Antibody to a High Prevalence Antigen

Objectives:

1. **Describe characteristic serologic reactivity of an antibody to a high prevalence antigen.**
2. **Explain how testing phenotype-matched RBCs differentiates multiple common antibodies from an antibody to a high prevalence antigen.**
3. **Discuss how the following factors provide clues in the identification of an antibody to a high prevalence antigen: patient ethnicity, testing treated reagent RBCs, antigen typing the patient's cells, testing rare cells.**

Quick lesson:

What kind of reactivity would alert you to the possibility that your patient has an antibody to a high prevalence antigen?

	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	IAT
1	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	**3+**
2	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	**3+**
3	+	0	+	+	0	0	+	+	0	+	+	+	0	+	+	**3+**
4	+	0	0	+	+	0	+	0								**3+**
5	0	+	0	+	+	0	+	+								**3+**
6	0	0	+	+	+	0	+	0								**3+**
7	0	0	0	+	+	+	+	0								**3+**
8	0	0	0	+	+	0	+	+								**3+**
9	0	0	0	+	+	0	+	+								**3+**
Auto																**0**

Characteristic reactivity:
- All cells reactive at the same strength
- Autocontrol negative (if not recently transfused)

Key Concept: Testing patient plasma against phenotype matched cells:

- If patient's phenotype is: D+,C+,E-,c-,e+; K-,k+; Fy(a-b+); Jk(a+b-); S+,s+
  - A phenotype-matched cells would be: **E-, c-; K-; Fy(a-); Jk(b-)**

(**phenotype-matched** = negative for the same antigens the patient is negative for)

Phenotype-matched cells are nonreactive:

	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	IAT
1	+	+	0	0	+	0	+	0	+	+	0	+	+	+	+	0
2	+	+	0	0	+	0	+	0	+	+	0	0	+	0	+	0
3	+	+	0	0	+	0	+	0	0	+	0	+	0	+	+	0

What do these results mean?
Previous reactivity must have been due to a combination of anti-E, anti-c, anti-K, anti-Fy^a and/or anti-Jk^b

Phenotype-matched cells are reactive:																
	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	IAT
1	+	+	0	0	+	0	+	0	+	+	0	+	+	+	+	**3+**
2	+	+	0	0	+	0	+	0	+	+	0	0	+	0	+	**3+**
3	+	+	0	0	+	0	+	0	0	+	0	+	0	+	+	**3+**

What do these results mean?
Reactivity persists, even when matching for all common antigens- this reactivity must be to an antigen not considered "common"

Review:

Testing phenotype-matched RBCs	
If phenotype-matched RBCs are…	**Reactivity is likely…**
Reactive with patient plasma	… antibody to a high prevalence antigen
Nonreactive with patient plasma	… a combination of multiple antibodies to common red cell antigens

Once you know the patient plasma contains antibody to a high prevalence antigen, there are many factors that can provide clues in the antibody identification process:

Factors providing clues when working up an antibody to a high prevalence antigen	
Factor	**Explanation**
Patient race/ethnicity	In some ethnicities, being negative for certain high prevalence antigens is more common (Example: U- in individuals of African descent)
Serologic phenotype/ genotype	Know which common alloantibodies a patient can make, test a phenotype-matched cell
Testing treated cells (papain/ficin, trypsin, DTT)	Treatment destroys some antigens/doesn't destroy others; narrows down reactivity into blood groups
Testing rare cells	When the patient's plasma is nonreactive with a rare cell that lacks a high prevalence antigen, this leads you to believe plasma might contain corresponding antibody
Testing patient's cells for high prevalence antigens	Patient's cells are expected to lack the antigen corresponding to the antibody in the plasma

Case Study:

Patient is group O, Rh positive with positive antibody screen

	Rh								MNSs				Lu		P	Lewis		Kell						Duffy		Kidd		Xg	Plasma	
	D	C	CW	E	c	e	f	V	M	N	S	s	Lua	Lub	P1	Lea	Leb	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Xga	5' RT	PEG IAT
1	+	+	+	0	0	+	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	3+
2	+	+	0	0	0	+	0	0	+	+	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	+	+	0	3+
3	+	0	0	+	+	0	0	0	+	0	0	+	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	0	0	3+
4	+	0	0	0	+	+	+	0	+	+	+	+	0	+	+	0	0	0	+	0	+	0	+	0	0	+	0	+	0	3+
5	0	+	0	0	+	+	+	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	0	+	0	3+
6	0	0	0	+	+	+	+	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	0	+	0	0	0	3+
7	0	0	0	0	+	+	+	0	0	+	+	+	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	3+
8	0	0	0	0	+	+	+	0	+	+	+	0	0	+	+	0	+	0	+	0	+	0	+	+	+	+	0	0	0	3+
9	0	0	0	0	+	+	+	0	+	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	0	3+
10	+	+	0	0	0	+	0	0	+	+	+	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	3+
AC																													0	0✓

1. What is the best next step?

a) Perform a DAT
b) Run a panel using prewarmed technique
c) Change to testing in gel
d) Run another panel in PEG
e) Serologically phenotype the patient

Patient phenotype											
	Anti-C	Anti-E	Anti-c	Anti-e	Anti-K	Anti-Fy^a	Anti-Fy^b	Anti-Jk^a	Anti-Jk^b	Anti-S	Anti-s
Positive Control	4+	4+	4+	4+	2+	2+	2+	3+	3+	4+	4+
Negative Control	0	0	0	0	0	0√	0√	0	0	0	0√
Patient Cells	0	0	4+	4+	0	0√	3+	3+	3+	0	4+

2. What antibodies can the patient make?

Testing antigen-matched cells:

	Rh								MNSs				Lu		P	Lewis		Kell						Duffy		Kidd		Xg	Plasma	
	D	C	C W	E	c	e	f	V	M	N	S	s	Lu a	Lu b	P1	Le a	Le b	K	k	Kp a	Kp b	Js a	Js b	Fy a	Fy b	Jk a	Jk b	Xg a	5' RT	PEG IAT
1	+	0	0	0	+	+	+	0	+	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	+	+	+	0	3+
2	0	0	0	0	+	+	+	0	+	+	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	+	0	3+
3	+	0	0	0	+	+	+	0	+	0	0	+	0	+	+	+	0	0	+	0	+	0	+	0	+	+	0	0	0	3+

3. What do these results tell us?

Further Testing:

	Rh								MNSs				Lu		P	Lewis		Kell						Duffy		Kidd		Xg	Plasma		
	D	C	C W	E	c	e	f	V	M	N	S	s	Lu a	Lu b	P1	Le a	Le b	K	k	Kp a	Kp b	Js a	Js b	Fy a	Fy b	Jk a	Jk b	Xg a	DTT treated PEG IAT	Ficin treated IAT	Trypsin-treated PEG IAT
1	+	+	0	0	0	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0√	3+	3+
2	+	0	0	+	+	0	0	0	+	+	+	0	+	+	0	0	+	0	+	0	+	0	+	+	0	+	+	+	+	0√	3+	3+
3	0	0	0	0	+	+	+	0	+	0	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0√	3+	3+

4. Do appropriate rule outs, using the nonreactive DTT results.

You can do rule outs with these reactions, but **you can't rule out Kell antibodies** (DTT treatment destroys Kell)

5. **Use the following table, adapted from *The Blood Group Antigen FactsBook*, to determine blood groups that are destroyed (or weakened) by DTT and resistant to ficin & trypsin treatment. List them:**

Ficin/Papain	Trypsin	0.2M DTT	Specificity
Neg	Neg	Pos	Ch/Rg; Xg^a; Bp^a
Neg	Neg	Neg	In, JMH
Neg	Neg	Pos	M, N, En^aTS; Ge2, Ge4
Neg	Pos	Pos	´N´; Fy^a, Fy^b
Variable	Pos	Pos	S, s
Variable	Pos	Weak o Neg	YT
Neg	Pos	Pos	En^aFS
Pos	Neg	Weak o Neg	LU, MER2
Ficin Weak or Neg Papain Pos	Neg	Neg	KN
Pos	Neg	Neg	DO
Pos	Pos	Weak	CROM
Pos	Pos	Pos	Some DI (Loop 3)
Pos	Pos	Neg	LW
Pos	Pos/Weak	Pos	SC
Pos	Pos	Neg	KEL
Pos	Pos	Pos	ABO, En^aFR P, LE, RH, JK, KEL, U, Fy3, DI, CO, H, Ge3, Kx, I/i, P1PK, ER, LKE, AnWj, At^a, Cs^a, EMM, ER, JR, LAN, OK, Vel, Sd^a, PEL, FORS, ABTI, MAM

Adapted from: Reid, M & Lomas-Francis, C. *The Blood Group Antigen FactsBook*, Elsevier Academic Press 2012.

Testing Rare Cells: choose cells negative for high prevalence antigens in the above blood group systems (based on availability of cells). If cells NULL for entire blood group system are available, those are also helpful.

	Rh								MNSs				Lu		P	Lewis		Kell						Duffy		Kidd		Xg	Plasma
	D	C	CW	E	c	e	f	V	M	N	S	s	Lua	Lub	P1	Lea	Leb	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Xga	PEG IAT
Yt(a-)	+	+	0	0	0	+	0	0	+	+	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	+	+	3+
$Kell_{null}$	0	0	0	0	+	+	+	0	+	0	+	+	0	+	0	0	+	0	0	0	0	0	0	+	0	+	0	+	0√
LW_{null}	0	0	0	0	+	+	+	0	+	+	+	+	0	+	+	0	0	0	+	0	+	0	+	+	+	+	+	+	0	3+

6. **What does the negative result with the $Kell_{null}$ cell tell us?**

7. **Name 3 high prevalence antigens (listed on many panels) within the Kell system:**

8. **We have found out that our patient is African American. Which of the above high prevalence antigens in the Kell system can be lacking in people of African descent?**

9. **How should we proceed?**

	Rh								MNSs				Lu		P	Lewis		Kell						Duffy		Kidd		Xg	Plasma
	D	C	CW	E	c	e	f	V	M	N	S	s	Lua	Lub	P1	Lea	Leb	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Xga	PEG IAT
Js(b+)	+	+	0	0	0	+	0	0	+	+	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	+	+	3+
Js(b-)	+	0	0	+	+	0	0	0	+	0	0	+	0	+	+	+	0	0	+	0	+	+	0	0	+	0	+	0	0√
Js(b-)	+	0	0	0	+	+	+	0	0	+	+	+	0	+	+	+	0	0	+	0	+	+	0	0	+	+	+	+	+	0√
Js(b-)	+	+	0	0	0	+	0	0	+	+	+	0	0	+	+	+	0	0	+	0	+	+	0	+	0	0	+	+	0√

10. What antibody have we identified? Is it clinically significant?

11. Which antibody to a common RBC antigen can't we rule out? (remember you can do rule outs on ALL negative cells ($Kell_{null}$, Js(b-), DTT-treated)

12. What blood will we choose for transfusion?

13. **How could HEA testing (molecular genotype panel) have helped us on this patient workup?**

Case Study Answers:

1. e
2. Anti-E, anti-C, anti-K, anti-Fy^a, anti-S
3. Antibody to a high prevalence antigen
4.

	Rh								MNSs				Lu		P	Lewis		Kell					Duffy		Kidd		Xg	Plasma		
	D	C	CW	E	c	e	f	V	M	N	S	s	Lua	Lub	P1	Lea	Leb	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Xga	DTT treated PEG IAT	Ficin treated IAT	Trypsin-treated PEG IAT
1	+w	+w	0	0	0	+w	0	0	0	+w	0	+w	0	+w	+w	0	+w	0	+	0	+	0	+	0	+w	+w	0	+w	0	3+	3+
2	+w	0	0	+w	+w	0	0	0	+	+	+w	0	+	+	0	0	+w	0	+	0	+	0	+	+w	0	+	+	+w	0	3+	3+
3	0	0	0	0	+w	+w	+w	0	+w	0	0	+w	0	+w	+w	+w	0	+	+	0	+	0	+	0	+w	0	+w	0	0	3+	3+

5. YT, CROM, LW, KEL
6. Antibody is to an antigen in the Kell system
7. k, Kp^b, Js^b
8. Js^b
9. Test Js(b-) cells
10. Anti-Js^b. Yes, Kell system antibodies are clinically significant.
11. Anti-K
12. K-negative, Js(b-) units, compatible with the patient's plasma at IAT
13. We would have known the patient's cells are Js(b-), which could have expedited antibody identification.

Assessing Understanding

1. **Which of the following patient results is consistent with an antibody to a high prevalence antigen?** (none of these patients have been recently transfused)

	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	Patient 1 IAT	Patient 2 IAT	Patient 3 IAT	Patient 4 IAT
1	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	2+	0v	2+	4+
2	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	2+	4+	2+	4+
3	+	0	+	+	0	0	+	+	0	+	+	+	0	+	+	2+	1+	2+	4+
4	+	0	0	+	+	0	+	0	0	+	0	+	+	0	+	2+	1+	2+	4+
5	0	+	0	+	+	0	+	+	0	+	0	+	+	0	0	2+	1+	2+	4+
6	0	0	+	+	+	0	+	0	+	+	+	0	+	0	+	2+	1+	2+	4+
7	0	0	0	+	+	+	+	0	+	+	0	+	0	+	+	2+	4+	2+	0v
8	0	0	0	+	+	0	+	+	+	0	+	0	+	+	+	2+	1+	2+	0v
9	0	0	0	+	+	0	+	+	+	0	+	+	0	0	+	2+	1+	2+	0v
10	+	+	0	0	+	0	+	+	0	+	+	+	+	+	0	2+	0v	2+	4+
11	+	0	0	+	+	+	+	0	0	+	+	0	+	+	+	2+	4+	2+	4+
Auto																2+	0v	0v	0v

   a. Patient 1
   b. Patient 2
   c. Patient 3
   d. Patient 4

2. **Which of the following is true regarding testing a reagent red cell phenotype-matched to a patient?**
   a. Phenotype-matched cells are positive for the all antigens that patient's cells express
   b. Phenotype-matched cells are negative for all the antigens that a patient's cells lack
   c. If the patient's plasma is reactive with phenotype matched cells, it indicates the presence of multiple common antibodies
   d. If the patient's plasma is nonreactive with phenotype matched cells, it indicates the presence of antibody to a high prevalence antigen

3. **How does testing enzyme- and chemically-treated red cells aid in the investigation of antibody to a high prevalence antigen?**
   a. Pattern of reactivity with treated cells helps narrow down specificity to certain blood groups
   b. Pattern of reactivity with treated cells helps to distinguish allo- from autoantibody
   c. Pattern of reactivity with treated cells helps to distinguish clinically significant from clinically insignificant antibody
   d. Pattern of reactivity with treated cells helps to distinguish multiple common antibodies from antibody to a high prevalence antigen

Answers: 1. c; 2. b; 3. a