New York Blood Center Enterprises

Community Blood Center

IRL Experience:

MLS Rotation in the Immunohematology Reference Laboratory

Student Handbook

innovation • experience • expertise

Required Pre-Rotation Modules

- Print out the handbook for this rotation.
- Access modules at www.nybc.org/educationresources
    - Click on “Laboratory Science Student Resources”
- Complete the following modules and fill out the associated pages in this handbook:
    - **Comparing Blood Bank Methods** (p. 2-5 of this handbook)
    - **ABO Discrepancies** (p. 7 of this handbook)
    - **DATs, Eluates and Rh Nomenclature** (p. 9-14 of this handbook)
    - **Warm Autoantibodies** (p. 15-17 of this handbook)

Learning Objectives

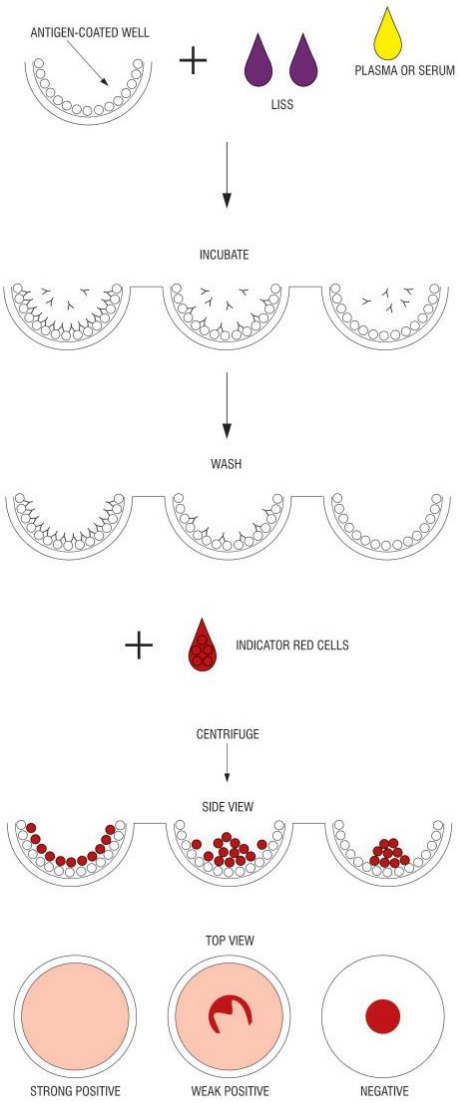
1. Perform antibody testing using the three blood bank methodologies: Tube, gel and solid phase.
    a. Describe how each methodology works to demonstrate antibodies in patient plasma.
    b. Compare and contrast gel, solid phase, and tube testing in terms of procedure, interpretation, strengths and limitations.
2. Resolve ABO discrepancies.
    a. Discuss causes of discrepancies and laboratory methods used to resolve discrepancies of various causes.
3. Perform pre-transfusion testing to rule out alloantibodies in a sample containing warm autoantibody.
    a. Describe common reactions in a sample containing warm autoantibody.
    b. Compare and contrast allo- and autoadsorption in terms of criteria for performing, choosing adsorbing cells, treatment of adsorbing cells, and limitations of the procedure
    c. List transfusion options for patients with warm autoantibody.
    d. Compare and contrast methods to determine the phenotype of recently transfused patients.

Comparing Blood Bank Methods

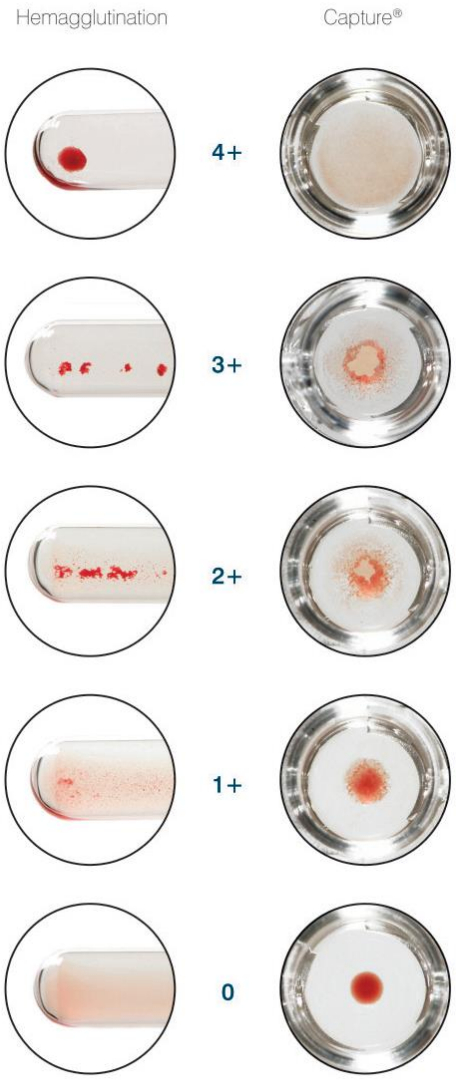
Use the "Comparing Blood Bank Methods" Module to review pages 2-4 and then to complete the chart on p. 5.

Solid Phase Red Cell Adherence Assay (SPRCA)

Test Procedure



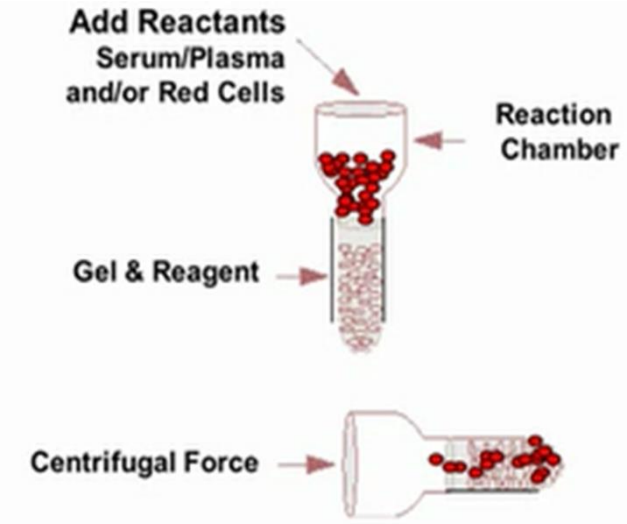
Grading Chart

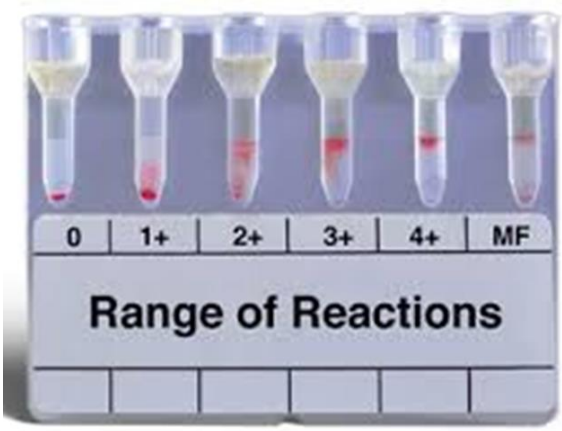


Solid Phase Red Cell Adherence Assay (SPRCA) continued

- Antigens coat the well
- Plasma is added to wells, incubated at 37C, antibodies (if present) attach to antigens
- Wash step: unbound antibody is removed
- Indicator cells (RBCs coated with anti-IgG) are added
- Centrifugation
- Interpretation: a tight cell button indicates a negative reaction, effacement of the cell button indicates a positive reaction

Gel (Column Agglutination Testing)

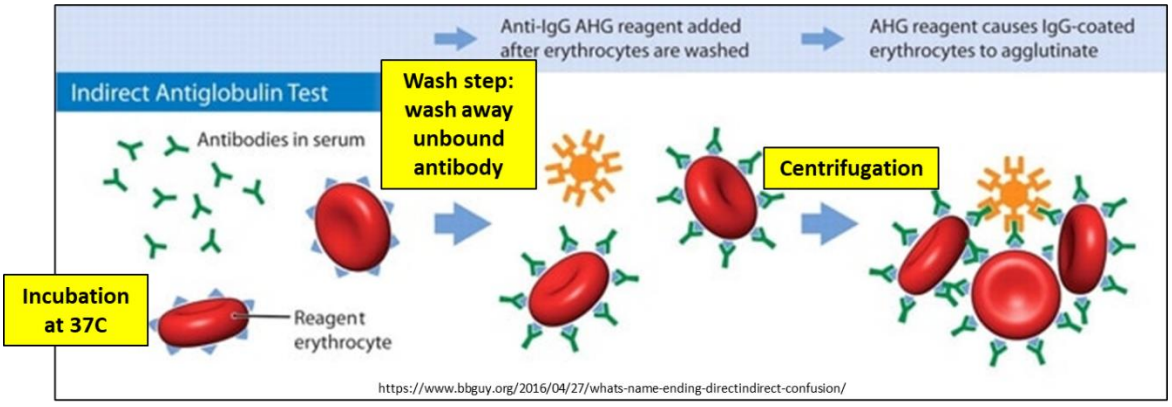




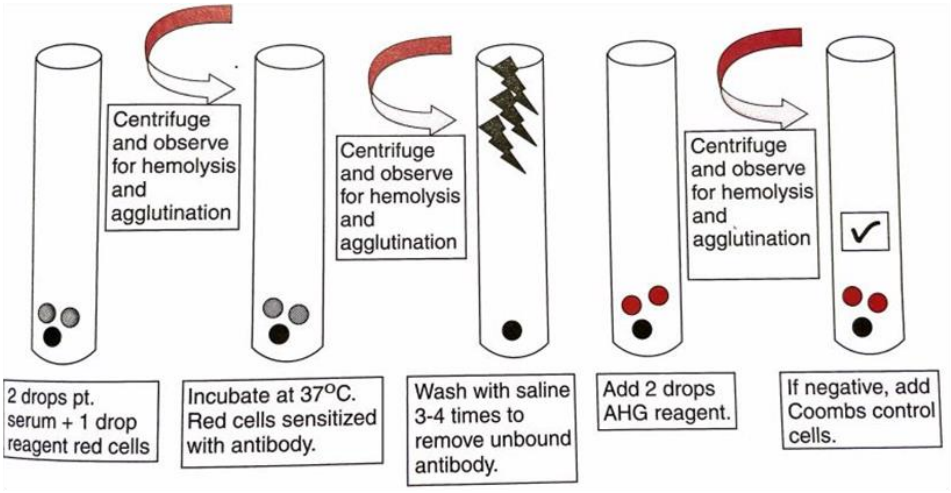
https://labs-inc.org/pdf/358_1.pdf

- Plasma and (0.8%) RBC suspension added to reaction chamber
- Incubation at 37C, antibodies (if present) attach to antigens
- Centrifugation pulls RBCs through gel that contains anti-IgG
- RBCs coated with antibody will get stuck in gel; RBCs without coated antibodies fall down to a pellet at the bottom of the column.
- Interpretation: RBCs stuck in the gel indicate a positive reaction; RBCs at the bottom of the column indicate a negative reaction.

Tube Testing



Tube Testing continued



Harmening DM. Modern Blood Banking & Transfusion Practices. 5th ed. Philadelphia, PA: F.A. Davis Company; 2005.

- Add plasma and cells to tube; spin and read for immediate spin phase (alternatively, incubate at room temperature before spinning and reading)
- Add enhancement media, if applicable
- Incubate at 37C. Following incubation, spin and read for 37C phase
- Wash
- Add antihuman globulin reagent (anti-IgG)
- Spin and read (may read microscopically)
- To all negative tests, add check cells. Spin and read. Check cells should produce positive result for valid test.

Comparing Blood Bank Methods

Complete this chart prior to arriving at CBC and come ready to discuss.

Methodology	What does a positive reaction look like?	What does a negative reaction look like?	Tests for IgG, IgM, or both?	Includes incubation at 37C (yes/no)?	Includes centrifugation (yes/no)?	Advantages of this methodology	Disadvantages of this methodology
Tube	Antibody coated red cells visibly agglutinate after adding anti-IgG reagent						
Gel	Antibody coated cells get stuck in gel matrix following centrifugation						
Solid phase	Indicator cells adhere to the surface of the microwell when antibody is bound to red cell antigens coating the microwell.						

Laboratory Testing: 3 Methods

The table below will be used to record results of testing at CBC.

	Tube Testing				Gel
	IS	LISS			
		37C	IAT		IgG
Screening cell I					
Screening cell II					
Screening cell III					

SPRCA testing results will be recorded on a panel provided by the instructor.

ABO Discrepancies

Use the "ABO Discrepancies" Module to complete this page and come to CBC ready to discuss. Use the choices available in the boxes to explain and resolve the following ABO discrepancies. You may use any answer more than once or not at all. Please note: sometimes, there will be more than one possible explanation and/or more that one way to resolve.

Possible Explanations

Cold Autoantibody
Recent Transfusion
Cold reacting alloantibody
A subgroup with anti-A1
BMT (group A+ to O+)
Rouleaux
Immunosuppression

Ways to Resolve

Increase 22C incubation time of back type
Prewarm back type
Inquire about patient history
No further testing required
Saline replacement of back type
Warm wash front type
Increase plasma/cell ratio
Identify alloantibody
Test with anti-A1 lectin

	Front Type: Testing patient cells					Back Type: Testing patient plasma					Possible Explanation	Ways to Resolve
	Anti-A	Anti-B	Anti-A,B	Anti-D	Rh Cont.	A_1 cells	A_2 cells	B cells	O cells	Auto Cont.		
1	4+	0	4+	4+	0	1+	0	4+	0	0		
2	0	4+	4+	4+	0	4+	3+	1+w	1+w	1+w	• •	• •
3	0	0	0	4+	0	0	0	3+	0	0	• •	• •
4	4+	2+mf	4+	4+	0	0	0	0	0	0		• •
5	1+	4+	4+	4+	1+	4+	4+	4+	4+	4+		• •
6	0	4+	4+	4+	0	4+	4+	4+	4+	0		
7	0	0	0	0	0	0	0	0	0	0		• •

Laboratory Testing: ABO Discrepancies

Use the following table to record results on samples:

Testing Method	**Front Type**							**Back Type**					**Interpretation**
(IS, 37C incubation, saline replacement, etc)	Sample ID	Anti-						A_1 cells	A_2 cells	B cells	O cells	Auto Cont.	
		A	B	A,B	A1	D	Rh Cont						

DATs, Eluates & Rh Nomenclature

Use the "DAT, Eluates & Rh Nomenclature" Module to complete pages 9-14.

Name reasons an individual might have a positive DAT:

-
-
-
-
-

What is an eluate?

The eluate procedure begins with WASHING the RBCs. What is the purpose of washing?

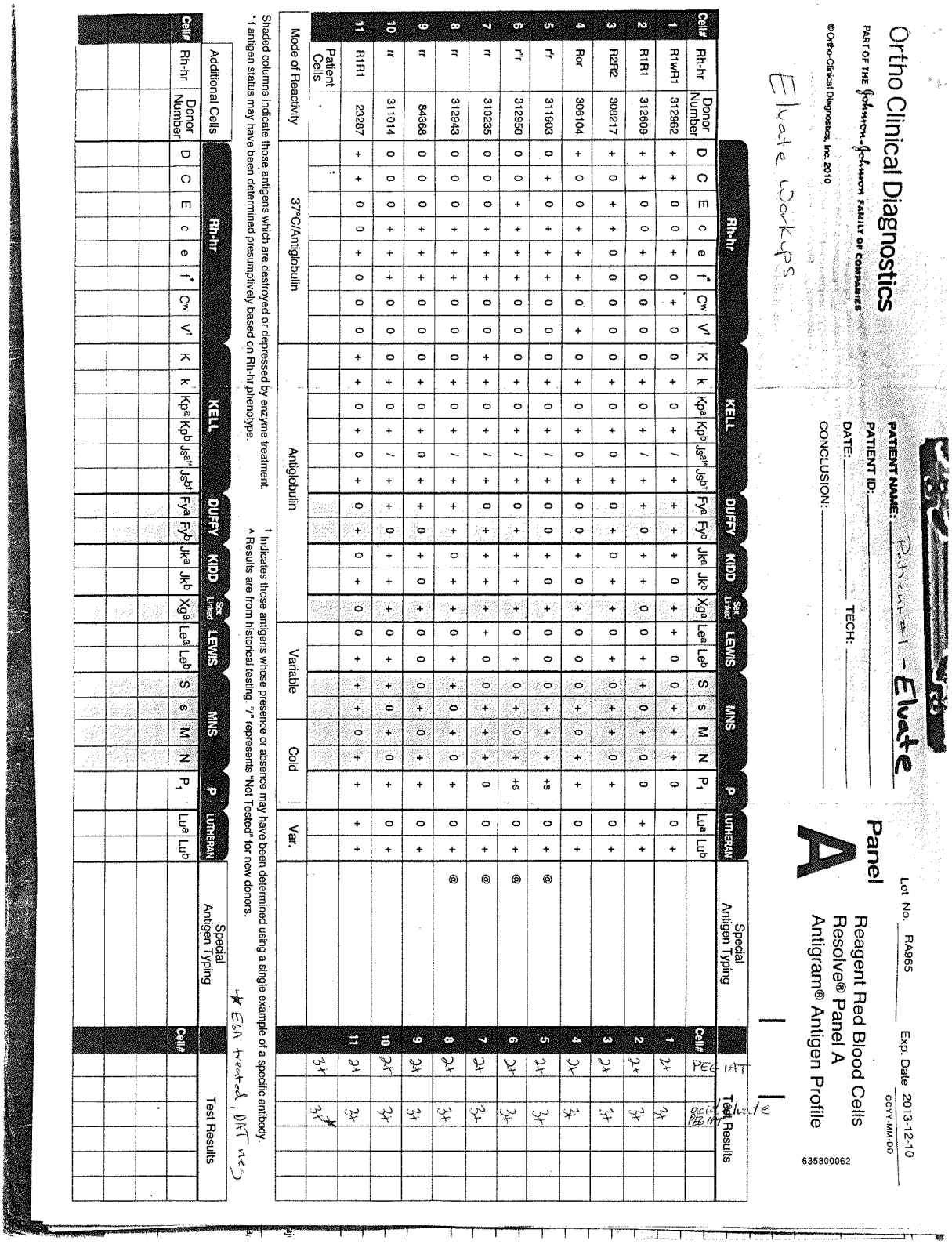
The "last wash" is tested as a control. What results are expected when testing the last wash? What if there are unexpected results?

Reactivity of the Eluate	Possible cause of the positive DAT
Nonreactive	
Panreactive, reactive with autocontrol	
Contains alloantibody	

At CBC you will prepare and test an eluate from the sample provided.

STUDENT HANDBOOK: IRL Experience

Study the following dry cases and determine the most likely cause of the positive DAT. Come to CBC prepared to discuss these cases.



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PATIENT NAME: Patient 1 - Elute

PATIENT ID:

DATE:

TECH:

CONCLUSION:

Elute workups

Lot No. RA965 Exp. Date 2013-12-10 CCYY-MM-DD

A Panel

Reagent Red Blood Cells
Resolve® Panel A
Antigram® Antigen Profile

635800062

Cell #	Rh-hr	Donor Number	Rh-hr D	Rh-hr C	Rh-hr E	Rh-hr c	Rh-hr e	Rh-hr f*	Rh-hr Cw	Rh-hr V'	KELL K	KELL k	KELL Kpa	KELL Kpb	KELL Jsa*	KELL Jsb†	DUFFY Fya	DUFFY Fyb	KIDD Jka	KIDD Jkb	Sex Linked Xga	LEWIS Lea	LEWIS Leb	MNS S	MNS s	MNS M	MNS N	P P1	LUTHERAN Lua	LUTHERAN Lub	Special Antigen Typing	Cell #	Test Results
1	R1wR1	312962	+	+	0	+	0	+	0	0	0	+	0	/	+	+	+	0	+	0	+	+	0	+	+	+	+	0	+		1	2+
2	R1R1	312609	+	+	0	+	0	+	0	0	0	+	0	/	+	+	+	0	+	0	0	0	+	0	+	0	0	+	+		2	2+
3	R2R2	308217	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	+		3	3+
4	Ror	306104	+	0	0	+	+	0	+	0	0	+	0	+	0	+	0	0	+	0	+	0	0	+	0	+	+	+	0		4	2+
5	r'r	311903	0	+	0	+	+	+	0	0	0	+	0	/	+	+	0	0	+	0	+	0	0	0	+	+	+	+s	0	@	5	2+
6	r"r	312950	0	0	+	+	+	+	0	0	0	+	0	/	+	+	0	+	+	+	+	0	+	0	+	0	+	+s	0	@	6	2+
7	rr	310235	0	0	0	+	+	+	0	0	0	+	0	/	+	+	0	+	+	+	+	0	+	0	+	+	+	0	0	@	7	2+
8	rr	312943	0	0	0	+	+	+	0	0	0	+	0	/	+	+	+	0	0	+	+	0	+	0	+	0	+	0	0	@	8	2+
9	rr	84368	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	0	+	0	+	+	0	+		9	2+
10	rr	311014	0	0	0	+	+	+	0	0	0	+	0	+	/	+	+	0	+	+	+	0	+	0	+	0	+	+	0	+		10	2+
11	R1r1	23287	+	+	0	+	+	0	0	0	+	+	0	+	0	+	0	+	0	+	0	0	+	+	+	0	+	+	+	+		11	2+
	Patient Cells	-																														
	Mode of Reactivity		37°C/Antiglobulin								Antiglobulin											Variable					Cold		Var.				

Shaded columns indicate those antigens which are destroyed or depressed by enzyme treatment.

* f antigen status may have been determined presumptively based on Rh-hr phenotype.

† Indicates those antigens whose presence or absence may have been determined using a single example of a specific antibody.

^ Results are from historical testing. "-" represents "Not Tested" for new donors.

★ EGA treated, DAT neg

Additional Cells	Rh-hr							KELL						DUFFY		KIDD		Sex Linked	LEWIS			MNS			P	LUTHERAN		Special Antigen Typing	Cell #	Test Results
Cell #	Rh-hr	Donor Number	D	C	E	c	e	f*	Cw	V'	K	k	Kpa	Kpb	Jsa*	Jsb†	Fya	Fyb	Jka	Jkb	Xga	Lea	Leb	S	s	M	N	P1	Lua	Lub			

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PATIENT NAME: Patient #2 Eluate

PATIENT ID:

DATE: TECH:

CONCLUSION:

Lot No. RA965 Exp. Date 2013-12-10 (CCYY-MM-DD)

Panel A

Reagent Red Blood Cells
Resolve® Panel A
Antigram® Antigen Profile

635800062

			Rh-hr								KELL						DUFFY		KIDD		Sex Linked	LEWIS		MNS				P	LUTHERAN		Special Antigen Typing		Test Results					
Cell#	Rh-hr	Donor Number	D	C	E	c	e	f*	C^w	$V^†$	K	k	Kp^a	Kp^b	$Js^{a†^}$	$Js^{b†}$	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	Le^a	Le^b	S	s	M	N	P_1	Lu^a	Lu^b		Cell#	PEG IAT				acid eluate PEG IAT	
1	R1wR1	312962	+	+	0	0	+	0	+	0	0	+	0	+	/	+	+	+	+	0	+	+	0	0	+	+	+	0	0	+		1	2+				0✓	
2	R1R1	312609	+	+	0	0	+	0	0	0	0	+	0	+	/	+	+	0	+	+	0	0	+	+	0	+	0	0	+	+		2	2+				0✓	
3	R2R2	308217	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	0	+		3	2+				0✓	
4	Ror	306104	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	0	0	+	0	+	+	0	+		4	2+				0✓	
5	r'r	311903	0	+	0	+	+	+	0	0	0	+	0	+	/	+	0	0	+	0	+	0	0	0	+	+	+	+s	0	+	@	5	2+				0✓	
6	r''r	312950	0	0	+	+	+	+	0	0	0	+	0	+	/	+	0	+	+	+	+	0	+	0	+	0	+	+s	0	+	@	6	2+				0✓	
7	rr	310235	0	0	0	+	+	+	0	0	+	+	0	+	/	+	0	+	+	+	+	+	0	0	+	+	+	0	0	+	@	7	2+				0✓	
8	rr	312943	0	0	0	+	+	+	0	0	0	+	0	+	/	+	+	+	0	+	+	0	+	+	0	+	0	+	0	+	@	8	2+				0✓	
9	rr	84368	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	0	+	0	+	+	0	+		9	2+				0✓	
10	rr	311014	0	0	0	+	+	+	0	0	0	+	0	+	/	+	+	0	+	+	+	0	+	+	0	+	0	+	0	+		10	2+				0✓	
11	R1R1	23287	+	+	0	0	+	0	0	0	+	+	0	+	0	+	0	+	0	+	0	0	+	+	+	0	+	+	+	+		11	2+				0✓	
	Patient Cells																																4+					
Mode of Reactivity			37°C/Antiglobulin								Antiglobulin											Variable				Cold			Var.									

Shaded columns indicate those antigens which are destroyed or depressed by enzyme treatment.

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† Indicates those antigens whose presence or absence may have been determined using a single example of a specific antibody.

^ Results are from historical testing. "/" represents "Not Tested" for new donors.

	Additional Cells		Rh-hr								KELL						DUFFY		KIDD		Sex Linked	LEWIS		MNS				P	LUTHERAN		Special Antigen Typing		Test Results					
Cell#	Rh-hr	Donor Number	D	C	E	c	e	f*	C^w	$V^†$	K	k	Kp^a	Kp^b	$Js^{a†^}$	$Js^{b†}$	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	Le^a	Le^b	S	s	M	N	P_1	Lu^a	Lu^b		Cell#						

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PATIENT NAME: Patient #3 Elvate

PATIENT ID:

DATE: TECH:

CONCLUSION:

Panel A

Reagent Red Blood Cells
Resolve® Panel A
Antigram® Antigen Profile

Lot No. RA965 Exp. Date 2013-12-10 CCYY-MM-DD

PEG IAT 635800062

Cell #	Rh-hr Donor Number	D	C	E	c	e	f*	Cw	V†	K	k	Kpa	Kpb	Jsa†^	Jsb†	Fya	Fyb	Jka	Jkb	Xga	Sex Linked	Lea	Leb	S	s	M	N	P1	Lua	Lub	Special Antigen Typing	Cell #	Test Results
1	R1wR1 312962	+	+	0	0	+	0	0	0	0	+	0	+	/	+	+	+	+	0	+		0	+	0	+	+	+	0	0	+		1	0
2	R1R1 312609	+	+	0	0	+	0	0	0	0	+	0	+	/	+	+	0	+	0	+		0	+	+	0	+	0	0	+	+		2	0
3	R2R2 308217	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	0	+		0	+	0	+	+	0	+	0	+		3	0
4	Ror 306104	+	0	0	+	+	0	0	+	0	+	0	+	0	+	0	0	+	0	+		0	0	0	+	+	+	+	0	+		4	0
5	r'r 311903	0	+	0	+	+	0	0	0	0	+	0	+	/	+	0	0	+	0	+		0	0	0	+	+	+	+s	0	+	@	5	0
6	r''r 312950	0	0	+	+	+	0	0	0	0	+	0	+	/	+	0	+	+	+	+		0	+	0	+	0	+	+s	0	+	@	6	0
7	rr 310235	0	0	0	+	+	0	0	0	+	+	0	+	/	+	0	+	+	+	+		0	+	0	+	+	+	+	0	+	@	7	0
8	rr 312943	0	0	0	+	+	0	0	0	0	+	0	+	/	+	+	+	0	+	+		0	+	+	0	+	0	+	0	+	@	8	0
9	rr 84368	0	0	0	+	+	0	0	0	0	+	0	+	0	+	+	0	+	0	+		0	+	0	0	+	0	+	+	0	+		9	0
10	rr 311014	0	0	0	+	+	0	0	0	0	+	0	+	/	+	+	0	+	+	+		0	+	0	+	0	+	0	+	0	+		10	0
11	R1R1 23287	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	0	+	+		0	+	0	+	+	+	0	+	+	+		11	0
	Patient Cells																																
	Mode of Reactivity	37°C/Antiglobulin								Antiglobulin												Variable		Cold					Var.				

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Additional Cells	Rh-hr							KELL				DUFFY		KIDD		Sex Linked	LEWIS		MNS			P	LUTHERAN		Special Antigen Typing	Test Results
Cell #	Donor Number	D	C	E	c	e	f*	Cw	V†	K	k	Kpa	Kpb	Jsa†^	Jsb†	Fya	Fyb	Jka	Jkb	Xga	Lea	Leb	S	s	M	N	P1	Lua	Lub		Cell #	

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PATIENT NAME: Patient 21 Elvate

PATIENT ID:

DATE: TECH:

CONCLUSION:

Panel A

Reagent Red Blood Cells
Resolve® Panel A
Antigram® Antigen Profile

Lot No. RA965 Exp. Date 2013-12-10 CCYY-MM-DD

635800062

Cell #	Rh-hr								KELL				DUFFY		KIDD	Sex Linked	LEWIS		MNS			P	LUTHERAN		Special Antigen Typing	Test Results	
	Rh-hr	Donor Number	D	C	E	c	e	f*	Cw	V	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Xga	Lea	Leb	S	s	M	N	P1	Lua	Lub		PEG	IAT
1	R1wR1	312962	+	+	0	0	+	0	0	0	+	0	+	/	+	+	+	+	0	+	+	0	+	+	+	+	0	+	+		2+	3+
2	R1R1	312609	+	+	0	0	+	0	0	0	+	0	+	/	+	+	0	+	+	0	0	+	0	+	0	+	0	+	+		2+	3+
3	R2R2	308217	+	0	+	+	0	0	0	0	+	0	+	/	+	+	0	+	+	0	+	0	+	0	+	+	0	+	0		0	0
4	Ror	306104	+	0	0	+	+	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	0	0	+	0	+	+	0			0	0
5	r'r	311903	0	+	0	+	+	+	0	0	0	+	0	/	+	+	0	0	+	0	+	0	0	0	+	+	+	+s	0	+	@	1+	0
6	r'r	312950	0	0	+	+	+	+	0	0	0	+	0	/	+	+	0	+	+	0	+	0	+	0	+	0	+	+s	0	+	@	0	0
7	rr	310235	0	0	0	+	+	+	0	0	+	+	0	/	+	+	0	+	+	0	+	0	+	0	+	+	+	+	0	+	@	0	0
8	rr	312943	0	0	0	+	+	+	0	0	0	+	0	/	+	+	0	+	+	0	+	0	+	0	+	0	+	+	0	+	@	0	3+
9	rr	84368	0	0	0	+	+	+	0	0	0	+	0	+	+	+	0	+	+	0	+	0	0	0	+	0	+	+	0	+		0	3+
10	rr	311014	0	0	0	+	+	+	0	0	0	+	0	+	+	+	+	0	+	+	+	0	+	+	0	+	0	+	0	+	+		0	3+
11	R1R1	23287	+	+	0	0	+	0	0	0	+	+	0	+	+	+	0	+	+	0	+	0	0	+	+	0	+	+	+	+	+		2+	0
	Patient Cells																															3+	
	Mode of Reactivity		37°C/Antiglobulin								Antiglobulin											Variable				Cold			Var.				

Shaded columns indicate those antigens which are destroyed or depressed by enzyme treatment.

* I antigen status may have been determined presumptively based on Rh-hr phenotype.

† Indicates those antigens whose presence or absence may have been determined using a single example of a specific antibody.

^ Results are from historical testing. "/" represents "Not Tested" for new donors.

Additional Cells	Rh-hr							KELL				DUFFY		KIDD	Sex Linked	LEWIS		MNS			P	LUTHERAN		Special Antigen Typing	Test Results	
Cell #	Rh-hr	Donor Number	D	C	E	c	e	f*	Cw	V	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Xga	Lea	Leb	S	s	M	N	P1	Lua	Lub		

Rh Nomenclature

After completing the online module, complete the table below.

Common Rh Haplotypes	
Fisher-Race	**Wiener**
DcE	
DCe	
	R_0
	r
dCe	
	r”

Warm Autoantibodies

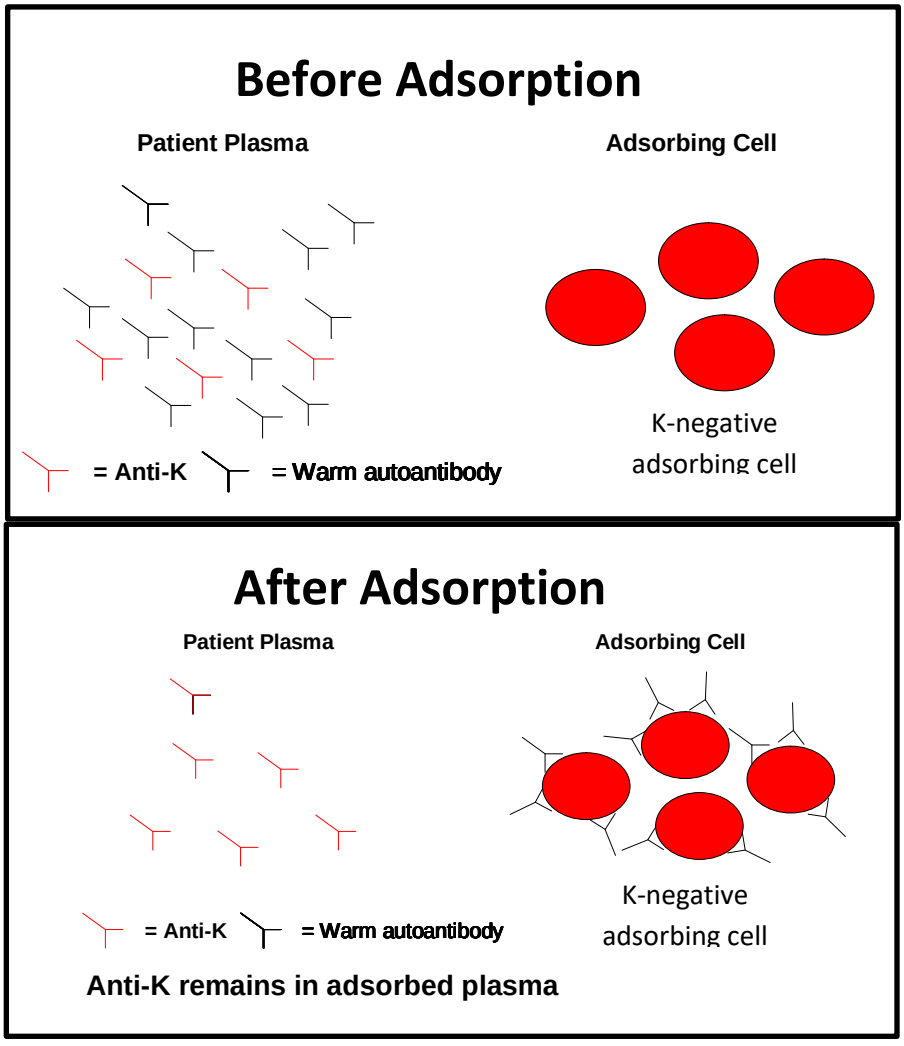
Use the "Warm Autoantibodies" Module to complete pages 15-17.

What are typical reactions for samples containing a strong warm autoantibody?

Test		Positive or Negative?
Antibody Screen		
Antibody Panel		
Autocontrol		
DAT	Polyspecific	
	Anti-IgG	
	Anti-complement	
	Saline Control	
Eluate		

****Prepare and test an eluate from the sample provided.**

How are clinically significant alloantibodies to common red cell antigens ruled out in cases of warm autoantibodies that react with all cells tested?



List 2 criteria for performing <u>auto</u>adsorptions

1.

2.

What is ZZAP? Why do you ZZAP autologous cells prior to adsorption?

When we perform <u>allo</u>adsorptions, we typically use a cell that is phenotypically matched to the patient.

- **Describe what we mean by "phenotypically matched."**

- **Why do we use phenotypically matched RBCs for adsorbing cells?**

What is the biggest risk when doing <u>allo</u>adsorptions?

Why do you ficin-treat adsorbing cells? (Give two reasons)

1.

2.

Which adsorbing cell would you use for performing alloadsorptions for patients with the following phenotypes? (Adsorbing cells are ficin-treated) Choose the correct cell(s).

<u>Adsorbing cells available:</u>

Adsorbing Cell A= R_1R_1, K-, Jk(a-)
Adsorbing Cell B= R_1R_1, K-, Jk(b-)
Adsorbing Cell C= R_2R_2, K-, Jk(a-)
Adsorbing Cell D= R_2R_2, K-, Jk(b-)
Adsorbing Cell E= rr, K-, Jk(a-)
Adsorbing Cell F= rr, K-, Jk(b-)

1. **R_1r, K+, Fy(a+b-), Jk(a+b-), S+s-**
   _____
2. **rr, K-, Fy(a-b-), Jk(a-b+), S-s+**
   _____
3. **R_2R_2, K-, Fy(a-b+), Jk(a+b+), S-s+**
   _____
4. **rr, K-, Fy(a-b+), Jk(a+b+), S+s+**
   _____

How do you obtain a phenotype if the patient has been recently transfused?

- **Hypotonic Wash:**
    - **Principle:**
    - **Limitations:**

- **Molecular Phenotype**
    - **Principle:**
    - **Limitations:**

The information below will be covered during your rotation at CBC.

Name 3 options for transfusion of patients with warm autoantibody and no underlying alloantibodies:

1.

2.

3.

Will crossmatches be compatible?

Procedures

Solid Phase Ready Screen Procedure

1. Add two drops of LISS to each well.
2. Add one drop of positive control to the positive control well. Add one drop of negative control to the negative control well.
3. Add one drop of patient plasma to each of the testing wells (the purple color of the LISS will change to blue).
4. Incubate at 37C for 20-60 minutes.
5. Wash wells 4 times.
6. Add 1 drop of indicator cells to each well.
7. Centrifuge. Refer to job aid for centrifugation specifications.

Gel Testing Procedure

1. Visually inspect gel card and verify the presence of gel and liquid layer above the gel. GEL CARD MUST REMAIN UPRIGHT. Label the card appropriately. Remove the foil from only the wells you will be using.
2. Add 50ul of 0.8% RBC suspension to each appropriate well. The RBC suspension should remain in the upper reaction chamber.
3. Add 25ul of patient plasma.
4. Incubate gel card(s) at 37C for 15 minutes.
5. Centrifuge the gel card for 10 minutes. Read, record, and interpret results.

Tube Testing Procedure

1. Label sufficient tubes for each cell tested.
2. Add 2 drops of plasma (or eluate/or alloadsorbed plasma) to each tube.
3. Add one drop of panel cells or autologous cells to appropriate tubes.
4. Add 2 drops of PEG (or LISS), mix, and incubate at 37C for 10 minutes.
5. Wash 4 times. Add 2 drops of anti-IgG.
6. Centrifuge. Read and record results. Check all negative reactions microscopically.
7. Add 1 drop of check cells to all negative tubes.
8. Centrifuge. Read. Make a check mark beside the IAT result if positive.
9. If negative, repeat IAT procedure.

ABO/Rh Typing

1. Label tubes for front type (anti-A, anti-B, anti-D, Rh control- test anti-A,B and anti-A1 only if necessary to resolve discrepancy) and back type (A_1 cells, A_2 cells, B cells, O cells, autocontrol)
2. Add 1 drop of the corresponding antisera into the front type tubes.
3. Add 2 drops of patient plasma to each back type tube and 1 drop of patient cells to each front type tube.
4. Add 1 drop of corresponding reagent red cells into the back type tubes.
5. Centrifuge for 15 seconds and read.

Eluate Procedure (using acid eluate kit)

1. Wash patient cells one time with saline. Wash 4 additional times with Working Wash Solution.
2. Remove the supernatant from the last wash, saving 1 ml from just above the red cells. Place in a tube labeled “last wash.”
3. Add 20 drops of Eluting Solution to 20 drops of washed red blood cells. Gently invert the red cells 4 times to mix.
4. Immediately centrifuge for 45-60 seconds.
5. Transfer the eluate to a clean 12X75 tube.
6. Add Buffering Solution until the eluate turns pale blue.
7. Mix and centrifuge eluate to eliminate debris. Transfer to a clean 12X75 labeled tube.
8. Test the last wash against antibody screening cells and the eluate against a panel by PEG IAT. If reactivity is detected in the last wash, prepare a new eluate after washing the cells more times with Working Wash Solution.

Procedures continued

Alloadsorption Procedure

The number of adsorptions needed to remove the autoantibody is equal to the antibody strength (i.e. an autoantibody reacting 2+ is adsorbed 2 times.)

1. Prepare 2 ml aliquots of appropriate ficin-treated adsorbing cells. Wash until the supernatant is clear.
2. On the last wash, centrifuge for 3 minutes. Remove as much remaining saline as possible (use small glass pipet).
3. To the tube of adsorbing cells, add 1 ml of patient plasma.
4. Mix and incubate at 37C for 10 minutes.
5. Centrifuge the tubes for 3 minutes.
6. Carefully remove the plasma and add to the next tube of adsorbing cells.
7. Repeat steps 2-4 as needed.
8. After final adsorption, remove the serum/plasma and place it into labeled tubes.
9. Test the alloadsorbed plasma against a panel by LISS IAT.

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