Blood Bank, We Have a Problem: Working together to Optimize Antibody Resolution

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Immunohematology Reference Laboratory

“Is there anything I can do here?”

Phone consultation

- Supervisor/Lead Tech
- If patient was previously evaluated by ARC, ARC# or date tested (if recent)
- Fax worksheets for review
- Based on serologic problem, you may be able to avoid sending sample to Ref Lab

Recognizing the problem is always the first step….

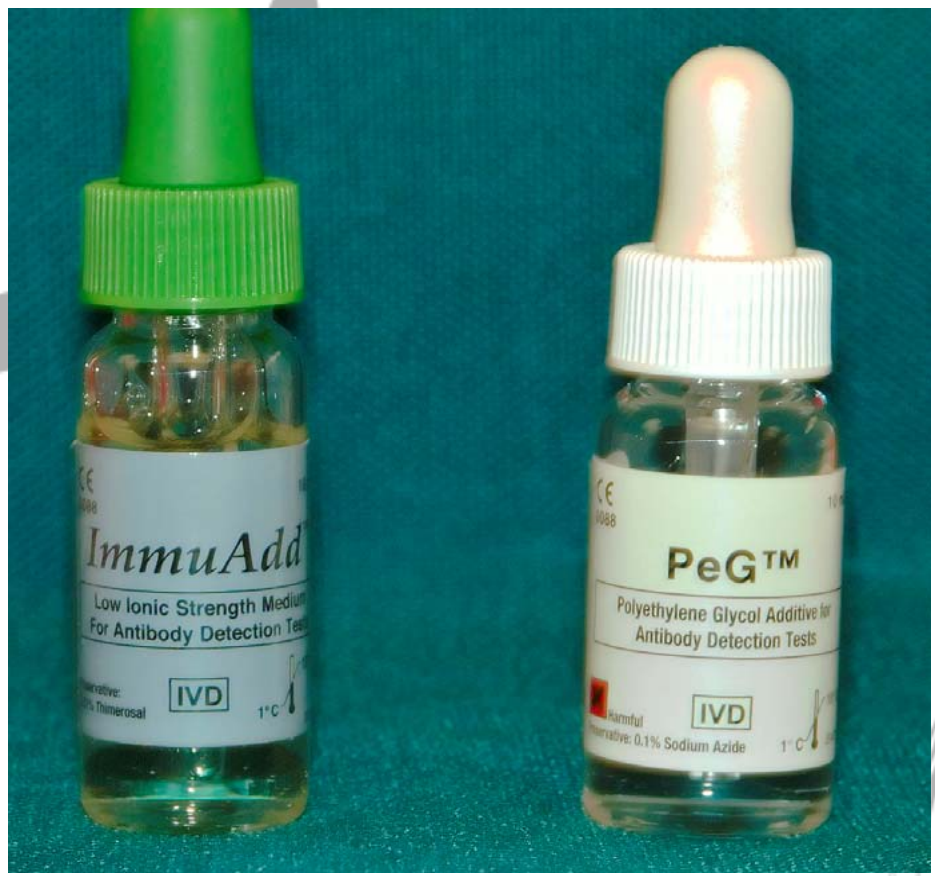
Evaluating the problem

- Patients with previous evaluations
  - Review report or call Ref Lab for consultation
    - What was identified? Has reactivity changed?
    - What test method was used for identification and for ruling out other antibodies?
    - What resources do you have for testing?
    - Do you need antigen-negative units?

Evaluating the problem

- Patients with no previous evaluations
  - Obtain thorough patient history
  - What antibodies can be ruled out? Are there any obvious specificities? If crossmatches were done, what percent are compatible?
  - Is there a way to safely provide transfusion pending results of antibody ID?
    - Have common alloantibodies been excluded?
    - Are there compatible crossmatches?

Other resources I would highly recommend



“Unexplained Reactivity”

Reactions with few cells on the panel

- Strong reactions most likely due to antibody to a LIA
- Weaker reactions
  - Antibodies to white cell antigens (Bg)
  - Antibody reacting only with cells that have stronger expression of antigen (I, IH, P_1, D or other Rh specificities)
  - Antibodies showing dosage (-M, -Jk^a)
  - Developing antibodies, ???

What to do…

- Are you able to rule out the presence of common alloantibodies?
  - Use cells with double dose expression
    - r/o of anti-K an exception. K+k+ cells are acceptable.
- Do you have compatible crossmatches?
  - Crossmatch/screen using method by which the reactivity was detected

If **Yes to both**, it is acceptable to transfuse the patient….investigation of the reactivity may continue (get adequate samples first!)

The Low-Down…

D	C	E	c	e	K	S	s	Fy^a	Fy^b	Jk^a	Jk^b	Gel
+	0	0	+	+	0	0	+	0	+	0	+	0
+	0	+	+	0	0	0	+	0	+	0	+	3+
0	0	0	+	+	+	0	+	+	0	+	0	0
0	0	0	+	+	0	+	0	0	+	0	+	0
+	0	+	+	0	0	0	+	0	+	+	0	0
+	+	0	0	+	0	+	+	0	+	0	+	0
0	0	+	+	+	0	0	+	0	+	0	+	3+

The Low-Down…

D	C	E	c	e	K	S	s	Fy^a	Fy^b	Jk^a	Jk^b	Gel
+	+	0	0	+	0	0	+	0	+	0	+	0
+	0	+	+	0	0	0	+	0	+	0	+	3+
0	0	0	+	+	+	0	+	+	0	+	0	0
0	0	0	+	+	0	+	0	0	+	0	+	0
+	0	+	+	0	0	0	+	0	+	+	0	0
+	+	0	0	+	+	+	0	+	0	0	+	0
0	0	+	+	+	0	0	+	0	+	0	+	3+

The Low-Down…on lows

DONOR	(Bg^a, Bg^b, Bg^c)	Coa	Cob	Sda	Ytb
101984	0		0		
107952	0		0		
108226	0		0		
111208	0				
111209	0				
111247	0				
111302	0		0		
111329	0		+		
111375	0		0		
111444	0		0		
111931	0				
112858	0		0		

Common LIA on your panels

- Rh: V, Go^a, C^w
- Kell: Kp^a, Js^a
- Lu^a
- Yb^b
- Co^b
- He
- Di^a
- And hundreds more that aren’t listed!

Case of Mistaken Identity

- 15 y/o male with sickle cell disease
- Admitted with “sickle cell crisis”
- ABO/Rh: A pos, rec’d only C-E-K- units per PFL protocol
- Last transfusion in Florida, 10 days before sample submitted for Reference evaluation
- Per hospital in Florida, anti-E and –Jk^a were identified at a Florida Ref Lab. Patient rec’d C-E-K-Jk(a-) red cells.

D.C.

- Initial results:
  - DAT negative (weak pos DAT reported by Florida hospital)
  - Anti-Jk^a detected in PEG. Anti-E was not detected in PEG.
  - Gel panel subsequently tested to try and detect anti-E reported by Florida Reference Lab.

D	C	E	c	e	K	S	s	Fy^a	Fy^b	Jk^a	Jk^b	Gel
+	0	0	+	+	0	0	+	0	+	0	+	2+
+	0	+	+	0	0	0	+	0	+	0	+	3+
0	0	0	+	+	+	0	+	+	0	0	+	0
0	0	0	+	+	0	+	0	0	+	0	+	0
+	0	+	+	0	0	0	+	0	+	0	+	3+
+	+	0	0	+	0	+	+	0	+	0	+	2+
0	0	+	+	+	0	0	+	0	+	0	+	0

D	C	E	c	e	K	S	s	Fy^a	Fy^b	Jk^a	Jk^b	Gel
+	0	0	+	+	0	0	+	0	+	0	+	2+
+	0	+	+	0	0	0	+	0	+	0	+	3+
0	0	0	+	+	+	0	+	+	0	0	+	0
0	0	0	+	+	0	+	0	0	+	0	+	0
+	0	+	+	0	0	0	+	0	+	0	+	3+
+	+	0	0	+	0	+	+	0	+	0	+	2+
0	0	+	+	+	0	0	+	0	+	0	+	0

D	C	E	c	e	K	S	s	Fy^a	Fy^b	Jk^a	Jk^b	Gel
+	0	0	+	+	0	0	+	0	+	0	+	2+
+	0	+	+	0	0	0	+	0	+	0	+	3+
0	0	0	+	+	+	0	+	+	0	0	+	0
0	0	0	+	+	0	+	0	0	+	0	+	0
+	0	+	+	0	0	0	+	0	+	0	+	3+
+	+	0	0	+	0	+	+	0	+	0	+	2+
0	0	+	+	+	0	0	+	0	+	0	+	0

Estimated number of available antigen sites on D+ red cells

Issitt PD, Anstee DJ. Applied Blood Group Serology, 4th edition

Phenotype		# of D antigen sites
cDE/cDE	R_2R_2	16000-33500
CDe/cDE	R_1R_2	23000-31000
CDe/CDe	R_1R_1	14500-19500
cDE/cde	R_2r	14000-16500
cDe/cde	R_Or	12000-20000

D.C.

- Antibody ID (plasma): anti-D and –Jk^a
- Eluate prepared (even though tube DAT was negative), demonstrated anti-D
  - Auto control in Gel was 2+
- Patient is D+
  - Auto vs. Alloimmune anti-D?
  - Eluate performed on patient cells obtained after cell separation was non-reactive, suggestive of alloimmune anti-D.

D.C.

- Samples sent for molecular testing to determine if patient has variant D gene.
- Molecular lab determined patient inherited a partial D antigen, and anti-D was determined to be alloimmune.
- When identified in Florida, anti-D was likely weaker and only reactive with R_2R_2 cells (thus appeared to be anti-E)
- **Patient may have been having a delayed transfusion reaction due to anti-D**

More Unexplained Reactions

- Reactions with most or all panel cells
  - Antibody to HIA
  - Warm or Cold-reactive autoantibody
  - Multiple alloantibodies
  - Extraneous reactivity (antibody to component of test system: preservatives, diluent solutions, antibiotics, enhancement, etc.)

Recognizing Media-dependent reactivity

- Test sample using other methods, similar in sensitivity and reaction phase
  - LISS dependent antibodies – test @ 37C phase using albumin
  - Gel dependent antibodies
    - test using PEG or other enhancement media, at antiglobulin-phase
    - Test pre-diluted Ortho cells vs 0.8% cells prepared in lab from another source (donors, other screening cells or panel cells)

Much Ado about Nothing
Case of Missing Reactions

	IS	PEG IAT
SI	**0**	**0**
SII	**0**	**0**
SIII	**0**	**0**
AC	**0**	**0**

	LISS 37	LISS IAT
SI	**0**	**0**
SII	**0**	**0**
SIII	**0**	**0**
AC	**0**	**0**

0.8% Panel

D	C	E	c	e	K	S	s	Fy^a	Fy^b	Jk^a	Jk^b	Gel
+	0	0	+	+	0	0	+	0	+	0	+	3+
+	0	+	+	0	0	0	+	0	+	0	+	3+
0	0	0	+	+	+	0	+	+	0	+	0	3+
0	0	0	+	+	0	+	0	0	+	0	+	3+
+	0	+	+	0	0	0	+	0	+	+	0	3+
+	+	0	0	+	0	+	+	0	+	0	+	3+
Auto Control												0

Other reagent cells diluted to 0.8%

	Phenotype	Gel
1	**Phen. Sim Reagent cell**	**0**
2	**Phen. Sim. Donor unit**	**0**
3	**Kp(b-)**	**0**
4	**Lu(a-b-)**	**0**
5	**Knops null**	**0**

- Reactions only noted with pre-diluted cells from manufacturer
- Antibody most likely directed at preservative or other component of pre-diluted panel cells

Reading the ARC final report

Serum Antibody: Warm reactive autoantibody

Reactive By: LISS 37C/antiglobulin; PEG/antiglobulin

- **Additional Comments:** The patient's serum was adsorbed x2 at 37C with selected allogeneic red cells following papain treatment. The adsorbed serum was then tested with reagent red cells and was non-reactive. The presence of antibodies to common red cell antigens was excluded by PEG/IAT. However, antibodies to high incidence antigens may not be detected when testing allogeneic-adsorbed serum.

Reading the ARC Final Report

Serum Antibody: Warm reactive autoantibody

Reactive By: PEG/antiglobulin, Gel test (IgG)

The presence of alloantibodies to common red cell antigens was excluded by LISS/IAT.

Reading the ARC Final Report

Serum Antibody: Anti-Fy^a

Reactive By: PEG/antiglobulin

Previously identified anti-C, -E, -S, and –Jk^b were not reconfirmed at this time. The presence of alloantibodies to common red cell antigens was excluded by PEG/IAT.

What to consider…

- What needs to be ruled out?
  - Patient has anti-C, -E, -S, -Fy^a, Jk^b already
- Patient's phenotype:

  C-E-c+e+; K-; Fy(a-b+); Jk(a+b-); S-s+

What to consider…

- What needs to be ruled out?
  - Patient has anti-C, -E, -S, -Fy^a, Jk^b already
- Patient's phenotype:

  C-E-c+e+; **K-**; **Fy(a-**b+); **Jk**(a+**b-**); **S-**s+

- Anti-K needs to be ruled out…..or does it?
- Other potential problems to be considered
  - Autoantibodies, "HTLA" antibodies, lows, etc.
  - If you don't have a negative cell on the screen, test one or more phenotypically similar cells from a panel. If negative, it is probably "safe" to order phenotyped units w/o sending sample

Submitting Samples to a Reference Laboratory

What is important to know??

- Patient history
  - Previously identified antibodies
  - Transfusion history, esp. date of last trx
  - Diagnosis
  - Has the patient been seen at another hospital? – when, where
  - Pregnancy history
  - Medications / IV therapy

Look away from the computer

Other resources for information:

- Ask the patient or the patient's family
  - "have you ever been transfused?"
  - "have you had any problems being transfused"
  - "where have you been transfused?"
- Physician's admitting H&P may be on patient's chart - lots of info here!
- Nursing may be able to assist

Other important information…

- Patient demographics (sex, age, race)
- What is the serologic problem you are seeing?
  - Send copies of worksheets (panels, ABSC)
  - Describe testing you have done (method(s) used, strength of reactivity, estimated prevalence of reactivity, phase(s) of reactivity, etc.)

Things that bring work to a halt

- Mislabeled samples/ discrepant requests
  - Patient name must match exactly
  - ID# must match exactly
  - Collection date must be on sample, must match date on request
- Insufficient sample
  - Slows work down
  - Transit time for additional samples adds to overall turn-around-time

More Speed Bumps….

- Missing information
- Inaccurate information
  - Especially transfusion history (basis for cell separations, eluates, interpretation of screen…)
- Multiple phone calls
  - Call us if you have more information or the patient's status has changed
- Changing the order multiple times
  - If you're unsure about how many units are needed, or if special requirements (CMV, IRR, AG-) are needed, PLEASE get this worked out before placing the order!

New ARC Request Form!!

American Red Cross
Biomedical Services
Washington, DC 20006

Immunohematology Consultation Request

Southern Region

CLIA # 11-D0699333

Reference Lab contact numbers: **Phone:** (404) 253-5580 or 1-800-884-2710 ext. 5580 **Fax:** (404) 876-6984

See page 2 for instructions, sample types and tube labeling requirements

❶ Call Reference Laboratory before sending sample

Reference Lab person contacted: _____ Date/Time contacted: _____

❷ Submitting Facility Information

Facility Name/ID: _____ Request Date: _____

Facility Address: _____ City/State: _____ Zip: _____

Blood Bank Contact: _____

Blood Bank Phone #: _____ Requesting Physician: _____

Blood Bank Fax #: _____

❸ Patient Information

Patient Name: _____ **Patient ID:** _____

Birth Date/Age: _____ Race: _____ Gender: **M** ☐ **F** ☐

Specimen Date: _____ ABO/Rh: _____

Diagnosis: _____ Hgb/Hct: _____

Medications: _____

Additional information: _____

Transfusion History: **No record** ☐

Within last 3 months: **No** ☐ **Yes** ☐ ▶ **Dates / products:** _____

Prior to last 3 months: **No** ☐ **Yes** ☐ ▶ **Dates / products:** _____

Pregnancy History: **Number:** ____ Currently pregnant? **No** ☐ **Yes** ☐ ▶ Due date: _____

Known RBC antibody(ies) Anti -D ☐ -C ☐ -c ☐ -E ☐ -e ☐ -K ☐ -Fy^a ☐ -Fy^b ☐ -Jk^a ☐ -Jk^b ☐ -S ☐ -s ☐

Other (list): _____

❹ Test Request

Note: STAT and/or after-hours charges may apply. **Date/time needed:** _____

Routine ☐ Non-urgent, stable situations (examples include routine dialysis or elective surgery).

ASAP ☐ Non-emergent situations where a shortened turn around time would be beneficial (example: low hemoglobin).

STAT ☐ Status of the patient is extremely unstable. This is an urgent request (example: actively bleeding patient).

Investigation Requested: (Check all that apply)

ABO/Rh typing ☐	Incompatible crossmatch ☐	ABO/Rh typing Discrepancy ☐
Positive DAT ☐	Suspected transfusion reaction ☐	Partners for Life Evaluation ☐
Antibody identification ☐	Hemolytic Disease of the Fetus & Newborn ☐	**OTHER SPECIFY:**

Products Requested for this patient: (Check all that apply)

CROSSMATCHED RBCs

 # units: _____ **Date/Time needed:** _____

OTHER (SPECIFY):

ANTIGEN-NEGATIVE RBCs

 # units: _____ **Date/Time needed:** _____

Negative for: (Circle) C c E e K Fy^a Fy^b Jk^a Jk^b S s

Other: _____

Product Attributes: (Check all that apply)

CMV-negative ☐

Leukoreduced ☐

Irradiated ☐

Hemoglobin S-negative RBC ☐

OTHER SPECIFY: _____

❺ Summary of Antibody Testing Results: Please provide copies of blood bank test results.

Tube: **LISS** ☐ **PEG** ☐ **ENZ** ☐

Other: ☐ _____

Gel ☐

Solid Phase ☐

	IS	37C	AHG
I	____	_____	_____
II	____	_____	_____
III	____	_____	_____

AHG Used: **Polyspecific** ☐ **IgG** ☐

CROSSMATCH RESULT

 # Compatible Donors _____

 # Incompatible Donors _____

DAT: _____

American Red Cross
Biomedical Services
Washington, DC 20006

Immunohematology Consultation Request

Southern Region

CLIA # 11-D0699333

Instructions for submitting samples for a Reference Laboratory investigation:

1. Obtain an appropriate sample and label it according to the instructions in the table below.
2. Prepare the sample for shipping according to the instructions in the table below.

Sample and Tube Label Requirements

Sample Type:

20 mL EDTA or combination of clotted and EDTA

- Serum separator tubes are **NOT** acceptable
- If recently transfused, include pre-transfusion red cell sample, if available

Label Requirements:

Sample Label **MUST** include:

- Patient's First and Last Names
- Patient's ID Number
- Date collected

No special preparation of the patient is needed prior to collecting samples.

NOTE: IMPROPERLY LABELED SAMPLES WILL NOT BE TESTED!!

Shipping Instructions:

Contact the Reference Lab if assistance is needed for sample transportation.

3. Complete the form on the reverse side.
4. Contact the reference lab at the phone number on the top of the form prior to sending the sample.
5. Record the name of the person contacted and the date and time of the notification.
6. Follow any additional instructions noted in the ARC Facility-specific area below.
7. Submit the sample and a completed Consultation Request to the ARC.

Segment Compatibility Test Request/Report

Patient Name:_____

Patient ID #_____

A printed label may be used to provide this information.

When submitting segments for testing, please verify the following:

1. If possible, two segments have been submitted for each unit.
2. Each segment submitted has a complete segment number.
3. Each segment is labeled with the corresponding whole blood number.
4. The first three columns in the table below have been completed.

NOTE: If segments are from units tagged antigen negative by American Red Cross, please include a photocopy of units with tag to prevent duplicate testing and charges.

NOTE: shaded area for ARC use only

Unit number	Segment number	ABO & Rh	Crossmatch Result: ☐ Compatible with ☐ Least Incompatible with ☐ Incompatible with	Clerical Review	Date
1			☐ neat serum ☐ allo absorbed ☐ auto absorbed	/	
2			☐ neat serum ☐ allo absorbed ☐ auto absorbed	/	
3			☐ neat serum ☐ allo absorbed ☐ auto absorbed	/	
4			☐ neat serum ☐ allo absorbed ☐ auto absorbed	/	
5			☐ neat serum ☐ allo absorbed ☐ auto absorbed	/	
6			☐ neat serum ☐ allo absorbed ☐ auto absorbed	/	

Next, the fun stuff!

Case J.G.

- Patient J.G.
  - **African American male, DOB 12/7/53**
  - **Dx: Multi-organ failure, Hemolytic process**
  - **No transfusions in last 3 months**
  - **Hgb 7.9; submitted for antibody identification and 2 units crossmatched red cells**

J.G. Initial Results

Anti-A	Anti-B	Anti-D	Rh Ctl	A_1 cells	B cells
4+	0	4+	0	3+	4+

DAT

PS	-IgG	-C3	Control
1+	1+	0	0

J.G. Initial Results

	IS	PEG IAT
SI	2+s	3+
SII	2+s	3+
SIII	0	0
AC	0	1+

	LISS 37	LISS IAT
SI	3+	4+
SII	3+	3+
SIII	0	0
AC	0	1+

To Summarize

- ABO discrepancy
- Positive DAT
- Positive Antibody Screen
  - Phenotype patient cells
  - Identify direct agglutinating/IAT antibody
  - Prepare and test eluate

J.G.

Phenotype:

C-E-c+e+; K-; Fy(a-b-); Jk(a+b-); S-s+; M-; Le(a-b-); A_1-

J.G.

	Rh	K	M	N	Le^a	Le^b	P_1
SI	**R1R1**	**+**	**+**	**0**	**0**	**+**	**0**
SII	**R2R2**	**0**	**+**	**+**	**+**	**0**	**+**
SIII	**rr**	**0**	**0**	**+**	**0**	**+**	**+**

J.G.

	Rh	K	M	N	Le^a	Le^b	P_1
SI	**R1R1**	**+**	**+**	**0**	**0**	**+**	**0**
SII	**R2R2**	**0**	**+**	**+**	**+**	**0**	**+**
SIII	**rr**	**0**	**0**	**+**	**0**	**+**	**+**

Panel Results - Summary

	Phenotype	IS	LISS 37	LISS IAT
1	**R1R1 M-**	**0**	**0**	**0**
2	**R2R2 M-**	**0**	**0**	**0**
3	**I- M-**	**0**	**0**	**0**
4	**rr M+**	**1+s**	**2+**	**2+**

Panel Results - Summary

	Phenotype	IS	LISS 37	LISS IAT
1	**R1R1 M-**	**0**	**0**	**0**
2	**R2R2 M-**	**0**	**0**	**0**
3	**I- M-**	**0**	**0**	**0**
4	**rr M+**	**1+s**	**2+**	**2+**

Final interpretation of Panel:

Anti-M reactive @ IS, 37 and IAT. Other common alloantibodies ruled out.

ABO Discrepancy

Reverse grouping	A_1 cells (M-)	B cells (M-)
Patient J.G.	**2+**	**4+**

ABO Resolution

	Anti-M	Anti-A_1	Pt. J.G.
Gp A #1	0	4+	2+
Gp A #2	0	0	0
Gp A #3	0	4+	3+

ABO Resolution

	Anti-M	Anti-A_1	Pt. J.G.
Gp A #1	0	4+	2+
Gp A #2	0	0	0
Gp A #3	0	4+	3+

Interpretation: Probable anti-A_1; unable to find additional M-, A_1- cells to fully resolve ABO discrepancy

J.G. Eluate

	Eluate	Last Wash
SI	**0**	**0**
SII	**0**	**0**
SIII	**0**	**0**
AC (EGA-tr)	**1+w**	**0**

J.G. Eluate

	Eluate	Last Wash
A_1 cells	**1+s**	**0**
B cells (x 2)	**0**	**0**
A_1 cells	**1+s**	**0**
A_2 cells	**1+w**	**0**

- **Interpretation: Anti-A eluted from patient cells**

Where did that come from?

- No known transfusion of blood products
- Medication history (extensive list):
  - Cefepime
  - Insulin
  - Diphenhydramine injection
  - Multiple antibiotic ointments
  - **IV Immune Globulin**

Antibodies in IVIG

- "Commercial immune globulins, including IVIG, have measurable levels of anti-A and -B (IgG class), as well as a variety of non-ABO antibodies…RBC antibodies have been found in 49% of 165 lots of IVIG, including anti-K, -C and – Le^b."
- "..examples have been reported of hemolytic anemias due to anti-D or anti-A."

Reference: Intravenous immune globulins: an update for clinicians. Transfusion 2003;43:1460-1480

J.G.

- Summary of results: Anti-A in eluate. Anti-M and apparent anti-A_1 in plasma.
  - Anti-A_1 in plasma may be from the IVIG, and is only reacting with the A_1 cells because the antibody is weak
  - Recommended group O, crossmatch compatible units for transfusion

Case D.S.

- Patient D.S.
  - Female, race unknown, DOB 11/25/70
  - Dx: Uncontrolled hypertension, anemia
  - Never transfused
  - No medications
  - History of 1 pregnancy

D.S.

- Initial results:
  - Group A, Rh positive (no discrepancies)
  - DAT:

PS	-IgG	-C3	Control
2+	Micro+	2+	0

D.S.

	IS	PEG IAT
SI	**0**	**3+**
SII	**0**	**0**
SIII	**0**	**1+s**
AC	**0**	**3+**

D.S.

- Phenotype
  (EGA-treated for AHG typings):
  C+E-c+e+; K-; Fy(a+b+); Jk(a+b+);
  S+s-; M+N-; Le(a-b-)

Antibody Screen

	Rh	K	S	s	Le^a	Le^b	M	N
SI	**R1R1**	**+**	**0**	**+**	**0**	**+**	**0**	**+**
SII	**R2R2**	**0**	**+**	**+**	**0**	**+**	**+**	**0**
SIII	**rr**	**0**	**+**	**0**	**+**	**0**	**+**	**0**

Antibody Screen

	Rh	K	S	s	Le^a	Le^b	M	N
SI	**R1R1**	**+**	**0**	**+**	**0**	**+**	**0**	**+**
SII	**R2R2**	**0**	**+**	**+**	**0**	**+**	**+**	**0**
SIII	**rr**	**0**	**+**	**0**	**+**	**0**	**+**	**0**

Rh/ other	K	Fy^a	Fy^b	Jk^a	Jk^b	S	s	M	N	Le^a	Le^b	**PEG IgG**
R2R2	0	+	+	0	+	+	0	+	0	0	+	**0**
rr	+	0	+	0	+	0	+	+	+	0	+	**0**
rr,Lu(a+)	+	+	+	+	0	+	+	+	0	0	+	**2+s**
rr	0	+	+	+	+	0	+	0	+	+	0	**2+**
R1R1	0	0	+	+	+	+	0	+	+	+	0	**2+**
rr, Lu(a+b-)	0	+	+	+	0	0	+	0	+	0	+	**2+s**

Rh/ other	K	Fy^a	Fy^b	Jk^a	Jk^b	S	s	M	N	Le^a	Le^b	**PEG IgG**
R2R2	0	+	+	0	+	+	0	+	0	0	+	**0**
rr	+	0	+	0	+	0	+	+	+	0	+	**0**
rr,Lu(a+)	+	+	+	+	0	+	+	+	0	0	+	**2+s**
rr	0	+	+	+	+	0	+	0	+	+	0	**2+**
R1R1	0	0	+	+	+	+	0	+	+	+	0	**2+**
rr, Lu(a+b-)	0	+	+	+	0	0	+	0	+	0	+	**2+s**

Rh/ other	K	Fy^a	Fy^b	Jk^a	Jk^b	S	s	M	N	Le^a	Le^b	**PEG IgG**
R2R2	0	+	+	0	+	+	0	+	0	0	+	**0**
rr	+	0	+	0	+	0	+	+	+	0	+	**0**
rr,Lu(a+)	+	+	+	+	0	+	+	+	0	0	+	**2+s**
rr	0	+	+	+	+	0	+	0	+	+	0	**2+**
R1R1	0	0	+	+	+	+	0	+	+	+	0	**2+**
rr, Lu(a+b-)	0	+	+	+	0	0	+	0	+	0	+	**2+s**
rr	+	+	+	+	+	0	+	0	+	0	+	**2+**
R2R2, Lu(a+)	0	0	+	0	+	0	+	+	+	+	0	**0**

Rh/ other	K	Fy^a	Fy^b	Jk^a	Jk^b	S	s	M	N	Le^a	Le^b	**PEG IgG**
R2R2	0	+	+	0	+	+	0	+	0	0	+	**0**
rr	+	0	+	0	+	0	+	+	+	0	+	**0**
rr,Lu(a+)	+	+	+	+	0	+	+	+	0	0	+	**2+s**
rr	0	+	+	+	+	0	+	0	+	+	0	**2+**
R1R1	0	0	+	+	+	+	0	+	+	+	0	**2+**
rr, Lu(a+b-)	0	+	+	+	0	0	+	0	+	0	+	**2+s**
rr	+	+	+	+	+	0	+	0	+	0	+	**2+**
R2R2, Lu(a+)	0	0	+	0	+	0	+	+	+	+	0	**0**

D.S.

	PEG/IAT
EGA-treated Autologous Cells + plasma	**3+**
EGA-tr. Cell control (tested w/ 6% alb)	**0**

- Additional Jk(a-) cells tested to complete rule outs
- Eluate demonstrated Anti-Jk^a
- **Conclusion: Autoimmune anti-Jk^a in serum and eluate**

On to more case studies with LeeAnn…

It’s not always what it seems…

- T&S ordered for patient having hysterectomy
- History: 3 pregnancies, transfused after 2nd (2 units)
- Initial results
  - O Rh positive, DAT negative

	Gel
SI	**1+**
SII	**1+**
SIII	**1+**
AC	**0**

	D	C	E	c	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s		Gel IgG
1	+	+	0	0	+	+	0	+	0	+	+	+	+	0	+	+	+	+	+		1+
2	+	+	0	0	+	0	+	+	+	+	+	0	0	+	+	+	0	+	0		1+
3	+	0	+	+	0	0	0	+	0	+	+	+	0	+	+	+	+	0	+		1+
4	+	0	0	+	+	0	0	+	0	0	+	+	0	0	+	+	0	+	+		1+
5	0	+	0	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	+		1+
6	0	0	+	+	+	0	0	+	+	+	+	+	0	+	+	+	0	0	+		1+
7	0	0	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	0	+		0
8	0	0	0	+	+	0	0	+	+	0	0	+	+	0	+	0	+	+	+		1+
9	0	0	0	+	+	0	0	+	0	+	+	+	0	+	0	+	0	+	0		1+
10	+	+	0	+	+	0	0	+	+	0	+	0	0	+	0	+	+	0	+		1+
11	0	0	0	+	+	0	0	+	+	+	0	+	0	0	+	0	+	+	+		1+
Auto																					0

- What would you do next?

Selected cells…

	D	C	E	c	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s		Gel IgG
1	+	+	0	0	+	+	+	0	+	+	+	+	+	0	+	0	+	+	+		1+
2	+	0	+	+	0	0	+	0	0	+	+	0	0	+	+	+	0	0	+		1+
3	+	0	0	+	+	0	+	0	0	0	+	+	0	0	+	+	0	+	0		1+
4	0	+	0	0	+	0	+	0	0	+	0	+	0	+	+	+	+	0	+		1+

- What now??
  - Phenotype
    - R_or, Kk, Fy(a-b+), Jk(a+b-), Le(a-b+), P1, MNs

	D	C	E	c	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s		Gel IgG	
1	+	0	0	+	+	0	+	+	+	0	+	0	+	0	+	+	0	0	+		0	
2	+	0	0	+	+	0	0	+	+	0	0	+	0	+	0	+	+	0	0		0	
3	+	+	0	0	+	0	0	+	+	+	+	0	0	0	+	0	+	0	+		1+	
4	+	0	0	+	+	0	0	+	+	+	+	+	0	+	+	+	0	+	0		1+	
5	+	0	+	+	+	0	+	+	0	+	0	+	0	+	0	+	+	0	+		1+	

- Summary
  - Anti-C, -E, -S present
  - Anti-Fy^{a}, -Jk^{a}, -Le^{a} ruled out
  - No anti-k!
  - Let’s look at the original panel…

The original panel…

	D	C	E	c	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s		Gel IgG
1	+	+	0	0	+	+	0	+	0	+	+	+	+	0	+	+	+	+	+		1+
2	+	+	0	0	+	0	+	+	+	+	+	0	0	+	+	+	0	+	0		1+
3	+	0	+	+	0	0	0	+	0	+	+	+	0	+	+	+	+	0	+		1+
4	+	0	0	+	+	0	0	+	0	0	+	+	0	0	+	+	0	+	+		1+
5	0	+	0	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	+		1+
6	0	0	+	+	+	0	0	+	+	+	+	+	0	+	+	+	0	0	+		1+
7	0	0	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	0	+		0
8	0	0	0	+	+	0	0	+	+	0	0	+	+	0	+	0	+	+	+		1+
9	0	0	0	+	+	0	0	+	0	+	+	+	0	+	0	+	0	+	0		1+
10	+	+	0	+	+	0	0	+	+	0	+	0	0	+	0	+	+	0	+		1+
11	0	0	0	+	+	0	0	+	+	+	0	+	0	0	+	0	+	+	+		1+
Auto																					0

You really can help us…

- Prenatal patient at XYZ Hospital
  - Dx: placenta previa
- Order for 4 RBCs
- Request form indicates:
  - A, Rh positive
  - IAT positive – 2 of 3 cells

Reference Lab performs normal workup

	D	C	E	c	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	IS	PEG IgG
1	+	+	0	0	+	+	0	+	0	+	+	+	+	0	+	+	+	+	+	0	0
2	+	+	0	0	+	0	+	+	+	+	+	0	0	+	+	+	0	+	0	0	0
3	+	0	+	+	0	0	0	+	0	+	+	+	0	+	+	+	+	0	+	0	0
4	+	0	0	+	+	0	0	+	0	0	+	+	0	0	+	+	0	+	+	0	0
5	0	+	0	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	+	0	0
6	0	0	+	+	+	0	0	+	+	+	+	+	0	+	+	+	0	0	+	0	0
7	0	0	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0	0
8	0	0	0	+	+	0	0	+	+	0	0	+	+	0	+	0	+	+	+	0	0
9	0	0	0	+	+	0	0	+	0	+	+	+	0	+	0	+	0	+	0	0	0
10	+	+	0	+	+	0	0	+	+	0	+	0	0	+	0	+	+	0	+	0	0
11	0	0	0	+	+	0	0	+	0	+	0	+	0	0	+	0	+	+	+	0	0
Auto																				0	0

- What should we do next?

Let’s call XYZ hospital!

- Routine technique in XYZ lab is gel!
- What do you think will happen if we test in gel?

Let’s call XYZ hospital!

- Routine technique in XYZ lab is gel!
- What do you think will happen if we test in gel?

	D	C	E	c	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s		Gel IgG
1	+	0	0	+	+	0	+	+	+	0	+	0	+	0	+	+	0	0	+		2+
2	+	0	0	+	+	0	0	+	+	0	0	+	0	+	0	+	+	0	0		1+
3	+	+	0	0	+	0	0	+	+	+	+	0	0	0	+	0	+	0	+		0
4	+	0	0	+	+	0	0	+	+	+	+	+	0	+	+	+	0	+	0		2+
5	0	0	0	+	+	0	0	+	0	0	0	+	+	0	0	0	+	+	+		0
6	+	0	+	+	0	0	+	+	0	+	0	+	0	+	0	0	+	0	+		0

Let’s call XYZ hospital!

- Routine technique in XYZ lab is gel!
- What do you think will happen if we test in gel?

	D	C	E	c	e	Cw	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s		Gel IgG
1	+	0	0	+	+	0	+	+	+	0	+	0	+	0	+	+	0	0	+		2+
2	+	0	0	+	+	0	0	+	+	0	0	+	0	+	0	+	+	0	0		1+
3	+	+	0	0	+	0	0	+	+	+	+	0	0	0	+	0	+	0	+		0
4	+	0	0	+	+	0	0	+	+	+	+	+	0	+	+	+	0	+	0		2+
5	0	0	0	+	+	0	0	+	0	0	0	+	+	0	0	0	+	+	+		0
6	+	0	+	+	0	0	+	+	0	+	0	+	0	+	0	0	+	+	0		0

Now what do we know?

- No reactivity in PEG-IgG tests
- Possible anti-M reactive in gel
  - Additional rule outs needed for:
    - Anti-C, -E, -K, -Fy^a, -Fy^b, -Jk^a, -S, -s
- Reactivity of anti-M enhanced in gel
  - Possibly due to slightly lower pH in gel
- Is this significant?
- Could we have arrived at this answer faster with XYZ's testing method?

Summary

- Working together allows everyone to achieve the best results and provide the best products for our patients.
  - Simple techniques can help with problem solving.
  - Proper sample labeling and completion of request form are critical to sample acceptance.