

# **Resident Rotation:** *Immunohematology Reference Laboratory*

# Handbook for Online Modules









**Nebraska** Community Blood Bank **A New York** Blood Center



# Immunohematology Reference Laboratory

## **Objectives:**

1.	Perform routine blood bank tests including type and screen, antibody identification, antigen typing of
rec	d cells, DAT, eluate, and alloadsorptions.
a) b) c) d)	Describe the purpose and methodology of each test performed. Demonstrate proper grading of reactions. Follow procedures exactly and interpret results correctly. Perform rule-outs appropriately using panel results, and correctly identify alloantibodies in laboratory samples.
e) f)	Compare and contrast the three testing platforms for blood bank testing: tube testing, MTS gel testing, and solid phase red cell adherence assay (SPRCA). List 5 possible explanations for a positive DAT and explain how laboratory testing can aid in determining the cause of a
a)	positive DAT. Define shorthand nomenclature for the common Rh antigens (e.g. R1R1,R2R2,rr) and discuss its use in alloadsorptions.
g) 2	List common red cell antigen groups and describe characteristics of alloantibodies directed against
	mmon red cell antigens.
a)	Distinguish between clinically significant and clinically insignificant alloantibodies.
	Recognize ABO discrepancies, and describe possible explanations and common resolutions.
a) b)	Identify ABO discrepancies for the following patients: bone marrow transplant recipient, immunodeficient patient, recipient of recent transfusion, A subgroup with anti-A1, patient with cold autoantibody or cold-reacting alloantibody, patient with rouleaux. Choose techniques to resolve ABO discrepancies including the following methods: increasing incubation time, decreasing incubation temperature, further testing with special reagents (anti-A,B, anti-A1, etc), prewarmed technique, investigating
	patient history.
4.	Explain the purpose of antigen typing patients and donors and the feasibility of antigen matching for
blo	bod transfusion.
a) b) c) d) e)	Discuss differences in approaches to ABO/Rh determination in patients and donors. Explain how antigen-typing is used to work up a patient sample with warm reactive autoantibody. Perform calculations to determine how difficult it will be to find antigen-negative units. Name 2 specific patient populations that benefit from transfusing antigen-matched blood. Discuss the difference between the phenotype and the genotype.
5.	Determine special requirements for selecting donor red blood cells, platelets, and plasma for
	insfusion to patients.
a) b) c) d) e)	Choose donor red cells and plasma of the appropriate ABO type for transfusion. Discuss how institutional policies may vary for transfusing non ABO-identical platelet products. List the requirements for transfusion of patients with clinically significant alloantibodies. Discuss how institutional policies may vary for selection of red blood cells to transfuse to patients with warm autoantibodies. List the indications for transfusing CMV-negative, irradiated, and washed blood products, and apply current recommendations for these special requests in case studies.
6.	Describe the process of procuring specially typed units.
a) b) c)	Discuss the ordering of routine antigen negative units. Describe the implications for ordering rare frozen units from Community Blood Center. Understand the availability of rare blood through the American Rare Donor Program (ARDP).

## **Blood Type**

I. Grading of reactions in tubes:



• Why is consistent reaction grading important?

## II. Definitions:

	Testing of patient's	Reagents commonly used
Front type		
Back type		

• What is the Rh control? Why do we use it?

## III. Interpreting blood types:

		Fron	t Type		Back	Туре	
				Rh			Interpretation
	Anti-A	Anti-B	Anti-D	Control	A <sub>1</sub> cells	B cells	
Sample 1	4+	0	4+	0	0	4+	A pos
Sample 2	0	0	0	0	3+	3+	
Sample 3	4+	4+	0	0	0	0	
Sample 4							B neg
Sample 5							O pos

How might interpreting blood types differ when testing donors or patients?

IV. Observe and perform blood type procedure

## **Blood Type Procedure**

- 1. Label 1 tube for the patient cell suspension and one tube for the patient plasma.
- 2. Add the patient plasma to the appropriately labeled tube. Add one drop of patient cells to the cell suspension tube.
- 3. Fill the cell suspension tube with saline, and centrifuge both tubes for 1 minute.
- 4. Remove the supernatant from the red cells and add saline to create a 2-5% red cell suspension.
- 5. Label 4 tubes for the front type: A, B, D, Rh Control.
- 6. Add one drop of reagent Anti-A, Anti-B, Anti-D, and Rh Control to the appropriate tube.
- 7. Label 2 tubes for the back type: A<sub>1</sub>, B.
- 8. Add 2 drops of the patient plasma to each back type tube.
- 9. Add one drop of patient cell suspension to each front type tube.
- 10. Add one drop of the appropriate reagent red cells to the A1 and B back type tubes.
- 11. Centrifuge the tubes for 15 seconds. Read and record results.

## **Blood Type Results**

### Record your results below.

		Front	Туре		Back	Туре	Interpretation
Sample ID	Anti-A	Anti-B	Anti-D	Rh Control	A1 cells	B cells	

## **ABO Discrepancies**

With a partner, 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.

## 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 incubation time of back type Prewarm back type Inquire about patient history No further testing required Saline replacement of backtype Warm wash front type Decrease incubation temperature Identify alloantibody Test with anti-A1 lectin

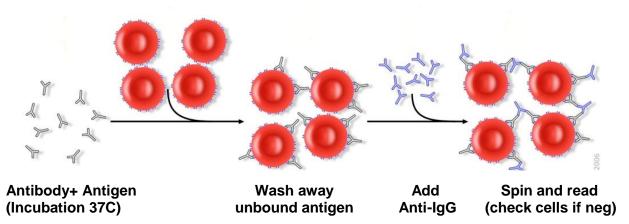
F	ront 7		Testi cells	ng pa	tient	Ba		ype: ent pla	Testir asma	ng		
	Anti- A	Anti- B	Anti- A,B	Anti- D	Rh Cont.	A1 cells	A2 cells	B cells	O cells	Auto Cont.	Possible Explanation	Ways to Resolve
1	4+	0	4+	4+	0	1+	0	4+	0	0	•	•
2		4+	4+	4+	0	4+	3+	1+w	1+w	1+w	•	•
	0	0	0	4+	0	0	0	3+	0	0	•	•
4		2+mf	4+	4+	0	0	0	0	0	0	•	•
5	1+	4+	4+	4+	1+	4+	4+	4+	4+	4+	•	•
	0	4+	4+	4+	0	4+	4+	4+	4+	0	•	•
7	0	0	0	0	0	0	0	0	0	0	•	•

## **Antibody Identification**

Why do some patients have "unexpected" antibodies?

What is the first step to determine if a patient has "unexpected" antibodies?

## Indirect antiglobulin test:



What is the purpose of adding check cells in the final step of the indirect antiglobulin test?

Observe and perform PEG antibody screen procedure.

## **PEG Antibody Screen Procedure**

- 1. Label three tubes: I, II, III
- 2. Add two drops of patient plasma to each tube.
- 3. Add one drop of appropriate screening cell to each tube.
- 4. Incubate the tubes for 5 minutes at room temperature.
- 5. Centrifuge 15 seconds. Read and record results.
- 6. Add two drops of PEG to each tube. Incubate at 37C for 10-30 minutes.
- 7. Wash 4 times with buffered saline. Add two drops of anti-IgG.
- 8. Centrifuge 15 seconds. Read and record results. Review negative tubes microscopically.
- 9. Add 1 drop of IgG-coated check cells to all negative tubes.
- 10. Centrifuge, read, and place a check mark beside the IAT result if positive.
- 11. If check cells are negative, repeat testing.

## **PEG Antibody Screen Results**

Record your results below.

Sample ID	Screening Cell	5 Minutes RT	PEG IAT
	I		
	II		
	III		
	I		
	II		
	III		
	II		

## **Antibody Identification (Continued)**

When a patient's antibody screen is positive, the next test that should be performed is the \_\_\_\_\_.

Do's and Don'ts for Rule-outs on an Antibody Panel

- a) Perform rule-outs on \_\_\_\_\_\_ (positive/negative) reactions.
  b) In most cases, use \_\_\_\_\_\_ (heterozygous/homozygous) expressions of antigens to rule out.
- c) In the IRL, we rule out all clinically significant antibodies to common red cell antigens \_\_\_\_\_ (once/twice) and we rule in \_\_\_\_\_ (once/twice).

In your own words, define DOSAGE:

Name two possible explanations for variable reactivity on panel cells.

1.

2.

Perform PEG antibody panel on a specimen with a positive antibody screen.

Practice ruling out on the following "dry" panels (Patients 1-9 on green sheets). For each patient, write suspected antibody and common clinically significant antibodies that you can't rule out.

## **Antibody Panel Procedure**

- 1. Label sufficient tubes for each panel cell and an auto control (usually 1-11 and AC).
- 2. Add 2 drops of plasma to each tube.
- 3. Add one drop of panel cells or autologous cells to appropriate tubes.
- 4. Incubate the tubes based on the enhancement procedure being used.

When testing with PEG (or LISS):

a) Incubate the tubes for 5 minutes at room temperature. Centrifuge. Read and record results.

- b) Add 2 drops of PEG (or LISS), mix, and incubate at 37C for 10 minutes.
- c) Wash 4 times. Add 2 drops of anti-IgG.
- d) Centrifuge. Read and record results. Check all negative reactions microscopically.
- e) Add 1 drop of check cells to all negative tubes.
- f) Centrifuge. Read. Make a check mark beside the IAT result if positive.
- g) If negative, repeat IAT procedure.

When testing ficin- treated cells:

- a) Incubate at 37C for 30 minutes.
- b) Read (no centrifugation) and record reactions.
- c) Wash 4 times. Add 2 drops of anti-IgG
- d) Centrifuge. Read and record results.
- e) Add 1 drop of check cells to all negative tubes.
- f) Centrifuge. Read. Make a check mark beside the IAT result if positive.
- g) If negative, repeat IAT procedure.

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rr,	1184	03	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+ +	+	0	+		90	
rr	3041	73	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	0	0	0	+	+	+	+s	0	+		10 O	
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\* f antigen status may have been determined presumptively based on Rh-hr phenotype.

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

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2	R1R1	302788	+	+	0	0	+	0	0	0	+	+	0	+	0	+		0 -	ł	0	+	+	0	+	+	+	0	+	+	0	+			3+			
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 $^{\star}$  f antigen status may have been determined presumptively based on Rh-hr phenotype.

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

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#### \*Screening Cells / Suchzellen / Cellules de dépistage / Pannello screening / Células de Screening

#### \*\*See reverse side / Siehe Rückseite / Voir au verso / Vedere sul retro / Ver parte posterior

Usually cold reactive antibody / Normalerweise kältereaktive Antikörper / En principe anticorps froids / Solitamente anticorpi freddi / Usualmente anticuerpo reactivo en frío Usually warm or Coombs reactive Ab / Normalerweise wärme- oder Coombs-reaktive Ak / En principe Ac chauds ou réagissant par Coombs / Solitamente anticorpi caldi o reattivi in coombs / Usualmente reactivo en prueba de Coombs o en calien vs = very strong / sehr stark / très fort / molto forte / muy fuerte s = strong / stark / fort / fort / fort / molto forte / muy fuerte w = very weak / sehr schwach / très faible / molto debole / muy débil w = weak / schwach / faible / debole / débil

1. The Difference of the constant star are to strong any manufacture

w = weak / schwach / faible / debole / débil

NT = not typed / nicht typisiert / non typé / non tipizzato / no tipificado

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\*Screening Cells / Suchzellen / Cellules de dépistage / Pannello screening / Células de Screening / Screeningsceller / Keresö (screening) sejtek / stanice za probir / Células de triagem / Screeningceller

Usually cold reactive antibody / Normaleweise kaltereaktive Antiköper / En principe anticorps froids / Solitamente anticorpi fredi / Usualmente anticorpi cadii o reactive antibody / Normalt kuldereaktive antistoffer / Altaläban hideg antitestek / Uobičajeno hiadno reaktivno antitijelo / Geralmente anticorpis froids / Normalt kuldereaktive antistoffer / Altaläban hideg antitestek / Uobičajeno hiadno reaktivno antitijelo / Geralmente anticorpis froids / Normalt kuldereaktive antistoffer / Altaläban hideg antitestek / Uobičajeno hiadno reaktivno antitijelo / Geralmente anticorpis froids / Normalt kuldereaktive antistoffer / Altaläban hideg antitestek / Uobičajeno hiadno reaktivno antitijelo / Geralmente anticorpos froids / Normalt kuldereaktive antistoffer / Altaläban hideg antitestek / Uobičajeno hiadno reaktivno antitijelo / Geralmente anticorpos froids / Normalt kuldereaktive antistoffer / Altaläban hideg antitestek / Uobičajeno hiadno reaktivno antitijelo / Geralmente anticorpos reativos ao teste de Coombs-reaktiv antitestek / Uobičajeno topio III Coombs/reaktive as / Altaläban meleg, illene Coombs-reaktiv antitestek / Uobičajeno topio III Coombs/reaktive as /







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INSTITUTION \_\_\_\_\_\_ BLOOD GROUP \_\_\_\_\_\_ ANTIBODY IDENTITY \_\_\_\_\_

IMMUCOR, INC. Norcross, GA 30071 USA

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\*Screening Cells / Suchzellen / Cellules de dépistage / Pannello screening / Células de Screening / Screeningsceller / Kereső (screening) sejtek / stanice za probir / Células de triagem / Screeningceller

Usually cold reactive antibody / Normaleviene kattereaktive Antikorper / En principe anticorps froids / Solitamente anticorps froids / Usualmente anticorps reactivo en frio / Normali kuldereaktive antistoffer / Altalaban hitleg antitesive / UobiCajeno hiadno reaktivno antitijelo / Geralmente anticorps froids / Normali kuldereaktive antistoffer / Altalaban hitleg antitesive / UobiCajeno hiadno reaktivno antitijelo / Geralmente anticorps froids / Normali kuldereaktive antistoffer / Altalaban hitleg antitesive / UobiCajeno hiadno reaktivno antitijelo / Geralmente anticorps froids / Normali kuldereaktive antistoffer / Altalaban hitleg antitesive / UobiCajeno hiadno reaktivno antitijelo / Geralmente anticorps froids / Normali kuldereaktive are used in the combis reactivo en prieba de Courbs o en calevite / Normali kuldereaktive as / Atalaban meleg, sleve Coonth-reactiv anticenek / Uobičajeno topio ili Coombsovo reaktivno anticijelo / Geralmente anticorpos reativos ao teste de Coombs ou anticorpos guentes / Normalt varme-rae (coontoy-reactive-un





atrent #6 part 2 NAME:

GROUP \_\_\_\_\_ Rh \_\_\_\_\_ TEST DATE

HISTORY\_\_\_\_\_

INTERPRETATION\_\_\_\_\_

2       RR1       B2461       +       +       0       0       +       +       0       + </th <th></th> <th>USA U.S. License No. 886</th> <th>Lot N</th> <th>lo:</th> <th>288</th> <th>41</th> <th></th> <th></th> <th></th> <th>E</th> <th>xp. C</th> <th>)ate:</th> <th></th> <th>2</th> <th>008</th> <th>-09</th> <th>-19</th> <th></th> <th>420-4</th> <th>Г</th> <th></th> <th></th> <th></th> <th>EST P</th> <th>ESIILI</th> <th>rs</th>		USA U.S. License No. 886	Lot N	lo:	288	41				E	xp. C	)ate:		2	008	-09	-19														420-4	Г				EST P	ESIILI	rs
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31       rr       E235       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       +       +       0       +       +       0       +       +       0       +       +       0       +       + <td></td> <td></td> <td>D</td> <td>c</td> <td>c</td> <td>E</td> <td>e</td> <td>P</td> <td>v</td> <td>C.</td> <td>к</td> <td>ĸ</td> <td>K,</td> <td>к;</td> <td>Ĵ,*</td> <td>J</td> <td>Ę,</td> <td>F</td> <td>J,</td> <td>J<sub>k</sub></td> <td>L,</td> <td>Ľ,</td> <td>P,</td> <td>M</td> <td>N</td> <td>s</td> <td>s</td> <td>F.*</td> <td>11</td> <td>ž,</td> <td>Ulla+1</td> <td></td> <td><u> </u></td> <td>r</td> <td>L</td> <td></td> <td>l</td> <td>10</td>			D	c	c	E	e	P	v	C.	к	ĸ	K,	к;	Ĵ,*	J	Ę,	F	J,	J <sub>k</sub>	L,	Ľ,	P,	M	N	s	s	F.*	11	ž,	Ulla+1		<u> </u>	r	L		l	10
31       r'r       F693       0       0       +       +       0       + </td <td>11</td> <td>r'r E235</td> <td>0</td> <td>+</td> <td>+</td> <td>0</td> <td>+</td> <td>+</td> <td>0</td> <td>0</td> <td>0</td> <td>+</td> <td>0</td> <td>+</td> <td>-</td> <td>-</td> <td>10000</td> <td></td> <td>+</td> <td>0</td> <td>0</td> <td>+</td> <td>(282.000) (282.000)</td> <td>(9996)</td> <td>5.007</td> <td>X82223</td> <td>39963</td> <td>12309300</td> <td>100000</td> <td>(2012) <b>2</b></td> <td></td> <td>Ŧ</td> <td><u> </u></td> <td><u> </u></td> <td>T T</td> <td><u> </u></td> <td></td> <td><u> </u></td>	11	r'r E235	0	+	+	0	+	+	0	0	0	+	0	+	-	-	10000		+	0	0	+	(282.000) (282.000)	(9996)	5.007	X82223	39963	12309300	100000	(2012) <b>2</b>		Ŧ	<u> </u>	<u> </u>	T T	<u> </u>		<u> </u>
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14       r'r       E627       0       +       +       0       +       0       +       0       +       0       +       0       +       0       +       0       +       0       +       0       +       0       +       0       +       0       +       0       +       0       +       0       +       0       +       0       +       0       +       0       + </td <td>13</td> <td>п G660</td> <td>0</td> <td>0</td> <td>+</td> <td>0</td> <td>+</td> <td>+</td> <td>0</td> <td>0</td> <td>+</td> <td>+</td> <td>0</td> <td>+</td> <td>0</td> <td>+</td> <td>+</td> <td>+</td> <td>+</td> <td>0</td> <td>+</td> <td>0</td> <td>+</td> <td>+</td> <td>0</td> <td>0</td> <td>+</td> <td></td> <td>+</td> <td>+</td> <td>Q-4L-3</td> <td>+</td> <td></td> <td></td> <td>+</td> <td>┼──┤</td> <td></td> <td>2222</td>	13	п G660	0	0	+	0	+	+	0	0	+	+	0	+	0	+	+	+	+	0	+	0	+	+	0	0	+		+	+	Q-4L-3	+			+	┼──┤		2222
15       r       N2560       0       0       +       0 <td>14</td> <td>r'r E627</td> <td>0</td> <td>+</td> <td>+</td> <td>0</td> <td>+</td> <td>+</td> <td>0</td> <td>0</td> <td>0</td> <td>+</td> <td>0</td> <td>+</td> <td>0</td> <td>+</td> <td>0</td> <td>0</td> <td>+</td> <td>+</td> <td>+</td> <td>0</td> <td>+</td> <td>0</td> <td></td> <td></td> <td>+</td> <td></td> <td></td> <td>0</td> <td><u>L0(D+)</u></td> <td>┽</td> <td></td> <td>┝─-</td> <td>–</td> <td><math>\left  - \right </math></td> <td></td> <td>13</td>	14	r'r E627	0	+	+	0	+	+	0	0	0	+	0	+	0	+	0	0	+	+	+	0	+	0			+			0	<u>L0(D+)</u>	┽		┝─-	–	$\left  - \right $		13
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PATIENT'S CELLS       PATIENT'S CELLS         In those instances where a patient's serum is know to contain anti-D, it may be desirable to perform antibody screening tests with D-red cells. The panel cells 11, 12, and 13 can be used together to form a D- negative antibody screening reagent.       REVERSE GROUPING CELLS       Ar         All cells are positive for I, Ge, Yta*, Tja, Vel, Coa*, Dib and negative for Mg, Vw, Dia and Wra except where noted. * indicates those antigens whose presence or absence may have been determined using only a single example of a specific antibody.       * indicates those       PANOSCREEN LOT:       1       1	20	Ror D691	+	0	+	0	+	+	+	0	0	+	0	+	0			+	+							-				-	Yt(b+)	+		┣		<b> </b>		19
In those instances where a patient's serum is know to contain anti-D, it may be desirable to perform antibody screening tests with D- red cells. The panel cells 11, 12, and 13 can be used together to form a D- negative antibody screening reagent.  All cells are positive for I, Ge, Yta*, Tja, Vel, Coa*, Dib and negative for Mg, Vw, Dia and Wra except where noted. * indicates those antigens whose presence or absence may have been determined using only a single example of a specific antibody.  The f antigen status may have been determined presumptively based on Rh-hr phenotype.  PANOSCREEN LOT: PANOSCR			+		†		┢─				_		-		Ť	-	Ť	-		· ·	-		<u> </u>	-		Ť	-	-	+	+		$\downarrow$		L	$\bot$			20
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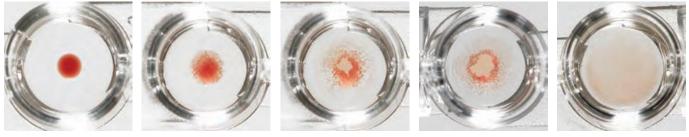
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# **Different Methodologies**

The following pages describe the solid phase red cell adherence assay (SPRCA) and gel column agglutination. Use the information to compare and contrast the different methodologies following the explanations.

## Solid Phase Red Cell Adherence Assay (SPRCA)

- Red cell antigens coat microwell
- Antibodies present adhere to antigens during 37C incubation
- Unbound antibody washed away
- Indicator cells (cells with attached anti-IgG) added
- Centrifugation
- Grading reactions:



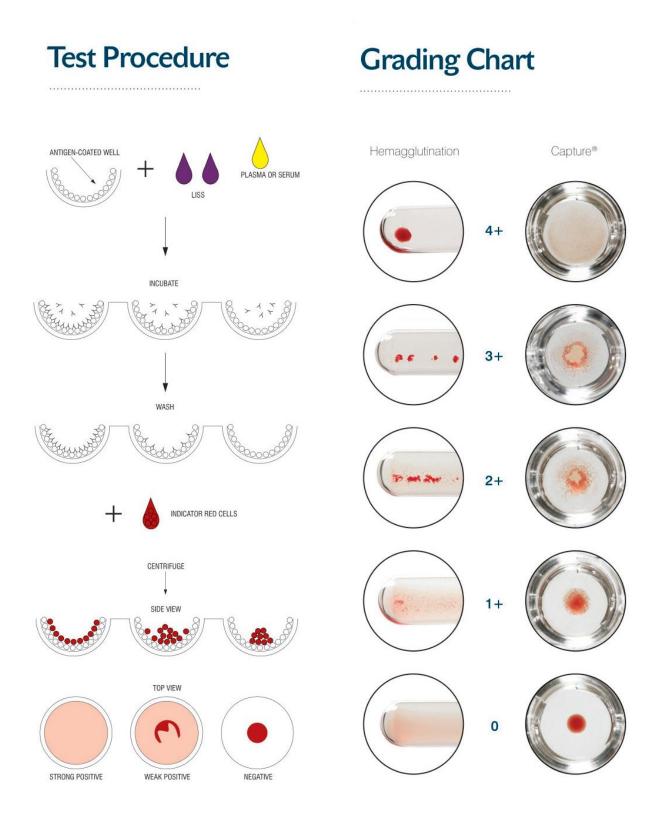
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## **SPRCA (Continued) Six Simple Steps**

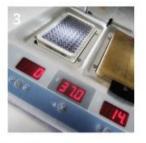


Add LISS Remove strip from foil pouch. Appropriate antigens for the test are pre-coated on the well.\* Add two drops of Capture\* LISS.

\*If using Capture-P° or Capture-R Select, prepare platelet or red cell monolayer first.



**Add Patient Sample** Using a routine blood bank pipette, add one drop of donor/patient plasma. Add one drop of controls.



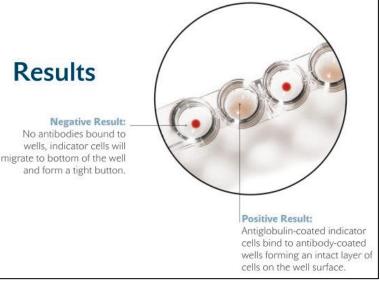
Incubate Incubate test strips in P2.\*

\*except Capture\*-CMV which requires incubation at room temperature.



Results

Wash Wash test strips in the automated washer.





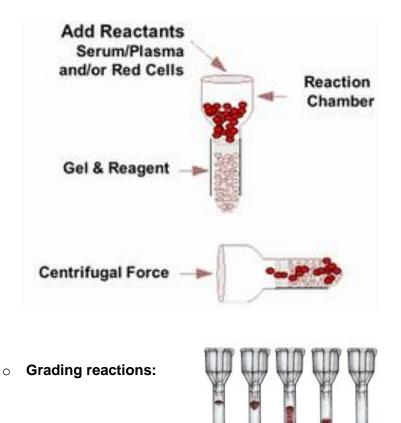
**Add Indicator Cells** Add one drop of indicator cells to each of the test wells.



**Centrifuge and Read Results** Centrifuge test strips. Read reactions.

## **MTS Gel Column Agglutination**

- Cells and plasma incubates at 37C in upper reaction chamber, cells coated with IgG
- Centrifuge
- Bottom column is a gel matrix with a density gradient of anti-IgG. Cells coated with IgG become stuck in the matrix. Negative cells fall to the bottom of the column in a single pellet.



4+ 3+ 2+ 1+

0

## Tube Testing, SPRCA, and Gel: A Comparison

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.						

## Common Red Cell Antigens and Their Corresponding Antibodies:

					Rh							Kell			Du	ıffy	Ki	dd	Lev	wis	Luth	eran		MI	NS		Р	Xg
Ι	)	С	E	С	e	f	C W	V	K	k	Кр а	Kp b	Js a	Js b	Fy a	Fy b	Jk a	Jk b	Le a	Le b	Lu a	Lu b	Μ	N	S	S	Р 1	Xg a

Antigen Group	Clinical Significance of Antibody	High Prevalence/ Low Prevalence Antigens	Special Characteristics
Rh	Significant	Low prevalence: C <sup>W</sup> , V	<ul> <li>Some autoantibodies have Rh specificity</li> <li>Some drug antibodies have Rh specificity</li> <li>Patients with partial antigens may make alloantibodies to the epitopes of the antigen they are missing. This occurs more frequently in blacks.</li> </ul>
Kell	Significant	Low prevalence: K (9% Caucasians), Kp <sup>a</sup> , Js <sup>a</sup> High prevalence: k, Kp <sup>b</sup> , Js <sup>b</sup>	<ul> <li>Antibodies to Kell system antigens may be clinically significant in HDFN at lower titers than other antibodies as Kell antigens are expressed on early RBCs in development</li> </ul>
Duffy	Significant		<ul> <li>Fy antigen serves as receptor for <i>Plasmodium vivax</i></li> <li>68% of individuals of African descent are Fy(a-b-).</li> <li>Individuals of African descent whose cells test Fy(a-b-) invariably have an allele that encodes Fy<sup>b</sup> on cells other than RBCs (the GATA mutation silences antigen production on the RBCs). These individuals do not make anti-Fy<sup>b</sup>.</li> </ul>
Kidd	Significant		<ul> <li>Antibodies to Jk<sup>a</sup> and Jk<sup>b</sup> are known to decrease in titer over time.</li> </ul>
Lewis	Anti-Le <sup>a</sup> – rare cause of hemolytic reactions Anti-Le <sup>b</sup> – insignificant		<ul> <li>Lewis antigens are found in the secretions and are absorbed onto red cells from the plasma. Transfused cells eventually acquire the recipient's Lewis typing.</li> <li>Lewis antibodies may be found naturally occurring, especially during pregnancy.</li> </ul>
Lutheran	Anti-Lu <sup>a</sup> – insignificant Anti-Lu <sup>b</sup> - significant	Low prevalence: Lu <sup>a</sup> High prevalence: Lu <sup>b</sup>	
MNS	Anti-M – often insignificant, may be significant Anti-N – insignificant Anti-S, anti-s – significant		• Anti-M is sometimes naturally occurring, sometimes occurring in children. It often is cold reacting (22C). Reactivity of anti-M that persists at IAT using prewarmed technique indicates clinical significance.
Ρ	Anti-P1 – insignificant		• The P1 antigen is expressed in varying strengths on red cells
Xg	Anti-Xg <sup>a</sup> - Insignificant		<ul> <li>Anti-Xg<sup>a</sup> is an uncommon antibody.</li> <li>The Xg<sup>a</sup> antigen is more prevalent on female cells as it is encoded by a locus on the X chromosome.</li> </ul>

**Antigen Typing ("Full phenotype")** 

Why would you antigen type donors?

Why would you antigen type patients?

What antigens do we type for?

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What cells are used for the positive control when antigen typing? Why?

What is the difference between a phenotype and a genotype?

Name 2 instances when molecular testing is advantageous over serologically phenotyping a patient.

- 1.
- 2.

What patient populations may benefit from receiving antigen-matched blood? (name at least 2)

- 1.
- 2.

# The following (shaded antigens) are considered "common" RBC antigens for which one may develop clinically significant antibodies:

Γ					Rh							Kell			Du	ffy	Ki	dd	Lev	wis	Luthe	eran		M	NS		Р	Xg
	D	С	E	С	e	f	C W	V	K	k	Кр а	Kp b	Js a	Js b	Fy a	Fy b	Jk a	Jk b	Le a	Le b	Lu a	Lu b	М	N	S	S	Р 1	Xg a

The following (shaded antibodies) are known to be "cold" reacting (react at room temperature/<37C):

				Rh							Kell			Du	ffy	Kie	dd	Lev	wis	Luthe	eran		MI	٧S		Р	Xg
D	С	E	С	e	f	C W	V	К	k	Кр а	Kp b	Js a	Js b	Fy a	Fy b	Jk a	Jk b	Le a	Le b	Lu a	Lu b	М	N	S	S	Р 1	Xg a

Т	h	e	fo	ollo	w	in	g (s	ha	de	ed	ant	ige	ns)	are	of	nigh	pre	eval	enc	e:								
					Rh							Kell			Du	lffy	Ki	dd	Le	wis	Luth	eran		MI	NS		Р	Xg
Ι	D	С	E	с	e	f	C W	V	K	k	Кр а	Kp b	Js a	Js b	Fy a	Fy b	Jk a	Jk b	Le a	Le b	Lu a	Lu b	М	N	S	S	Р 1	Xg a

The following (shaded antigens) are of low prevalence (you will probably not rule them out on your panel):

	Rh							Kell			Du	ffy	Ki	dd	Le	wis	Luth	eran		MN	IS		Р	Xg
D C E	c e	f	C W	V	К	k	Кр а	Kp b	Js a	Js b	Fy a	Fy b	Jk a	Jk b	Le a	Le b	Lu a	Lu b	М	N	S	S	Р 1	Xg a

## **Antigen Typing Procedures**

Anti-E, anti-c, anti-C, anti-e, anti-K, anti-S

- 1. Add one drop of the antisera to a labeled test tube.
- 2. Add one drop of patient red cell suspension.
- 3. Mix and incubate 5 minutes at room temperature.
- 4. Centrifuge, read and record results.

Anti-Jk<sup>a</sup>, anti-Jk<sup>b</sup>

- 1. Add one drop of antisera to a labeled test tube.
- 2. Add one drop of patient red cell suspension.
- 3. Mix and incubate for 15 minutes at room temperature.
- 4. Centrifuge for 60 seconds. Read and record results.

#### Anti-Fy<sup>a</sup>, anti-Fy<sup>b</sup>

- 1. Add one drop of antisera to a labeled test tube.
- 2. Add one drop of patient red cell suspension.
- 3. Mix and incubate for 15 minutes at 37C.
- 4. Wash 4 times.
- 5. Add 2 drops of anti-IgG.
- 6. Centrifuge, read and record results.
- 7. Add one drop of check cells to negative tests. Make a check mark next to IAT result if positive.
- 8. If negative, repeat testing.

#### Anti-s

- 1. Add one drop of antisera to a labeled test tube.
- 2. Add one drop of patient red cell suspension.
- 3. Mix and incubate for 30 minutes at 37C.
- 4. Wash 4 times.
- 5. Add 2 drops of anti-IgG.
- 6. Centrifuge, read and record results.
- 7. Add one drop of check cells to negative tests. Make a check mark next to IAT result if positive.
- 8. If negative, repeat testing.

## Antigen Typing Results

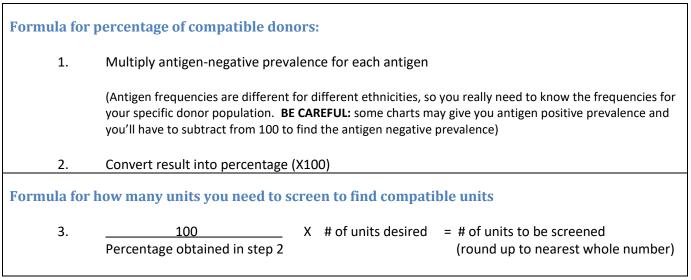
Record your results below.

		Front	Туре		Back	Туре	Interpretation
Sample ID	Anti-A	Anti-B	Anti-D	Rh Control	A1 cells	B cells	

		Rh S	ystem		Kell	Du	uffy	Ki	dd	M	ISs
	Anti-E	Anti-c	Anti-C	Anti-e	Anti-K	Anti-Fy <sup>a</sup>	Anti-Fy <sup>b</sup>	Anti-Jk <sup>a</sup>	Anti-Jk <sup>b</sup>	Anti-S	Anti-s
Positive											
Control cell #											
Result											
Negative Control cell #											
Result											
Sample ID:											

My phenotype is:

## How difficult will it be to obtain blood for your patient?



Example: Patient has anti-E and anti-Fy<sup>a</sup>

**Percentage of compatible donors:** 

- 1. 0.70 X 0.33 = 0.231
- 2. 0.231 X 100 = **23% of donor population will be compatible.**

How many units should you screen to find 2 E-, Fy(a-) units?

### 1. 100/23 = 4.34 X 2 = 8.68 You should screen 9 units

Antigen	% of donor population negative for antigen
D	15
E	70
С	30
е	3
С	20
K	90
Fy <sup>a</sup>	33
Fy <sup>b</sup>	20
Jk <sup>a</sup>	25
Jk <sup>b</sup>	25
S	45
S	11

Given the same donor population, what percent of donor units will be compatible with patients with the following antibodies:

- 1. Anti-E, anti-c, anti-K, anti-Fy<sup>a</sup>
- 2. Anti-Fy<sup>a</sup> and anti-Jk<sup>a</sup>
- 3. Anti-e and anti-Jk<sup>a</sup>

# How many units would you have to screen to find the appropriate number of units for each patient:

- 1. 1 unit for a patient with anti-E and anti-K
- 2. 4 units for a patient with anti-C and anti-Jk<sup>a</sup>
- 3. 2 units for a patient with anti-e and anti-J $k^b$

Direct Antiglobulin Test (I Antigen	DAT)	
	Anti-IgG AHG reagent added after erythrocytes are washed	AHG reagent causes IgG-coated erythrocytes to agglutinate
The 3 reagents that are used v 1. 2. 3. The 4 <sup>th</sup> tube is tested in (negative/pe	and serves as a control.	It should always be
5 reasons a patient may have 1.	a positive DAT:	
2.		
3.		
4.		
5.		

Observe and perform DAT procedure.

## **DAT Procedure**

- 1. Label 4 test tubes: polyspecific reagent, anti-IgG, anti-C3b,C3d, saline control.
- 2. Add 1 drop of patient cell suspension to each tube.
- 3. Wash 4 times.
- 4. Add 2 drops of the appropriate reagent (or saline) to each tube.
- 5. Mix, centrifuge, read and record results. Observe negative results microscopically.
- 6. Add one drop of check cells to negative tests. Use IgG-coated check cells for the polyspecific and anti-IgG tubes. Use complement-coated check cells for the anti-C3b,C3d tube. Centrifuge. Read. Make a check mark next to the result if positive. If negative, repeat testing.

## **DAT Results**

#### Record your results below.

Sample ID	Polyspecific	Anti-IgG	Anti-C3b,-C3d	Saline control

Eluates

What is an eluate?

When is an eluate prepared?

How is an eluate prepared?

Explain when the following might occur:

Reactivity of the Eluate	Explanation
Eluate contains alloantibody(ies)	•
	•
Eluate reacts with all cells tested, including patient's autologous cells	•
Eluate is completely nonreactive	•
	•
	•

Why is it necessary to test the last wash? What should the result of this testing be?

What is the purpose of EGA treating the patient's cells before testing with the eluate?

Prepare and test an eluate on a sample with a positive DAT.

Review "dry" panels of eluate results. Write explanation/interpretation for each patient (green sheets).

## **Eluate Procedure (using acid eluate kit)**

- 1. Wash patient cells one time with saline in a labeled, plastic 16X100 tube. 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.

Ortho-Clinical	) Clin Johnson I Diagnosace	-Jol	••••• 2010	<b>* Fai</b>	WILY	0 <b>7</b> C(		9128					PA1 DA1	<b>1EN</b> TE:	T ID	NE LAN		<u>P</u> e	in de la composition br>Francé de la composition de la compositio	1997) 1986 -	с. Трум	1.61451	- E	<u> </u>	<u>10</u>	<u>}</u>			P	an	el Reagent Re Resolve® P Antigram®	ed Blood ( anel A	0062
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Bh-hr	Donor	D	c	E	c	e	f*	Cw	V <sup>†</sup>	ĸ	k	Kp <sup>a</sup>		.lsat*	Js <sup>b†</sup>	Fya		Jk <sup>a</sup>	Jkb	Xg				S			L F	建設生成	un: Lua	(900)	Antigen Typing	Cell#	Test Results
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R2R2	308217	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	•	0		+	0	+		3 24	34
Ror	306104	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+	0	4	0	0	0	+	0	++		+	Q	+		4 22-	34
r'r	311903	0	+	0	+	+	+	0	0	0	+	0	+	7	+	0	0	+	0	() () ()	0	0	0	+	+	+	+	5	0	+	@	5 2+	3+
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rr	310235	0	0	0	+	+	+	0	0	+	+	0	+	1	+	0,	+	+	+	1+	+	0	0	+	+	1+		3	0	+	0	7 2+	3+
rr	312943	0	0	0	+	+	+	0	0	0	+	0	+	7	+	+	<u>+</u> :	0	+	100 +	0	+		0	+	0		ł	0	+	@	8 2+	34
fr	84368	0	0	0	+	+	+	0	0	.0	+	0	+	0	÷	+,	0	+	0		0	0	0	+	0	•		+	0	+		9 24	31
rr	311014	0	0	0	+	+	• +	0	0	0	+	0	+	1	+	+.;	0	+	+	+	0	+	+	0	+	0		ŧ	0	+		10 21	31
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Ror	306104	+	0	0	+	+	+	O	+	0	+	0	+	0	+	0	0	+	0	2. 1.	0	0	0	+	0	+	+	0	+		4 25		04
r'r	311903	0	+	0	+	+	+	0	0	0	+	0	÷	1	+	0	0	+	0	1. <b>+</b>	0	0	0	+	+		+s . }	0	+	e	5 2×		0 <sup>i</sup>
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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.

<sup>†</sup> 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.

A	ddition	al Cells				Rh-	hΓ						K	119			DU	eav.	KI	D	Sex Linked	ιe/	VIS		MN	4S		P	LUT	HERAN	Special Antigen Typing		Tes	t Res	ults
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rr	84368	0	0	0	+	+	+	0	0	0	+	0	+	0	+	· +.	0	+	0		0	0	0	+	0	+	+	0	+		9	ov		04
rr	311014	0	0	0	+	+	• +	0	0	0	+	0	+	1	+	(33) (***	0	+	+		0	+		0	+	0	+	0	+		10	04		01
R1R1	23287	+	+	0	0	+	0	0	0	+	+	0	+	0	+	0	+	0	+	0	0	+		+	0	+	+	+	+		11	OV		2+
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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.

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<sup>†</sup> 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.

Additior	nal Cells				Rh-	hr					c		KEL				ŀŀ	UFF	Y (	KID	D	Sex Linked	1	EWIS			MN	3		Р	LU	THERAN	Special Antigen Typing		Te	est F	lesu	lts
Rh-hr	Donor Number	D	с	E	с	ė	f	Cw	v'	к	k	άĸ	p <sup>a</sup> ł	٢þ٥	Js <sup>a†</sup>	* Jst	p' E	/ <sup>a</sup> F	yb J	ik <sup>a</sup>	Jkb	Xg <sup>a</sup>	Le	a Le <sup>t</sup>	S	S	N	M	N	P <sub>1</sub>	Ĺ	u <sup>a</sup> Lu <sup>b</sup>		Cell#				
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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.

<sup>†</sup> 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.

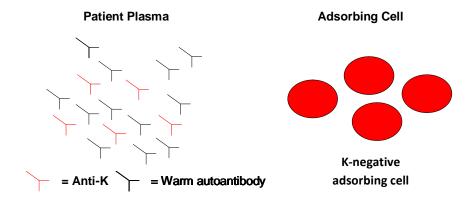
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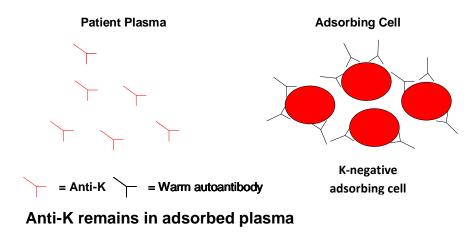
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## **Adsorptions**

## **Before Adsorption**



## **After Adsorption**



List 2 criteria for performing autoadsorptions

- 1.
- 2.

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

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

What is the biggest risk when doing <u>allo</u>adsorptions? Which adsorbing cell would you use for performing alloadsorptions for patients with the following phenotypes? (Adsorbing cells are ficin-treated)

## Adsorbing cells available:

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<sub>1</sub>r, K+, Fy(a+b-), Jk(a+b-), S+s-

- 2. rr, K-, Fy(a+b-), Jk(a-b+), S-s+
- 3. R<sub>2</sub>R<sub>2</sub>, K-, Fy(a-b+), Jk(a+b+), S-s+
- 4. rr, K-, Fy(a-b+), Jk(a-b+), S+s+

If you don't know the patient's phenotype, how do you perform alloadsorptions?

Ficin-treated adsorbing cell	What antigens are present on this adsorbing cell? (antibodies to these antigens will be removed from adsorbed plasma)	What antigens are not present on this adsorbing cell? (antibodies to these antigens will remain in adsorbed plasma)
R <sub>1</sub> R <sub>1</sub> , K-, Fy(a-b+), Jk(a-b+), S+s+		
R <sub>2</sub> R <sub>2</sub> , K-, Fy(a+b+), Jk(a+b-), S-s+		
rr, K- Fy(a+b-), Jk(a-b+), S+s-		

Enzyme-treat (ficin or papain) a 3-cell set of adsorbing cells. Perform adsorption(s) on a patient's sample that contains warm reactive autoantibody. Test the three sets of adsorbed plasma.

Review the "dry" panes provided (green sheets) and give your best interpretations of the testing results.

## **Alloadsorption Procedure**

Allogeneic adsorption is used to remove autoantibody from plasma so that it can be tested for underlying alloantibodies. Alloadsorptions are used when the patient has been recently transfused, when the transfusion history is unknown, or when an inadequate volume of autologous cells is available. The number of adsorptions needed to remove the autoantibody is one greater than the antibody strength (i.e. an autoantibody reacting 2+ is adsorbed 3 times.)

Ficin-treating adsorbing cells (may also use papain)

- 1. Transfer the R<sub>1</sub>R<sub>1</sub>, R<sub>2</sub>R<sub>2</sub>, and rr cells to labeled large plastic test tubes (16 X 100).
- 2. Wash the cells with saline until no hemolysis persists.
- 3. Add 2 ml of ficin to each tube.
- 4. Incubate at 37C for 15 minutes.
- 5. Wash cells twice with saline.
- 6. Aliquot 2 ml of the ficin-treated cells into labeled 12X75 tubes, 1 set for each adsorption.
- 7. Wash once more and centrifuge for 3 minutes to pack the cells.
- 8. Remove supernatant.

#### Alloadsorption

- 1. To the first set of  $R_1R_1$ ,  $R_2R_2$ , and rr cells, add 1 ml of patient plasma.
- 2. Mix and incubate at 37C for 10 minutes.
- 3. Centrifuge the tubes for 3 minutes.
- 4. Carefully remove the plasma and add to the next set of respective tubes of adsorbing cells.
- 5. Repeat steps 2-4 as needed.
- 6. After final adsorption, remove the serum/plasma and place it into labeled tubes.
- 7. Test each alloadsorbed aliquot against screening cells by LISS IAT.

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