



Resident Rotation: *Immunohematology Reference Laboratory*

Handbook for Online Modules



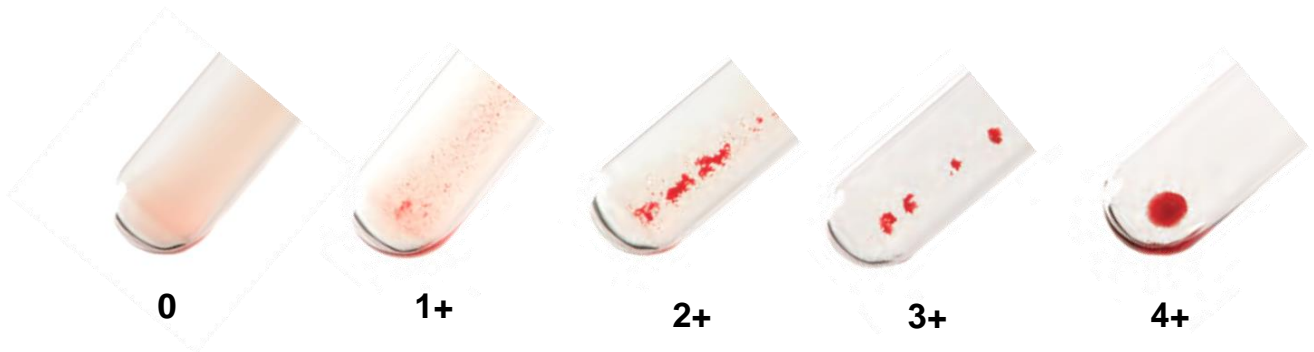
Immunohematology Reference Laboratory

Objectives:

1. Perform routine blood bank tests including type and screen, antibody identification, antigen typing of red cells, DAT, eluate, and alloadsorptions.
<ul style="list-style-type: none">a) Describe the purpose and methodology of each test performed.b) Demonstrate proper grading of reactions.c) Follow procedures exactly and interpret results correctly.d) Perform rule-outs appropriately using panel results, and correctly identify alloantibodies in laboratory samples.e) Compare and contrast the three testing platforms for blood bank testing: tube testing, MTS gel testing, and solid phase red cell adherence assay (SPRCA).f) List 5 possible explanations for a positive DAT and explain how laboratory testing can aid in determining the cause of a positive DAT.g) Define shorthand nomenclature for the common Rh antigens (e.g. R_1R_1, R_2R_2, rr) and discuss its use in alloadsorptions.
2. List common red cell antigen groups and describe characteristics of alloantibodies directed against common red cell antigens.
<ul style="list-style-type: none">a) Distinguish between clinically significant and clinically insignificant alloantibodies.
3. Recognize ABO discrepancies, and describe possible explanations and common resolutions.
<ul style="list-style-type: none">a) 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.b) 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 blood transfusion.
<ul style="list-style-type: none">a) Discuss differences in approaches to ABO/Rh determination in patients and donors.b) Explain how antigen-typing is used to work up a patient sample with warm reactive autoantibody.c) Perform calculations to determine how difficult it will be to find antigen-negative units.d) Name 2 specific patient populations that benefit from transfusing antigen-matched blood.e) Discuss the difference between the phenotype and the genotype.
5. Determine special requirements for selecting donor red blood cells, platelets, and plasma for transfusion to patients.
<ul style="list-style-type: none">a) Choose donor red cells and plasma of the appropriate ABO type for transfusion.b) Discuss how institutional policies may vary for transfusing non ABO-identical platelet products.c) List the requirements for transfusion of patients with clinically significant alloantibodies.d) Discuss how institutional policies may vary for selection of red blood cells to transfuse to patients with warm autoantibodies.e) 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.
<ul style="list-style-type: none">a) Discuss the ordering of routine antigen negative units.b) Describe the implications for ordering rare frozen units from Community Blood Center.c) 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:

	Front Type				Back Type		Interpretation
	Anti-A	Anti-B	Anti-D	Rh Control	A ₁ 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: A₁, 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 Type				Back Type		Interpretation
Sample ID	Anti-A	Anti-B	Anti-D	Rh Control	A ₁ 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

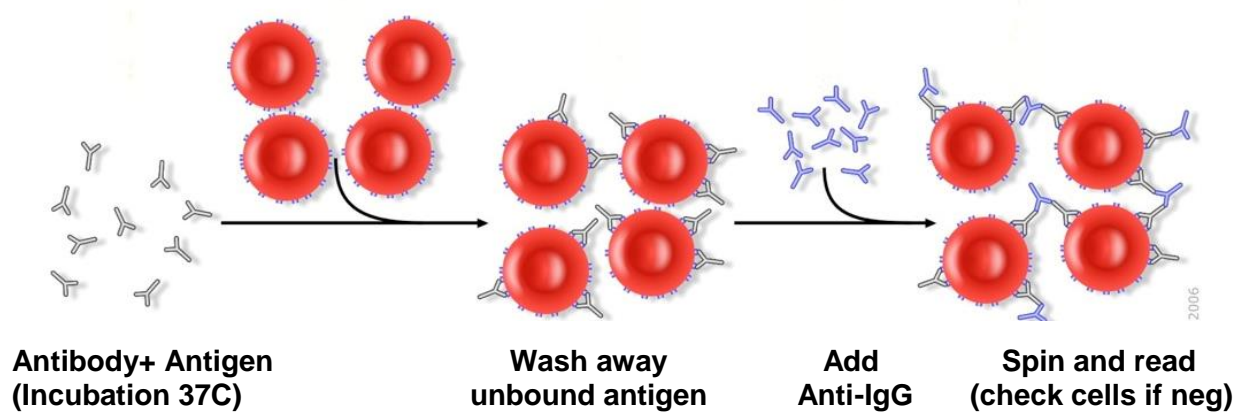
Front Type: Testing patient cells						Back Type: Testing patient plasma						
	Anti-A	Anti-B	Anti-A,B	Anti-D	Rh Cont.	A1 cells	A2 cells	B cells	O cells	Auto Cont.	Possible Explanation	Ways to Resolve
1	4+	0	4+	4+	0	1+	0	4+	0	0	•	•
2	0	4+	4+	4+	0	4+	3+	1+w	1+w	1+w	• •	• •
3	0	0	0	4+	0	0	0	3+	0	0	• •	• •
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	•	• •

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		
	I		
	II		
	III		

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

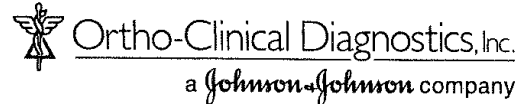
1. Label sufficient tubes for each panel cell and an auto control (usually 1-11 and AC).
2. Add 2 drops of plasma to each tube.
3. Add one drop of panel cells or autologous cells to appropriate tubes.
4. Incubate the tubes based on the enhancement procedure being used.

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.



©OCD 1989 Baritan, NJ 08869

Cell 6 of this lot is designated with an A.

E: Patient #1

PATIENT ID:

DATE: _____ TECH: _____

CONCLUSION:

Lot No. RC345

Exp. Date 2008-09-23

CCYY-MM-DD

Panel
C

REAGENT RED BLOOD CELLS
ORTHO FICIN PANEL SYSTEM
Resolve® Panel C **Ficin Treated**
Resolve® Panel C Untreated
Antigram® Antigen Profile

535200343

* 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.

[illegible]

©OCD 1989 Raritan, NJ 08869

Cell 6 of this lot is designated with an A.

PATIENT NAME:

PATIENT ID:

DATE:

TECH-

CONCLUSION:

Patient #2

Lot No. RC345

Exp. Date 2008-09-23

CCYY-MM-DD

Panel

C

REAGENT RED BLOOD CELLS
ORTHO FICIN PANEL SYSTEM
Resolve® Panel C **Ficin Treated**
Resolve® Panel C Untreated
Antigram® Antigen Profile

535200343

Cell#	Rh-ir	Donor Number	Rh-ir								KELL					DUFFY		KIDD	Sex Linked	LEWIS		MNS				P	LUTHERAN		Special Antigen Typing	Cell#	Rhesus	Test Results					
			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			
1	R1wR1	101526	+	+	+	0	0	+	0	+	0	+	0	+	+	0	0	+	+	0	+	+	+	+	0	+	0	+		1	3+						
2	R1R1	302788	+	+	0	0	+	0	0	+	+	0	+	0	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+		2	3+				
3	R2R2	304215	+	0	+	+	0	0	0	0	+	0	+	0	+	+	0	+	+	+	+	0	0	+	+	+	+	+	0	+		3	0v				
4	Ror	104635	+	0	0	+	+	+	0	+	0	+	0	+	0	0	+	0	0	0	0	0	0	+	+	+	+	+	0	+		4	0v				
5	r'r	118642	0	+	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	+	0	0	+	+	+	+	+	+	0	+	⊗	5	2+			
6	r'r	304019	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	+	+	0	+	+	+	+	+	0	+	⊗	6	0v				
7	rr	304206	0	0	0	+	+	+	0	0	+	+	0	+	0	+	+	0	+	+	+	0	+	0	+	+	+	+	0	+	⊗	7	0v				
8	rr	304217	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	0	+	0	0	0	+	+	⊗	8	0v				
9	rr	118403	0	0	0	+	+	+	0	0	0	+	0	+	0	0	+	0	+	+	0	+	0	+	0	+	+	+	0	+		9	0v				
10	rr	304173	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	0	0	0	+	+	+	+	+	+	0	+		10	0v			
11	R1R1	111483	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	0	+	+	0	0	+		11	3+					
	Patient Cells																																				
Mode of Reactivity			37°C/Antiglobulin								Antiglobulin								Variable				Cold		Var.												

* 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.

[illegible]

Medion Diagnostics GmbH
Data-Cyte® Plus
Reagent Red Blood Cells 3 ± 1%
LOT DC-1001
2005-06-11
Name/I.D. Nomme Nombre Patient #3
Date of birth Geb.-Datum Date de naissance Data di nascita Fecha de nacimiento
Drawn Entriahme Prélèvement Prelievo Estraido
Result Ergebnis Resultat Risultato Risultado
Signature Unterschrift Firma
Date Datum Data Fecha
Table with columns: No. Nr. N°, Rh., Donor Spender Donneur Donatore Donante, Other Andere Autres Altra Otros, Ag, Rhesus (D, C, E, c, e, f, Cw, V), MNS (M, N, S, s), P (P1), Lewis (Lea, Leb), Lutheran (Lua, Lub), Kell (K, k, Kpa, Jsa), Duffy (Fya, Fyb), Kidd (Jka, Jkb), Sex linked (Xga), No. Nr. N°, 5' RT, REG ID.

*Screening Cells / Suchzellen / Cellules de dépistage / Pannello screening / Células de Screening
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 / forte / fuerte
vw = very weak / sehr schwach / très faible / molto debole / muy débil
w = weak / schwach / faible / debole / débil
NT = not typed / nicht typisiert / non typé / non tipizzato / no tipificado
**See reverse side / Siehe Rückseite / Voir au verso / Vedere sul retro / Ver parte posterior

Reagent Red Blood Cells $3 \pm 1\%$

2008-11-22

For US customers only:

Exp. Date **22 NOV 08**

Name/I.D.
Nom
Nome
Nombre
Navn
Név
Ime/ID
Nome
Navn

Result
Ergebnis
Résultat
Risultato
Resultado
Resultat
Eredmény
Rezultat
Resultado
Resultat

Signature
Unterschrift
Firma
Underskrift
Aláírás
Potpis
Assinatura
Underskrift

Date of birth
Geb.-Datum
Date de naissance
Data di nascita
Fecha de nacimiento
Fødselsdato
Születési dátum
Datum rođenja
Data de nascimento
Fødselsdato

Drawn
 Entnahme
 Prélèvement
 Prelievo
 Estraido
 Prøve taget
 Mintavétel
 Uzimanje uzorka
 Colhido
 Prøve tatt

Date
Datum
Data
Fecha
Dato
Dátum
Datum
Data
Dato

IVD

CE 0123



②

*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 / Normalerweise kältereaktive Antikörper / En principe anticòrps froids / Solitamente anticòrps freddi / Usualmente anticòrpo reactivo en frío / Normalt kulderreaktive antistoffer / Általában hideg antitestek / Uobičajeno hladno reaktivno antitijelo / Geralmente anticòrpos frios / Normalt kulderreaktive antistoffer

☐ Usually warm or Coombs reactive Ab / Normalerweise wärme- oder Coombs-reaktive Ak / En principe Az chauds ou réagissant par Coombs / Solitamente anticòrpi caldi o reattivi in Coombs / Usualmente reactivo en prueba de Coombs o en caliente / Normalt varme- eller Coombs-reaktive as / Általában meleg, illetve Coombs-reaktív antitestek / Uobičajeno toplo ili Coombsovo reaktivno antitijelo / Geralmente anticòrpos reativos ao teste de Coombs ou anticòrpos quentes / Normalt varme- eller Coombs-reaktive as



IMMUCOR, INC. Norcross, GA 30071 USA

US LICENSE NO: 886

LOT NO: 28825

EXPIRES: 2008-09-19



PANOCELL - 16 MASTER LIST

NAME Patient #5
NO. _____
INSTITUTION _____
BLOOD GROUP _____
ANTIBODY IDENTITY _____

TECH _____ DATE _____

CELL	Special Type	Donor	Rh - Hr								Kell								Duffy		Kidd		Lewis		P	MN				Lutheran		X _g	PATIENT'S TEST RESULTS				
			D	C	c	E	e	f	V	C*	K	k	K ₁	K ₂	J _s ^a	J _s ^b	F _y ^a	F _y ^b	J _k ^a	J _k ^b	L _e ^a	L _e ^b	P ₁	M	N	S	s	L _u ^a	L _u ^b	X _g ^a	CELL	TEST	1	2	3	4	
1		RzR1 A3492	+	+	0	+	+	0	0	0	0	+	0	+	0	+	+	+	+	0	+	+	0	+	0	+	0	+	+	+	+	1	3+				
2	Sc2	R1wR1 B3586	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	+	+	+	0	0	+	0	+	+	+	2	3+					
3		R2R2 C3142	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	+	0	+	+	0	+	+	+	+	+	0	+	+	3	3+					
4		Ror D881	+	0	+	0	+	+	0	0	0	+	0	+	0	0	+	+	0	+	+	0	+	0	0	0	+	0	+	+	4	2+					
[5]		r'r E709	0	+	+	0	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	5	3+					
[6]		r''r F461	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	0	+	+	+	0	+	0	+	6	3+					
[7]		rr G1216	0	0	+	0	+	+	0	0	+	+	0	+	0	+	0	+	0	+	+	+	+	+	+	+	0	+	+	+	7	0+					
8		rr H1202	0	0	+	0	+	+	0	0	0	+	0	+	0	+	+	0	+	+	0	+	0	+	+	+	0	+	+	+	8	0+					
9	I, Bg(a+)	R1R2 A298	+	+	+	+	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	0	0	+	+	0	+	0	+	+	9	/					
10	Mg+, Lu:-8,14, Bg(a+)	R1R1 B1121	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	0	+	+	+	+	0	+	+	0	+	0	+	10	/				
11	D(a+), Yt(b+)	R1R1 B5161	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	0	+	0	0	0	+	+	0	+	+	0	+	+	11	/					
12	Co(b+), Yt(b+), Bg(a+)	rr N411	0	0	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	+	12	0+					
13		rr N1856	0	0	+	0	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	13	0+					
14		rr G242	0	0	+	0	+	+	0	0	+	0	0	+	0	+	+	+	0	+	0	+	+	+	+	0	0	+	+	+	14	0+					
15		rr V160	0	0	+	0	+	+	+	0	0	+	0	+	0	0	0	+	+	0	0	+	w	+	0	0	+	0	+	+	15	0+					
16		rr N1463	0	0	+	0	+	+	0	0	0	+	0	+	+	0	0	+	0	0	+	+	+	+	0	+	+	0	+	0	+	16	0+				
PC		PATIENT'S CELLS																													PC	0+					

DIRECT ANTIGLOBULIN TEST

POLY

IgG

C3

LOT

RESULT

ELUATE RESULT

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

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.

In those instances where a patient's serum is known to contain anti-D, it may be desirable to perform antibody screening tests with D-red cells. The panel cells whose vial numbers are set off by brackets [] can be used together to form a three- or four-vial D-negative antibody screening reagent. All bracketed cells must be used to construct a complete screening reagent.

PATIENT'S SERUM

REVERSE GROUPING SCREEN CELLS

PANO SCREEN LOT

I

II

III

A1

A2

B

14



IMMUCOR, INC. Norcross, GA 30071
USA
U.S. License No. 886

4B



PANOCELL-20 MASTER LIST

NAME: Patient #6 part 2 I.D. : _____
GROUP _____ Rh _____ TEST DATE _____ BY _____
HISTORY _____
INTERPRETATION _____

SYSTEM		Rh - Hr								Kell				Duffy		Kidd		Lewis		P	MN				Lutheran	Xg	Special Antigen Type	PATIENT'S TEST RESULTS								
Donor		D	C	c	E	e	F ^a	V	C ⁺	K	k	K ^a	K ^b	J ^a	J ^b	F ^a	F ^b	J ^a	J ^b	L ^a	L ^b	P ₁	M	N	S	s		L ₁	L ₂	X ₁	X ₂	1	2	3	4	
1	R1wR1 B6619	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	0	+	0	+	+	+	+	+	+	+	1	
2	R1R1 B2461	+	+	0	0	+	0	0	0	+	+	0	+	0	+	0	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	2	
3	R1R1 B2670	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	3	
4	R1R1 B6529	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	+	+	4	
5	RzR1 A3493	+	+	0	+	+	0	0	0	0	+	0	+	0	+	+	+	+	0	0	+	0	0	0	0	0	+	0	+	0	+	0	+	+	5	
6	RzR2 A2906	+	+	+	+	0	0	0	0	+	+	0	+	0	+	+	+	+	0	+	+	+	+	+	0	+	0	+	0	+	0	+	0	+	6	
7	R2R2 C1689	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	+	0	0	+	+	+	0	+	0	+	0	+	7	
8	R2R2 C2804	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	+	0	+	+	0	+	+	0	+	0	+	0	+	0	+	0	+	+	8	
9	R2R2 C2693	+	0	+	+	0	0	0	0	+	+	0	+	0	+	+	+	+	0	0	0	0	0	0	0	+	0	+	0	+	0	+	0	+	9	
10	R1R1 B3559	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	0	+	+	0	+	+	0	+	0	+	0	+	0	+	0	+	+	10	
11	r'r E235	0	+	+	0	+	+	0	0	0	+	0	+	0	+	+	+	+	0	0	+	+	+	+	0	+	0	+	0	+	0	+	0	+	11	
12	r'r F693	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	0	+	+	+	+	0	+	0	+	0	+	0	+	12	
13	rr G660	0	0	+	0	+	+	0	0	+	+	0	+	0	+	+	+	+	0	+	0	+	+	0	0	+	0	+	0	+	0	+	0	+	13	
14	r'r E627	0	+	+	0	+	+	0	0	0	+	0	+	0	+	0	0	+	+	+	0	+	0	+	+	+	0	+	0	+	0	+	0	+	14	
15	rr N2560	0	0	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	0	+	+	0	+	0	+	0	+	0	+	0	+	15	
16	rr N2553	0	0	+	0	+	+	0	0	0	+	0	+	0	+	0	0	+	+	+	0	+	+	0	0	+	0	+	0	+	0	+	0	+	16	
17	rr N1971	0	0	+	0	+	+	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	17	
18	rr H1157	0	0	+	0	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	18
19	rr V191	0	0	+	0	+	+	+	0	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	19
20	Ror D691	+	0	+	0	+	+	+	0	0	+	0	+	0	+	+	+	0	+	+	0	+	+	0	+	0	+	0	+	0	+	0	+	+	20	
PATIENT'S CELLS																																				
		PATIENT'S TEST RESULTS																																		
		REVERSE GROUPING CELLS																																		
		PANOSCREEN LOT:																																		

In those instances where a patient's serum is known 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.

Medion Diagnostics AG

Data-Cyte® Plus

Reagent Red Blood Cells $3 \pm 1\%$

IVD

CE 0123



LOT DC-1044



2008-09-27

For US customers only:

Exp. Date 27 SEP 08

Name/I.D.
Nom
Nome
Nombre
Navn
Név
Ime/ID
NomeResult
Ergebnis
Risultat
Risultato
Resultado
Eredmény
Rezultat
ResultadoSignature
Unterschrift
Firma
Underskrift
Aláírás
Potpis
AssinaturaDate of birth
Geb.-Datum
Date de naissance
Data di nascita
Fecha de nacimiento
Fødselsdato
Születési dátum
Datum rođenja
Data de nascimentoDrawn
Entnahme
Prélèvement
Prelievo
Estrado
Prave taget
Mintavétel
Uzimanje uzorka
ColhidoDate
Datum
Data
Fecha
Data
Datum
Datum

Patient #7

No. Nr. Nº Szám Br. Nº	Rh	Donor Spender Donneur Donatore Donante Davatelj Doador	Other Andere Autres Altri Outros	Ag Andet Egyéb Ostalo Outros	Rhesus							MNS				P	Lewis	Lutheran		Kell				Duffy		Kidd		Sex linked	No. Nr. Nº Szám Br. Nº	5'RT	AEGTAT	Faint tracked IAT	
					D	C	E	c	e	f	C ^w	V	M	N	S			s	P ₁	Le ^a	Le ^b	Lu ^a	Lu ^b	K	k	Kp ^a	Js ^a						Fy ^a
SC I*																												SC I*					
SC II*																												SC II*					
SC III*																												SC III*					
1	rr	L318BM			0	0	0	+	+	+	0	0	0	+	+	0	+	0	+	0	+	0	+	0	0	+	0	+	1	0	0v	0v	
2	rr	L498CC			0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	0	0	0	+	0	+	0	2	0	3r	3+
3	r'r	L589BC			0	+	0	+	+	+	0	0	0	+	0	+	+	0	+	0	+	0	+	+	0	+	+	0	3	0	2+	0v	
4	r'r	M2114CG			0	0	+	+	+	+	0	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	+	0	4	0	3+	3r	
5	rr	M2029CG	Co(b+)		0	0	0	+	+	+	0	0	+	+	0	+	+	0	+	+	0	0	+	0	+	+	+	0	5	0	3+	3r	
6	R ₀ r	M1909BC			+	0	0	+	+	+	0	+	+	+	0	+	+	0	0	+	0	+	0	0	+	0	+	+	6	0	0v	0v	
7	R ₁ R ₁	M559CC	Kp ^a		+	+	0	0	+	NT	0	0	+	+	0	+	0	+	0	+	0	+	+	0	+	+	+	+	7	0	1r	0v	
8	R ₁ R ₁	M2206CC			+	+	0	0	+	NT	0	0	+	+	+	+	0	0	+	0	+	+	+	0	0	+	0	+	8	0	3+	3r	
9	R ₁ R ₁ ^w	M1859CR			+	+	0	0	+	NT	+	0	+	+	0	+	+	0	0	+	+	0	0	+	+	0	+	0	9	0	1+	0v	
10	R ₂ R ₂	M2180CR			+	0	+	+	0	NT	0	0	+	0	0	+	+	0	+	0	+	0	0	+	+	0	+	0	10	0	1+	0v	
11	R ₁ r	M1459CC	Wr(a+)		+	+	0	+	+	+	0	0	+	+	+	+	+	0	0	+	0	+	0	0	+	0	+	+	11	0	2+	0v	
Auto																												Auto	0	0v	/		
Other cells / Andere Zellen / Autres cellules / Altre cellule / Outras células / Andere celler / Egyéb sejtek / Ostale stanice / Outras células					D	C	E	c	e	f	C ^w	V	M	N	S	s	P ₁	Le ^a	Le ^b	Lu ^a	Lu ^b	K	k	Kp ^a	Js ^a	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Xg ^a			
12																													12				
13																													13				
14																													14				

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

- ☐ Usually cold reactive antibody / Normalerweise kalterektive Antikörper / En principe anticorps froids / Solitamente anticorpi freddi / Usualmente anticuerpo reactivo en frío / Normalt kulderektive antistoffer / Általában hideg antitestek / Uobičajeno hladno reaktivno antitijelo / Geralmente anticorpo
Usually warm or Coombs reactive Ab / Normalerweise warme- oder Coombs-reaktive Ak / En principe Ac chauds ou réagissant par Coombs / Solitamente anticorpi caldi o reattivi in Coombs /
Usualmente reaktivno prijetelo de Coombs o en caliente / Normalt varme- eller Coombs-reaktive as / Általában meleg, direkt Coombs-reaktív antitestek / Uobičajeno toplo ili Coombsovo reaktivno antitijelo / Geralmente anticorpos reativos ao teste de Coombs ou anticorpos quentes

Medion Diagnostics AG

Data-Cyte® Plus

Reagent Red Blood Cells 3 ± 1%

LOT

DC-1044



2008-09-27

IVD

CE 0123



For US customers only:

Exp. Date 27 SEP 08

Name/I.D.
Nom
Nome
Nombro
Navn
Név
Ime/ID
NomeResult
Ergebnis
Résultat
Resultado
Resultat
Eredmény
Rezultat
ResultadoSignature
Unterschrift
Firma
Underskrift
Aláírás
Potpis
AssinaturaDate of birth
Geb.-Datum
Date de naissance
Data di nascita
Fecha de nacimiento
Fødselsdato
Születési dátum
Datum rođenja
Data de nascimentoDrawn
Entnahme
Prélèvement
Prelievo
Estraiido
Prøve taget
Mintavétel
Uzimanje uzorka
ColhidoDate
Datum
Data
Fecha
Dato
Datum
Datum
Data

Patient # 8

No. Nr. Nº Szám Br. №	Rh	Donor Spende Donneur Donatore Donante Davateļj Doador	Ag Andet Egyéb Ostalo Outros	Rhesus								MNS				P	Lewis		Lutheran		Kell				Duffy		Kidd		Sex linked	No. Nr. Nº Szám Br. №	S'RT	REG-IT		FLUO-TREATED	IT				
				D	C	E	c	e	f	C ^w	V	M	N	S	s		P _i	Le ^a	Le ^b	Lu ^a	Lu ^b	K	k	Kp ^a	Js ^a	Fy ^a	Fy ^b	Jk ^a									Jk ^b	Xg ^a	
SC I*																												SC I*											
SC II*																												SC II*											
SC III*																												SC III*											
1	rr	L318BM			0	0	0	+	+	+	0	0	0	+	0	+	+ ^w	+	0	0	+	0	+	0	+	0	0	+	0	+	1	0	+			2+			
2	rr	L498CC			0	0	0	+	+	+	0	0	+	+	0	+	0	0	+	0	+	+	+	0	0	0	+	0	+	0	2	0	0	✓		0	✓		
3	r'r	L589BC			0	+ ^w	0	+	+	+	0	0	0	+	0	+	+ ^s	0	+	0	+	0	+	0	+	+	0	+	+	0	3	0	0	✓		+			
4	r'r	M2114CG			0	0	+	+	+	+	0	0	+	0	+	0	+ ^w	+	0	0	+	+	+	0	0	+	0	+	+	0	4	0	0	✓		+			
5	rr	M2029CG	Co(b+)		0	0	0	+	+	+	0	0	+	+	0	+	+ ^w	+	0	0	+	+	+	0	0	+	0	+	+	+	5	0	0	✓		+			
6	R ₀ r	M1909BC			+	0	0	+	+	+	0	+	+	+	0	+	+	0	0	0	+	0	+	0	0	0	0	+	+	+	6	0	0	✓		+			
7	R ₁ R ₁	M559CC	Kp ^a		+	+	0	0	+	NT	0	0	+	+	0	+	0	+	0	0	+	0	+	+	0	+	+	+	+	0	7	0	0	✓		+			
8	R ₁ R ₁	M2206CC			+	+	0	0	+	NT	0	0	+	+	+	+	0	0	+	0	+	+	+	0	0	0	+	0	+	0	8	0	0	✓		0	✓		
9	R ₁ R ₁ ^w	M1859CR			+	+	0	0	+	NT	+	0	+	+	0	+	+	0	0	+	+	0	+	0	0	+	+	0	+	0	9	0	0	✓		0	✓		
10	R ₂ R ₂	M2180CR			+	0	+	+	0	NT	0	0	+	0	0	+	+ ^w	0	+	0	+	0	+	0	0	+	+	0	+	0	10	0	0	✓		0	✓		
11	R ₁ r	M1459CC	Wr(a+)		+	+	0	+	+	+	0	0	+	+	+	+	+ ^{vw}	0	0	0	+	0	+	0	0	+	0	+	+	+	11	0	0	✓		+			
Auto																													Auto	0	0	✓		/					
Other cells / Andere Zellen / Autres cellules / Altre cellule / Otras células / Andre celler / Egyéb sejtek / Ostale stanice / Outras células					D	C	E	c	e	f	C ^w	V	M	N	S	s	P _i	Le ^a	Le ^b	Lu ^a	Lu ^b	K	k	Kp ^a	Js ^a	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Xg ^a									
12																														12									
13																															13								
14																																14							

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

Usually cold reactive antibody / Normalerweise kältereaktive Antikörper / En principe anticorps froids / Solitamente anticorpi freddi / Usualmente anticuerpo reactivo en frío / Normalt kuldereaktive antistoffer / Általában hideg antitestek / Uobičajeno hladno reaktivno antitijelo / Geralmente anticorpos frios

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 reactivos en prueba de Coombs o en caliente / Normalt varme- eller Coombs-reaktive as / Általában meleg, illetve Coombs-reaktív antitestek / Uobičajeno toplo ili Coombsovo reaktivno antitijelo / Geralmente anticorpos reativos ao teste de Coombs ou anticorpos quentes

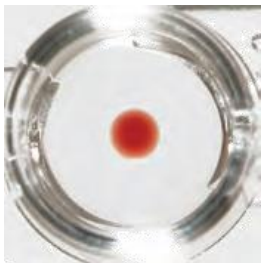
Usually cold reactive antibody / Normalerweise kältereaktive Antikörper / En principe anticorps froids / Solitamente anticorpi freddi / Usualmente anticuerpo reactivo en frío / Normált kuldereaktive antistoffer / Általában hideg antitestek / Uobičajeno hladno reaktivno antitijelo / Geralmente anticorpos frios / Normalt kuldereaktive antistoffer
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 caliente / Normalt varme- eller Coombs-reaktive as / Általában meleg, illetve Coombs-reaktív antitestek / Uobičajeno toplo ili Coombsovo reaktivno antitijelo / Geralmente anticorpos reativos ao teste de Coombs ou Coombs quentes / Normalt varme- eller Coombs-reaktive as

Different Methodologies

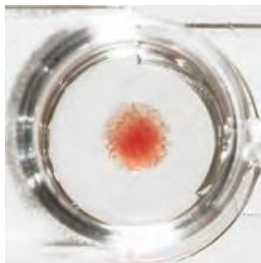
The following pages describe the solid phase red cell adherence assay (SPRCA) and gel column agglutination. Use the information to compare and contrast the different methodologies following the explanations.

Solid Phase Red Cell Adherence Assay (SPRCA)

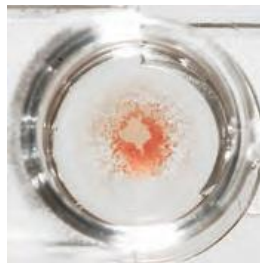
- Red cell antigens coat microwell
- Antibodies present adhere to antigens during 37C incubation
- Unbound antibody washed away
- Indicator cells (cells with attached anti-IgG) added
- Centrifugation
- Grading reactions:



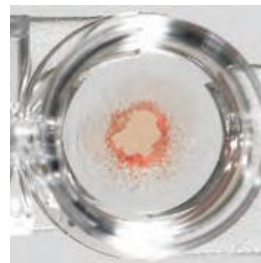
0



1+



2+

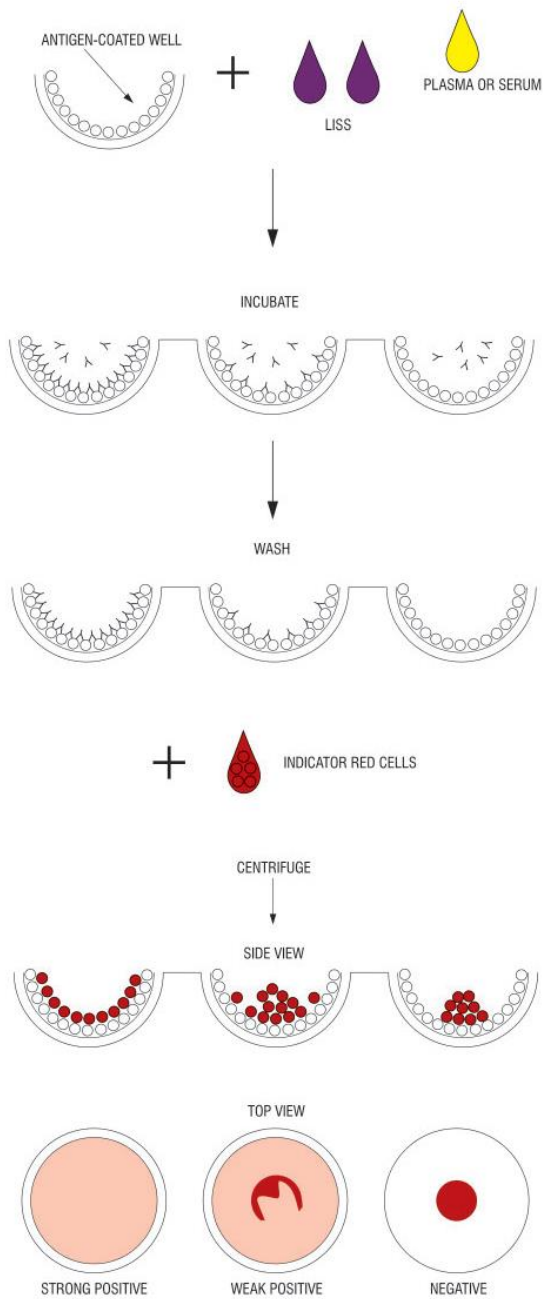


3+

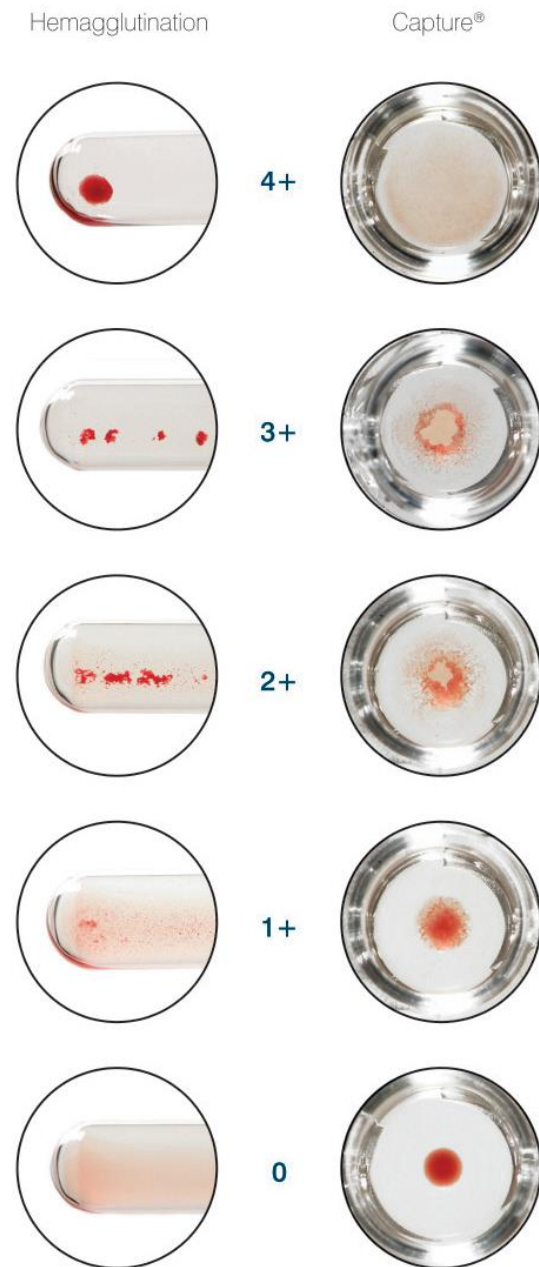


4+

Test Procedure



Grading Chart



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.



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.

Results

Negative Result:

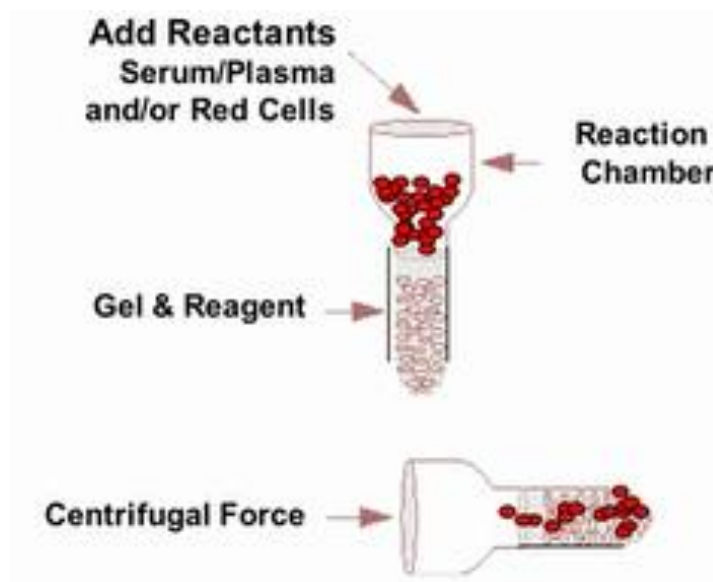
No antibodies bound to wells, indicator cells will migrate to bottom of the well and form a tight button.

Positive Result:

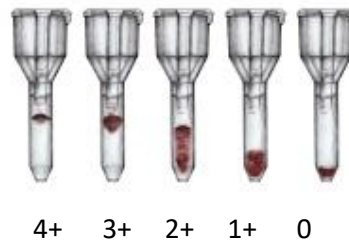
Antiglobulin-coated indicator cells bind to antibody-coated wells forming an intact layer of cells on the well surface.

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.



- Grading reactions:



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						Duffy		Kidd		Lewis		Lutheran		MNS				P	Xg
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	Le a	Le b	Lu a	Lu b	M	N	S	s	P 1	Xg a

Antigen Group	Clinical Significance of Antibody	High Prevalence/ Low Prevalence Antigens	Special Characteristics
Rh	Significant	Low prevalence: C ^w , V	<ul style="list-style-type: none"> Some autoantibodies have Rh specificity Some drug antibodies have Rh specificity Patients with partial antigens may make alloantibodies to the epitopes of the antigen they are missing. This occurs more frequently in blacks.
Kell	Significant	Low prevalence: K (9% Caucasians), Kp ^a , Js ^a High prevalence: k, Kp ^b , Js ^b	<ul style="list-style-type: none"> 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
Duffy	Significant		<ul style="list-style-type: none"> Fy antigen serves as receptor for <i>Plasmodium vivax</i> 68% of individuals of African descent are Fy(a-b-). Individuals of African descent whose cells test Fy(a-b-) invariably have an allele that encodes Fy^b on cells other than RBCs (the GATA mutation silences antigen production on the RBCs). These individuals do not make anti-Fy^b.
Kidd	Significant		<ul style="list-style-type: none"> Antibodies to Jk^a and Jk^b are known to decrease in titer over time.
Lewis	Anti-Le ^a – rare cause of hemolytic reactions Anti-Le ^b – insignificant		<ul style="list-style-type: none"> 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. Lewis antibodies may be found naturally occurring, especially during pregnancy.
Lutheran	Anti-Lu ^a – insignificant Anti-Lu ^b - significant	Low prevalence: Lu ^a High prevalence: Lu ^b	
MNS	Anti-M – often insignificant, may be significant Anti-N – insignificant Anti-S, anti-s – significant		<ul style="list-style-type: none"> 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.
P	Anti-P ₁ – insignificant		<ul style="list-style-type: none"> The P₁ antigen is expressed in varying strengths on red cells
Xg	Anti-Xg ^a - Insignificant		<ul style="list-style-type: none"> Anti-Xg^a is an uncommon antibody. The Xg^a antigen is more prevalent on female cells as it is encoded by a locus on the X chromosome.

Antigen Typing (“Full phenotype”)

Why would you antigen type donors?

Why would you antigen type patients?

What antigens do we type for?

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:

Rh								Kell						Duffy		Kidd		Lewis		Lutheran		MNS				P	Xg
D	C	E	c	e	f	C	V	K	k	Kp a	Kp b	Js a	Js b	Fy a	Fy b	Jk a	Jk b	Le a	Le b	Lu a	Lu b	M	N	S	s	P 1	Xg a

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

Rh								Kell						Duffy		Kidd		Lewis		Lutheran		MNS				P	Xg
D	C	E	c	e	f	C	V	K	k	Kp	Kp	Js	Js	Fy	Fy	Jk	Jk	Le	Le	Lu	Lu	M	N	S	s	P	Xg
						W				a	b	a	b	a	b	a	b	a	b	a	b					1	a

The following (shaded antigens) are of high prevalence:

Rh								Kell						Duffy		Kidd		Lewis		Lutheran		MNS				P	Xg
D	C	E	c	e	f	C	V	K	k	Kp a	Kp b	Js a	Js b	Fy a	Fy b	Jk a	Jk b	Le a	Le b	Lu a	Lu b	M	N	S	s	P 1	Xg a

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

Rh								Kell						Duffy		Kidd		Lewis		Lutheran		MNS				P	Xg
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	Le a	Le b	Lu a	Lu b	M	N	S	s	P 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^a, anti-Jk^b

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^a, anti-Fy^b

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

	Rh System				Kell	Duffy		Kidd		MNSs	
	Anti-E	Anti-c	Anti-C	Anti-e	Anti-K	Anti-Fy ^a	Anti-Fy ^b	Anti-Jk ^a	Anti-Jk ^b	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?

Formula for percentage of compatible donors:

1. Multiply antigen-negative prevalence for each antigen

(Antigen frequencies are different for different ethnicities, so you really need to know the frequencies for your specific donor population. **BE CAREFUL:** some charts may give you antigen positive prevalence and you'll have to subtract from 100 to find the antigen negative prevalence)

2. Convert result into percentage (X100)

Formula for how many units you need to screen to find compatible units

3.
$$\frac{100}{\text{Percentage obtained in step 2}} \times \text{\# of units desired} = \text{\# of units to be screened}$$

(round up to nearest whole number)

Example: Patient has anti-E and anti-Fy^a

Percentage of compatible donors:

1. $0.70 \times 0.33 = 0.231$
2. $0.231 \times 100 = \mathbf{23\% \text{ of donor population will be compatible.}}$

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

1. $100/23 = 4.34 \times 2 = 8.68$ **You should screen 9 units**

Antigen	% of donor population negative for antigen
D	15
E	70
C	30
e	3
c	20
K	90
Fy ^a	33
Fy ^b	20
Jk ^a	25
Jk ^b	25
S	45
s	11

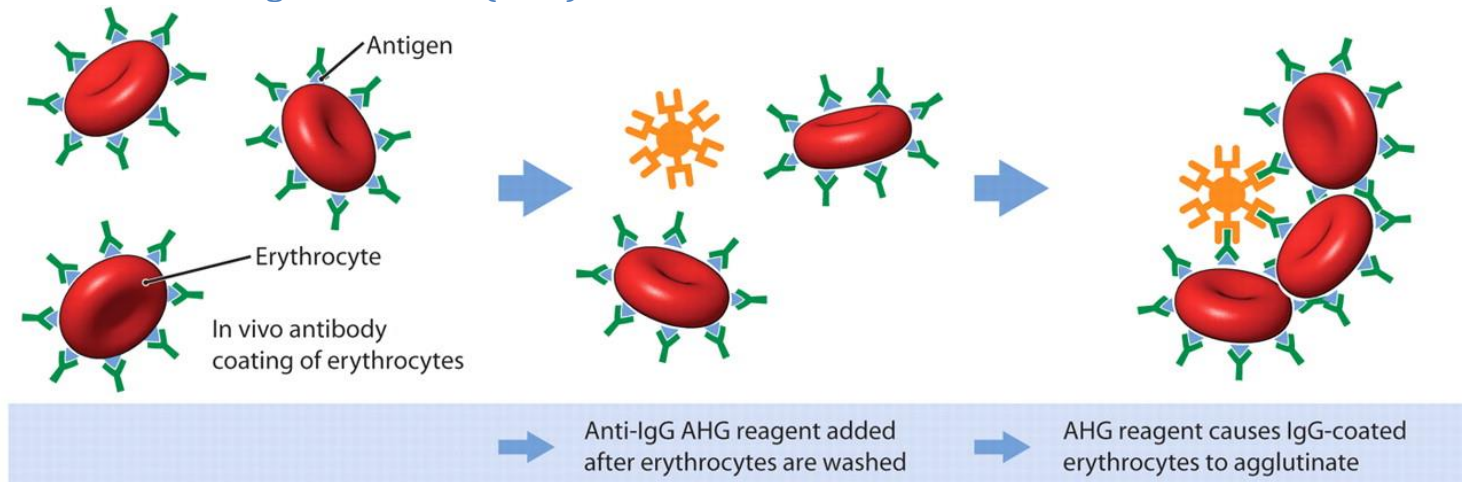
Given the same donor population, what percent of donor units will be compatible with patients with the following antibodies:

1. Anti-E, anti-c, anti-K, anti-Fy^a
2. Anti-Fy^a and anti-Jk^a
3. Anti-e and anti-Jk^a

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^a
3. 2 units for a patient with anti-e and anti-Jk^b

Direct Antiglobulin Test (DAT)



The 3 reagents that are used when performing a DAT are:

- 1.
- 2.
- 3.

The 4th tube is tested in _____ and serves as a control. It should always be _____ (negative/positive)

5 reasons a patient may have a positive DAT:

- 1.
- 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)	<ul style="list-style-type: none">••
Eluate reacts with all cells tested, including patient's autologous cells	<ul style="list-style-type: none">•
Eluate is completely nonreactive	<ul style="list-style-type: none">•••

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 Diagnostics, Inc. 2010

CONCLUSION: _____

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

† 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.

[illegible]

© Ortho-Clinical Diagnostics, Inc. 2010

CONCLUSION:

CCYY-MM-DD

A

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

035800062

† 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.

[illegible]

© Ortho-Clinical Diagnostics, Inc. 2010

CONCLUSION:

CCYY-MM-DD

A

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

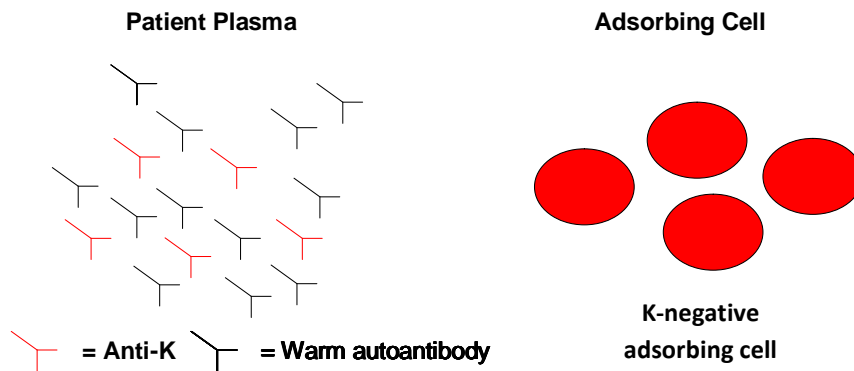
3580082

^a Results are from historical testing. "7" represents "Not Tested" for new donors.

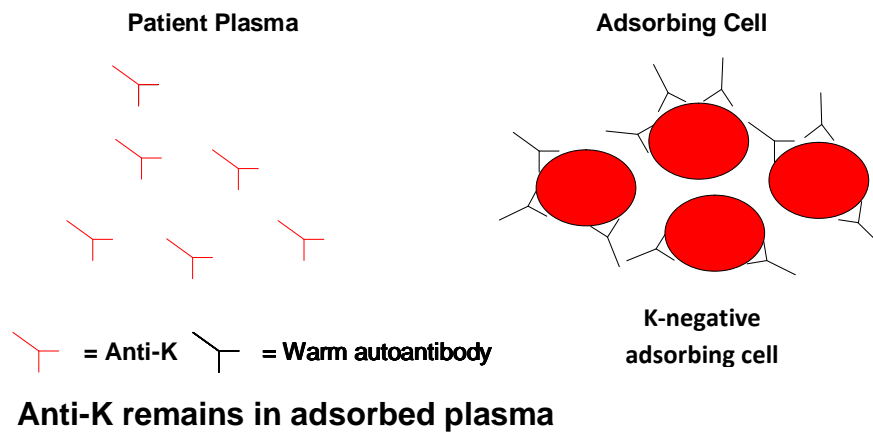
[illegible]

Adsorptions

Before Adsorption



After Adsorption



List 2 criteria for performing autoadsorptions

- 1.
- 2.

When you perform alloadsorptions, 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 alloadsorptions?

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= R₁R₁, K⁻, Jk(a⁻)
 Adsorbing Cell B= R₁R₁, K⁻, Jk(b⁻)
 Adsorbing Cell C= R₂R₂, K⁻, Jk(a⁻)
 Adsorbing Cell D= R₂R₂, K⁻, Jk(b⁻)
 Adsorbing Cell E= rr, K⁻, Jk(a⁻)
 Adsorbing Cell F= rr, K⁻, Jk(b⁻)

1. R₁r, K⁺, Fy(a+b⁻), Jk(a+b⁻), S+s⁻

2. rr, K⁻, Fy(a+b⁻), Jk(a-b⁺), S-s⁺

3. R₂R₂, 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 ₁ R ₁ , K ⁻ , Fy(a-b ⁺), Jk(a-b ⁺), S+s ⁺		
R ₂ R ₂ , 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 R₁R₁, R₂R₂, 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 R₁R₁, R₂R₂, 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.

RC-AID Worksheet

Patient Name: 2x allroads plasma	Patient ID #: Patient A	Patient DOB:	Date Drawn: 4/16/2014	Date Tested: 4/16/2014 Ads cell	Test:
-------------------------------------	----------------------------	--------------	--------------------------	---------------------------------------	-------

[illegible]

Adsorbing cells
from treated

$$\begin{aligned} R_1 R_1 &= R_1 R_1, K - F_1(a+b) JK(a-b) S - \bar{s} + \\ R_2 R_2 &= R_2 R_2, K - F_1(a-b) JK(a+b) S + \bar{s} + \\ r r &= r r, K - F_1(a+b) JK(a+b) S - \bar{s} + \end{aligned}$$

1 of 1

Immunohematology , Community Blood Center 4040 Main Street , Kansas City , MO 64111

RC-AID Worksheet

Patient Name:	Patient ID #:	Patient DOB:	Date Drawn:	Date Tested:	Tech:
2x allbads plasma	Patient B		4/16/2014	4/16/2014 Acds cell	

[illegible]

Adsorbing cells from treated

$$R_1 R_1 = R_1 R_1, K - F_1(a+b) J K(a-b) S - \bar{S} +$$

$$R_2 R_2 = R_2 R_2, K - F_1(a-b) J K(a+b) S + \bar{S} +$$

$$rr = rr, K - F_1(a+b) J K(a-b) S - \bar{S} +$$

1 of 1

RC-AID Worksheet

Patient Name:	Patient ID #:	Patient DOB:	Date Drawn:	Date Tested:	Tech:
2x allbads plasma	Patient C		4/16/2014	4/16/2014 A. cell	

[illegible]

Adsorbing cells
from treated

$$\begin{aligned} R_1 R_1 &= R_1 R_1, K - F_Y(a+b) J_K(a-b) S_{-3} + \\ R_2 R_2 &= R_2 R_2, K - F_Y(a-b) J_K(a+b) S_{+3} + \\ r &= r, K - F_Y(a+b) J_K(a-b) S_{-3} + \end{aligned}$$

1 of 1