *
	$(0)^{\sqrt{}}$ ♦		

* Warm washed x 4
♦ EGA treated cells

IRL confirms patient's ABO and Rh type as A positive. DAT is positive.

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Eluate Testing with Selected Cells

		Rh					Kell		Duffy		Kidd		Lewis		MNS				Acid Eluate	
		D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	M	N	S	s	5' RT	PEG IAT
1	R_1R_1	+	+	0	0	+	0	+	+	0	+	0	0	+	+	+	+	0	0	@
2	R_1R_1	+	+	0	0	+	+	+	+	+	0	+	0	0	0	+	+	0	2+	@
3	R_2R_2	+	0	+	+	0	0	+	0	+	+	0	0	+	0	+	0	+	2+	@
4	R_2R_2	+	0	+	+	0	0	+	+	0	0	+	0	+	+	+	0	+	2+	@
5	R_2R_2	+	0	+	+	0	0	+	+	+	+	0	+	0	+	0	0	+	2+	@
6	r'r	0	+	0	+	+	0	+	+	+	0	+	0	+	+	0	+	0	0	@
7	rr	0	0	0	+	+	0	+	+	0	+	+	+	0	+	0	+	0	2+	@
8	rr	0	0	0	+	+	+	+	0	+	+	+	0	+	+	0	0	+	0	@
	Auto EGA txd																		$3+^{S}$	@

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Cold Antibody Screen Testing

		Rh					Kell		Duffy		Kidd		Lewis		MNS				Plasma Results	
		D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	M	N	S	s	30' RT	30' 4 C
I	R_1R_1	+	+	0	0	+	0	+	0	+	+	0	+	0	+	+	+	+	$2+^S$	$3+^S$
II	R_2R_2	+	0	+	+	0	0	+	+	0	0	+	0	+	+	0	+	0	$2+^S$	$3+^S$
III	rr	0	0	0	+	+	+	+	0	+	0	+	0	+	0	+	0	+	$2+^S$	$3+^S$
Auto																			3+	4+

- Cold autoantibody present in patient's plasma

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Plasma Testing with Selected Cells

		Rh					Kell		Duffy		Kidd		Lewis		MNS				Plasma Results		
		D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	M	N	S	s	5' RT	PEG IAT	* IAT
1	R_1R_1	+	+	0	0	+	0	+	+	0	+	0	0	+	+	+	+	0	0	@	4+
2	R_1R_1	+	+	0	0	+	+	+	+	+	0	+	0	0	0	+	+	0	0	@	4+
3	R_2R_2	+	0	+	+	0	0	+	0	+	+	0	0	+	0	+	0	+	0	@	4+
4	R_2R_2	+	0	+	+	0	0	+	+	0	0	+	0	+	+	+	0	+	0	@	@
5	R_2R_2	+	0	+	+	0	0	+	+	+	+	0	+	0	+	0	0	+	0	@	@
6	r'r	0	+	0	+	+	0	+	+	+	0	+	0	+	+	0	+	0	0	@	$3+^S$
7	rr	0	0	0	+	+	0	+	+	0	+	+	+	0	+	0	+	0	0	@	$3+^S$
8	rr	0	0	0	+	+	+	+	0	+	+	+	0	+	+	0	0	+	0	@	$3+^S$
	Auto																		2+	@	NT

* = strict prewarming technique 30' at 37C

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Patient Phenotype

	Rh					Kell	Duffy		Kidd		MNS	
	D	C	E	c	e	K	Fy^a	Fy^b	Jk^a	Jk^b	S	s
Pt. Cells	+	+	+	+	+	$+^{MF}$	$+^{MF}$	+	$+^{MF}$	$+^{MF}$	$+^{MF}$	+

- Testing performed with warm washed x 4 RBCs
- Fy^a, Jk^a and Jk^b typings performed with warm washed EGA treated cells
- Patient was reportedly not transfused so why mixed field reactivity?
- Results not reported
- Sent sample to National Center for Blood Group Genomics (NCBGG) for Human Erythrocyte Antigen (HEA) genomic testing

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Human Erythrocyte Antigen (HEA) Phenotype by DNA Analysis Report

- Patient is R_1R_2 so can make anti-f
- Can also make anti-K, -Fy^a and -S

Blood Group	Antigen	Results	Comments
Rh	c	+	
	C	+	
	e	+	
	E	+	
	V	0	
	VS	0	
Kell	K	0	
	k	+	
	Kpa	0	
	Kpb	+	
	Jsa	0	
	Jsb	+	
Duffy	Fya	0	
	Fyb	+	
Kidd	Jka	+	
	Jkb	+	
MNS	M	+	
	N	+	
	S	0	
	s	+	
	U	+	
Lutheran	Lua	0	
	Lub	+	
Diego	Dia	0	
	Dib	+	
Colton	Coa	+	
	Cob	0	
Dombrock	Doa	+	
	Dob	+	
	Hy	+	
	Joa	+	
Landsteiner-Wiener	LWa	+	
	LWb	0	
Sclanna	Sc1	+	
	Sc2	0	

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What are the possibilities?

Panreactivity in eluate and plasma:

- Can be same cause of reactivity in eluate and plasma or different
  - Autoantibody
    - ❖Warm, cold, combo of both warm and cold
  - Multiple antibodies
    - ❖Alloantibodies
    - ❖Autoantibody and alloantibody
  - Antibody to high prevalence antibody
  - Drug antibody
  - Monoclonal antibody therapy

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Next Steps

Eluate testing:
- Test eluate without enhancement
- DTT treatment of eluate to determine if IgG, IgM or IgA antibodies

Plasma testing:
- Prewarm testing
- Test rare antigen negative cells to cold reacting abys
- Adsorb plasma
  - 4C
  - 37C
  - Both 4C and 37C
- DTT treatment of plasma to determine if IgG, IgM or IgA antibodies

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Eluate Testing

		Rh					Kell		Duffy		Kidd		Lewis		MNS				Neat Eluate		DTT treated Eluate		Saline control Eluate	
		D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	M	N	S	s	5' RT	saline IAT	5' RT	saline IAT	5' RT	saline IAT
1	R_2R_2	+	0	+	+	0	0	+	+	0	+	+	0	+	+	0	0	+	1+	@	0	3+	$1+^W$	@
2	R_o	+	0	0	+	+	0	+	0	0	+	0	0	0	+	+	0	+	1+	@	0	3+	$1+^W$	@
3	rr	0	0	0	+	+	0	+	+	0	0	+	0	+	0	+	0	+	1+	@	0	$2+^S$	$1+^W$	@
4	rr	0	0	0	+	+	+	+	+	0	+	0	0	+	+	+	+	0	1+	@	0	$2+^S$	$1+^W$	@
5	LW(a-)	+	+	+	+	+	0	+	+	+	+	+	0	+	+	+	+	0	NT	NT	0	4+	r	@
6	LW(a-b-)	0	0	0	+	+	0	+	+	0	+	0	0	+	0	+	0	+	NT	NT	0	4+	r	@

- DTT treatment of eluate removed 22C reactivity but IAT reactivity remained
- IAT is slightly weaker with DTT treatment
- Suggests the presence of IgM and/or IgA antibodies and IgG antibodies
- Panreactivity is consistent with presence of warm and cold autoantibodies

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Plasma Testing With Rare Cells

		Rh					Kell		Duffy		Kidd		Lewis		MNS				Plasma Results	
		D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	M	N	S	s	5' RT	PEG IAT
1	Vel -	+	0	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	0	@
2	Ge-	+	+	0	+	0	+	+	0	+	+	+	+	0	+	+	0	+	0	4+
3	$PP1P^k$-	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	4+
4	I-	+	+	0	+	+	0		0	0	+	0	0	+	0	+	0	+	0	4+

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Adsorbed Plasma Testing

		Rh					Kell		Duffy		Kidd		Lewis		MNS				R_1 Ads	R_2 Ads	r Ads
		D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	M	N	S	s	PEG IAT	PEG IAT	PEG IAT
I	R_1R_1	+	+	0	0	+	0	+	0	+	+	0	+	0	+	+	+	+	3+	3+	@
II	R_2R_2	+	0	+	+	0	0	+	+	0	0	+	0	+	+	0	+	0	3+	3+	@
III	rr	0	0	0	+	+	+	+	0	+	0	+	0	+	0	+	0	+	3+	3+	@

Alloadsorbed x 4 at 4C with papain treated cells
R_1 = E-c- K- Jk(a-) s-
R_2 = C-e- K- Jk(b-) s-
r = C-E- K-Jk(a-) s-

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Adsorbed Plasma Testing with Selected Cells

		Rh					Kell		Duffy		Kidd		Lewis		MNS				R_1 Ads	R_2 Ads	r Ads
		D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	M	N	S	s	Saline IAT	Saline IAT	Saline IAT
1	R_1R_1	+	+	0	0	+	0	+	+	0	+	0	0	+	+	+	+	0	(0)√	(0)√	4+
2	R_1R_1	+	+	0	0	+	+	+	+	+	0	+	0	0	0	+	+	0	(0)√	(0)√	4+
3	R_2R_2	+	0	+	+	0	0	+	0	+	+	0	0	+	0	+	0	+	(0)√	(0)√	4+
4	R_2R_2	+	0	+	+	0	0	+	+	0	0	+	0	+	+	+	0	+	(0)√	(0)√	4+
5	R_2R_2	+	0	+	+	0	0	+	+	+	+	0	+	0	+	0	0	+	(0)√	(0)√	3+
7	rr	0	0	0	+	+	0	+	+	0	+	+	+	0	+	0	+	0	(0)√	(0)√	(0)√
8	rr	0	0	0	+	+	+	+	0	+	+	+	0	+	+	0	0	+	(0)√	(0)√	(0)√

Alloadsorbed x 4 at 4C and x 2 at 37C with papain treated cells
R_1 = E-c- K- Jk(a-) s-
R_2 = C-e- K- Jk(b-) s-
r = C-E- K-Jk(a-) s-

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Adsorbed Plasma Testing with Selected Cells

		Rh					Kell		Duffy		Kidd		Lewis		MNS				R_1 Ads	R_2 Ads	r Ads
		D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	M	N	S	s	Saline IAT	Saline IAT	Saline IAT
1	R_1R_1	+	+	0	0	+	0	+	+	0	+	0	0	+	+	+	+	0	(0)√	(0)√	4+
2	R_1R_1	+	+	0	0	+	+	+	+	+	0	+	0	0	0	+	+	0	(0)√	(0)√	4+
3	R_2R_2	+	0	+	+	0	0	+	0	+	+	0	0	+	0	+	0	+	(0)√	(0)√	4+
4	R_2R_2	+	0	+	+	0	0	+	+	0	0	+	0	+	+	+	0	+	(0)√	(0)√	4+
5	R_2R_2	+	0	+	+	0	0	+	+	+	+	0	+	0	+	0	0	+	(0)√	(0)√	3+
7	rr	0	0	0	+	+	0	+	+	0	+	+	+	0	+	0	+	0	(0)√	(0)√	(0)√
8	rr	0	0	0	+	+	+	+	0	+	+	+	0	+	+	0	0	+	(0)√	(0)√	(0)√

Alloadsorbed x 4 at 4C and x 2 at 37C with papain treated cells
R_1 = E-c- K- Jk(a-) s-
R_2 = C-e- K- Jk(b-) s-
r = D-C-E- K-Jk(a-) s-

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Is This Anti-D or Something Else?

- There is phenotypic relationship between LW and D antigens
  - Adults: D- RBCs have lower expression of LW than D+ RBCs (1:1.5)
  - Cord: LW is strongly expressed in D- and D+ RBCs
- LW antigens may be depressed during pregnancy and in some diseases
  - Hodgkins, lymphoma, leukemia and sarcoma
- Autoanti-LW with suppression of LW antigens has been reported
  - Common in patients with warm AIHA
- Differentiate anti-D from anti-LW using DTT or Pronase treated D+ cells
  - Anti-D will be reactive
  - Anti-LW will be nonreactive
- Lw^a and Lw^{ab} are high prevalence antigens
  - LW(a-b+) rare
  - LW(a-b-) null of system

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Rare Testing

		Rh					Kell		Duffy		Kidd		Lewis		MNS				Plasma Results		
		D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	M	N	S	s	5' RT	LISS IAT	IAT
1	LW(a-)	+	+	+	+	+	0	+	+	+	+	+	0	+	+	0	+	0	0	1+	@
2	LW(a-b-)	0	0	0	+	+	0	+	+	0	+	0	0	+	0	+	0	+	0	1+	@

		Rh					Kell		Duffy		Kidd		Lewis		MNS				R_1 Ads Plasma	R_2 Ads Plasma	r' Ads Plasma
		D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	M	N	S	s	Saline IAT	Saline IAT	Saline IAT
1	LW(a-)	+	+	+	+	+	0	+	+	+	+	+	0	+	+	0	+	0	(0)√	(0)√	3+
2	LW(a-b-)	0	0	0	+	+	0	+	+	0	+	0	0	+	0	+	0	+	(0)√	(0)√	(0)√

Alloadsorbed x 4 at 4C and x 2 at 37C with papain treated cells

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DTT Treated Plasma Testing

		Rh					Kell		Duffy		Kidd		Lewis		MNS				DTT TX Plasma			Saline Control		
		D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	M	N	S	s	5' RT	LISS 37C	IAT	5' RT	LISS 37C	IAT
1	R_1R_1	+	+	0	0	+	+	+	+	+	0	+	0	0	0	+	+	0	0	0	$3+^S$	0	1+	$3+^S$
2	R_2R_2	+	0	+	+	0	0	+	+	0	0	+	0	+	+	+	0	+	0	0	4+	0	$1+^W$	4+
3	r'r	0	+	0	+	+	0	+	+	+	0	+	0	+	+	0	+	0	0	0	2+	0	$1+^W$	$2+^S$
4	rr	0	0	0	+	+	0	+	+	0	+	+	+	0	+	0	+	0	0	0	$1+^S$	0	$1+^W$	2+
5	rr	0	0	0	+	+	+	+	0	+	+	+	0	+	+	0	0	+	0	0	$1+^S$	0	$1+^W$	2+

- DTT treatment removed reactivity at LISS 37C but not IAT reactivity
- Indicates plasma contains some IgM or IgA and IgG antibodies.
- Panreactivity is consistent with presence of warm and cold autoantibodies

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What Else to Investigate?

- Apparent anti-D in a RhD positive patient
  - Autoantibody or Alloantibody?
- How do you distinguish between auto and alloantibodies?
  - Anti-D was not absorbed out with D negative cells suggesting maybe alloanti-D
- Sent patient sample for *RHD* Genotype testing
- Sample sent to NYBC for testing

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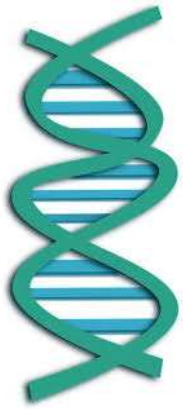
Rh Genotype Results from NYBC

Testing performed
- *RHD*: Automated RHD BeadChip
- Zygosity determination by hybrid box detection
- PCR-RFLP for *RHD* exon 8 c.1136C>T
- Sequencing of exon 2

RH alleles
- *RHD* homozygote
  - No changes associated with commonly reported partial D or weak D



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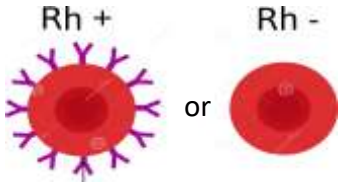
Genotype Results from NYBC



Predicted RhD Phenotype:
- D+

Comments:
- Testing consistent with two apparently conventional *RHD* alleles
- Patient would not be predicted to make alloanti-D
- If anti-D demonstrates characteristics of alloantibody, sequencing of remainder of *RHD* gene can be investigated for uncommon or new alleles

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Conclusion

ABO/Rh and DAT
- RBCs required several 37C washes to obtain valid results

Eluate
- No specificity with 22C reactivity
- Invalid IAT results due to agglutination after 37 C incubation and washing
- DTT treatment removed 22C but IAT reactivity remained
  - Suggests presence of IgM or IgA and IgG antibodies
  - Consistent with cold and warm autoantibodies
- Some patients with IgM on RBCs and/or eluate reactivity at 22C have been reported to have a more severe form of WAIHA

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Conclusion

Plasma

- Reactive with all cells at 4C and 22C
  - Consistent with cold autoantibody
- Reactive with all cells at 37 LISS
  - Reactivity circumvented by DTT treatment
- Invalid IAT results due to agglutination after 37 C incubation and washing
  - Strict prewarm and DTT treatment did not remove IAT reactivity
    - ❖ Consistent with warm autoantibody
- DTT treatment removed 22C but IAT reactivity remained
- Alloadsorbed plasma contained anti-D
- Predicted RhD phenotype is D+ by genotyping
  - Patient would not be predicted to make alloanti-D
  - If anti-D demonstrates characteristics of alloantibody more genotype testing may be needed

Conclusion

Units for transfusion:

- Sent 1 unit D-, K-, Fy(a-), S- (antigen matched) to hospital
- What would your institution transfuse?
  - Least incompatible?
    - ❖ D+ or D- units
  - Antigen matched units [K-, Fy(a-), S-]?
    - ❖ D+ or D- units?
    - ❖ What about f- units?
      - ➢ Can't give D- units if want to transfuse f-
      - ➢ R_1R_1, R_2R_2 and R_1R_2 are f-
  - Units compatible with adsorbed serum/plasma?
    - ❖ Antigen matched units?

Discussion

Institutions need to have a policy for what units to transfuse in presence of warm autoantibody

- Autoantibody in eluate only
- Autoantibody in eluate and plasma
- History of autoantibody but current sample has nonreactive eluate and plasma
- If normally give antigen matched units, what do you drop if antigen negative units not available

Objectives

List some benefits of obtaining a patient's genotype during a serologic investigation

- Ability to obtain patient's predicted phenotype despite recent transfusion or if the patient has a positive DAT
- Determining which antigens are lacking on the patient's red cells and therefore which antibodies the patient could potentially produce
- Determining if plasma reactivity is autoantibody or alloantibody
- Identifying rare or unusual phenotypes
- Resolving antigen typing discrepancies

Objectives

Describe some possible causes of panreactivity in a patient's plasma

- Autoantibody
- Monoclonal antibody therapy
- Multiple antibodies
- Antibody to high prevalence antigen
- Unusual antibody due to gene inheritance

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Objectives

Discuss selection of donor units for transfusion

- Give least incompatible ABO/RH compatible
- Give antigen matched
  - Partially antigen matched (only RH and K matched)
- Give units compatible with adsorbed serum/plasma
  - Give antigen matched and compatible with adsorbed serum/plasma
- Dependent on facility's policy

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References

- Reid ME, Lomas-Francis, C, Olsson ML. The Blood Group Antigen FactsBook. 3rd ed. Elsevier Ltd; 2012.
- isbtweb.org

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