Community Blood Center

Warm Autoantibodies

New York Blood Center Enterprises

EXPANDING OUR ORGANIZATION TO MEET CLINICAL, CELLULAR AND TRANSFUSION PRODUCT AND SERVICE NEEDS FOR PATIENTS. NOW PROVIDING ALMOST ONE MILLION BLOOD PRODUCTS, OVER 450,000 LABORATORY AND MULTI-ASSAY INFECTIOUS DISEASE TESTS AND OVER 12,500 SPECIALTY CLINICAL PROCEDURES ANNUALLY TO HOSPITALS NATIONWIDE.

COMPREHENSIVE CELL SOLUTIONS™

NATIONAL CENTER FOR BLOOD GROUP GENOMICS

NATIONAL CORD BLOOD PROGRAM

Objectives

- Describe common reactions in a sample containing warm autoantibody.
- Compare and contrast allo- and autoadsorptions.
- Compare and contrast methods used to determine the phenotype of recently transfused patients.

Common Reactions Seen with Warm Autoantibody

	Anti-IgG + C3d	Anti-IgG	Anti-C3d	Saline
DAT	3+	3+	0V	0

This is the first indication of a possible warm autoantibody

Common Reactions Seen with Warm Autoantibody

- Initial antibody screen:

		Rh									Kell						Duffy		Kidd		MNS					Xg	Results	
		D	C	E	c	e	f	V	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Xg^a	5' RT	LISS IAT
1	R_1R_1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	+	+	+	+	+	+	0	3+
2	R_2R_2	+	0	+	+	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	3+
3	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	3+

Initial screen is all positive

Common Reactions Seen with Warm Autoantibody

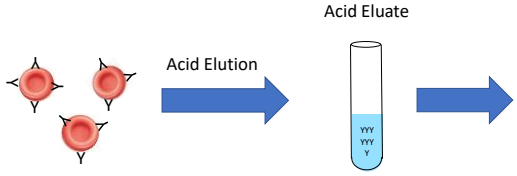
		Rh									Kell						Duffy		Kidd		MNS					Xg	Patient Plasma	
		D	C	E	c	e	f	V	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Xg^a	5' RT	LISS IAT
1	R_1R_1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	+	+	+	+	+	+	0	3+
2	R_1R_1	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	3+
3	R_1R_2	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	0	0	3+
4	R_0r'	+	0	0	+	+	+	+	0	0	+	0	+	0	+	0	0	+	0	+	+	0	+	+	0	3+
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	+	0	0	+	0	3+
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	0	3+
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	0	+	0	+	+	+	0	3+
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	+	+	0	0	3+
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	+	+	0	+	+	0	0	+	0	0	3+
10	R_2R_2	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	0	0	+	+	+	+	0	+	0	3+
11	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	0	+	+	0	+	+	+	+	0	3+
AC																									0	3+

Common Reactions Seen with Warm Autoantibody

- Common Test Results
  - DAT = positive with polyspecific and monospecific reagents
  - Antibody Screen = positive
  - Antibody Panel = positive
- Positive DAT requires an elution be performed and tested to discern what is causing the positive DAT

Warm Autoantibody - Elution

- Suspected warm autoantibody
  - Perform Acid Elution to detect bound antibody causing the positive DAT



Warm Autoantibody -Elution

		Rh								Kell						Duffy		Kidd		MNS				Xg	Eluate
		D	C	E	c	e	f	V	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Xg^a	PEG IAT
1	R_1R_1	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	+	+	+	+	+	+	+	3+
2	R_1R_1	+	+	0	0	+	0	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	3+
3	R_2R_2	+	0	+	+	0	0	0	0	+	0	+	0	+	0	+	0	+	+	+	0	+	+	0	3+
4	R_oR'	+	0	0	+	+	+	+	0	+	0	+	0	+	0	0	0	+	0	+	+	0	+	+	3+
5	r'r	0	+	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	0	+	3+
6	r^yr	0	0	+	+	+	+	0	0	+	0	+	0	+	0	0	+	+	+	0	+	0	+	+	3+
7	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	+	+	+	3+
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	+	+	0	3+
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	+	+	0	+	+	0	0	+	0	3+
10	R_2R_2	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	0	0	+	+	+	+	0	+	3+
11	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	0	+	+	0	+	+	+	+	3+
EGA AC																									3+

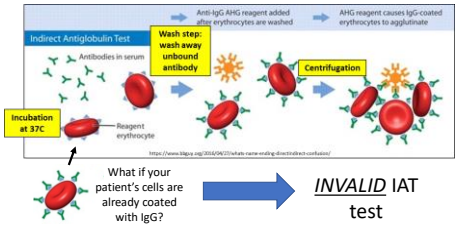
What is EGA??

Warm Autoantibody – EGA treated RBCs

EGA - EDTA Glycine Acid

- Removes the bound IgG while leaving the red cells intact for testing

Reminder: you can't test DAT-positive cells using the IAT!



Objectives

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What is adsorption?

- Adsorbing cell + patient plasma
- Warm autoantibody attaches to the adsorbing RBCs
- Alloantibody is left in adsorbed plasma

Adsorbing cell
(may be autologous or allogeneic)

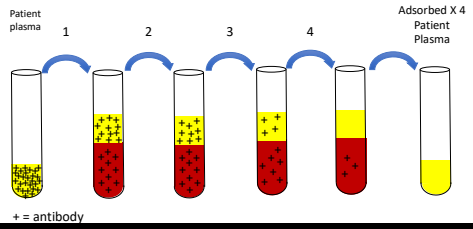
Patient Adsorbed plasma

Autoantibody

Alloantibody

Warm Autoantibody - Adsorptions

- So what does performing adsorptions look like??



Warm Autoantibody - Autoadsorptions

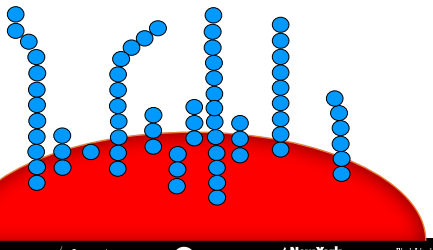
Autoadsorption – using the patient's own cells to diminish the warm auto reactivity in the patient's serum or plasma

- Requirements:
  - If performing autoadsorption, patient cannot have been transfused in the last 3 months
  - Need 1-2 mL of packed red blood cells per adsorption – meaning a lot of sample is required
  - Must ZZAP treat the patient's autologous cells prior to performing adsorptions – why?

ZZAP Treatment

- **What is ZZAP?** Combination of DTT and enzyme (papain)
  - DTT removes bound antibody and destroys certain antigens
  - Papain destroys other antigens
  - Autoantibodies react well with enzyme-treated cells

How Enzymes Work



Warm Autoantibody - Adsorptions

- **Alloadsorption** - using ***phenotypically similar*** *donor cells* to diminish the warm auto reactivity in the patient's serum or plasma
  - **Phenotypically similar** – RBCs that lack the same antigens as the patient. Does NOT need to be a perfect match

Alloadsorption: how it works

- Phenotype-matched adsorbing cells + patient plasma
- Warm autoantibody attaches to the RBCs
- Alloantibody is left in adsorbed plasma

Phenotype-matched adsorbing cell

Patient Alloadsorbed plasma

Autoantibody

Alloantibody

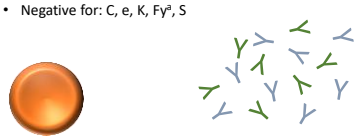
Warm Autoantibody - Alloadsorptions

Why use a phenotypically matched cell for alloadsorptions?

- The goal of adsorptions is to rid the plasma of the warm autoantibody reactivity while leaving behind any alloantibodies that may be lurking underneath
- Using phenotypically matched cells (cells negative for what the patient is negative for) will prevent accidently adsorbing out an alloantibody

Alloadsorption: Example

- Patient phenotype: R_2R_2, K-, Fy(a-b+), Jk(a+b+), S-s+
  - Negative for: C, e, K, Fy^a, S



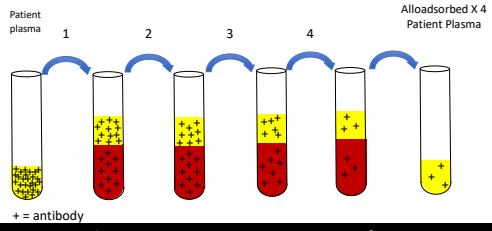
Adsorbing cell: C-, e-, K-, Fy(a-), S-

Patient Alloadsorbed plasma

Autoantibody

Anti-C

Alloadsorption: Example



Warm Autoantibody - Alloadsorptions

		Rh								Kell						Duffy		Kidd		MNS				Xg	Alloads X 4 Patient Plasma
		D	C	E	c	e	f	V	C^W	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Xg^a	LISS IAT
1	R_1R_1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	+	+	+	+	+	+	3+
2	R_1R_1	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	3+
3	R_2R_2	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	0	0√
4	R_0r	+	0	0	+	+	+	+	0	0	+	0	+	0	+	0	0	+	0	+	+	0	+	+	0√
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	+	0	0	+	3+
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	0√
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	0	+	0	+	+	+	0√
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	+	+	0	0√
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	+	+	0	+	+	0	0	+	0	0√
10	R_2R_2	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	0	0	+	+	+	+	0	+	0√
11	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	0	+	+	0	+	+	+	+	0√

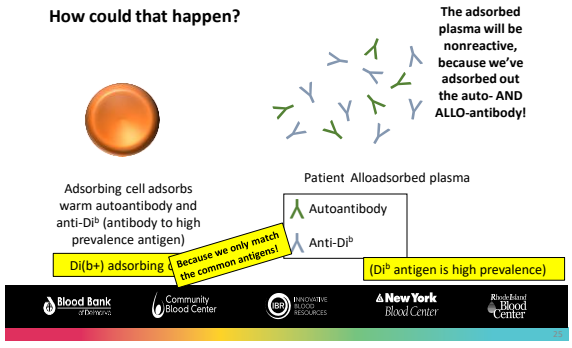
Warm Autoantibody - Alloadsorptions

What is a big risk when performing alloadsorptions?

- Can't match for everything (ie: Kp^b, Js^b, etc)
- There is a risk of adsorbing out an antibody to a high incidence antigen

The biggest risk of Alloadsorption

How could that happen?



The biggest risk of Alloadorption:

Adsorbing a clinically significant alloantibody onto the allogeneic adsorbing cells (especially against an antigen of high prevalence)

- Small probability of this happening
- Reports have disclaimer that this is a risk of alloadsorption

Warm Autoantibody - Adsorptions

Allogeneic red cells are treated with papain prior to performing alloadsorption – why?

- Papain destroys certain antigens: M, N, S, s (variable) as well as Fy^a and Fy^b which means we don't have to match for those antigens when choosing an adsorbing cell
- Warm autoantibodies react strongly with enzyme-treated cells

Practice choosing adsorbing cells

- What antigens are not expressed on my patient's cells?
  - Could potentially make corresponding antibodies
- Choose and adsorbing cell that lacks those same antigens (phenotypically similar)
- Consider papain treatment
  - M,N,S,s antigens destroyed
  - Fy^a, Fy^b antigens destroyed

Warm Autoantibody - Alloadsorptions

Choose which papain treated adsorbing cell(s) to use for a patient with the following phenotype. Choose all that apply.

R_1R_1, K+, Fy(a-b+), Jk(a-b+), S-s+

a) Adsorbing Cell 1) R_1R_1, K-, Jk(a-)
b) Adsorbing Cell 2) R_1R_1, K-, Jk(b-)
c) Adsorbing Cell 3) R_2R_2, K-, Jk(a-)
d) Adsorbing Cell 4) R_2R_2, K-, Jk(b-)
e) Adsorbing Cell 5) rr, K-, Jk(a-)
f) Adsorbing Cell 6) rr, K-, Jk(b-)

Warm Autoantibody - Alloadsorption

		Rh								Kell					Duffy		Kidd		MNS				Xg	Alloads Plasma
		D	C	E	c	e	f	V	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Xg^a	LISS IAT
1	R_1R_1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	+	+	+	+	+	0√
2	R_1R_1	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0√
3	R_2R_2	+	0	+	+															0	+	+	0	0√
4	R_0r	+	0	0	+																+	0	+	+	0√
5	r'r	0	+	0	+																+	0	0	+	0√
6	r''r	0	0	+	+																+	0	+	+	0√
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	0	+	0	+	+	+	0√
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	+	+	0	0√
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	+	+	0	+	+	0	0	+	0	0√
10	R_2R_2	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	0	0	+	+	+	+	0	+	0√
11	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	0	+	+	0	+	+	+	+	0√

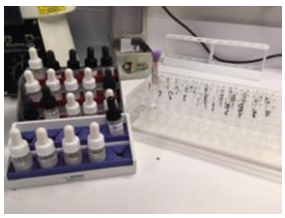
No alloantibodies *to common RBC antigens* were detected.

Objectives

- Describe common reactions in a sample containing warm autoantibody.
- Compare and contrast allo-and autoadsorptions.
- Compare and contrast methods used to determine the phenotype of recently transfused patients.

Warm Autoantibody - Obtaining a Phenotype

- Why is a phenotype important?
- What portion of the patient's phenotype does IRL test for?
  - D, E, c, C, e, K, Fy^a, Fy^b, Jk^a, Jk^b, S, s



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Warm Autoantibody - Obtaining a Phenotype

How is a phenotype obtained when the patient has been recently transfused?

- Donor cells in sample may interfere with serologic typing

Warm Autoantibody - Obtaining a Phenotype

There are two options when a patient has been recently transfused

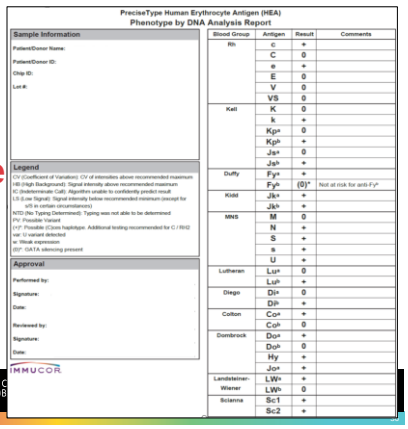
- Hypotonic wash:
  - Sickle Cell Disease (SCD) patients
  - Wash patient cells with hypotonic saline solution
    - SCD (autologous) cells resistant to lysis
    - Healthy donor cells lyse in hypotonic environment
  - Limitations:
    - Sample < 24 hours old
    - Transfusion >3 days ago
    - Only effective for SCD patients

Warm Autoantibody - Obtaining a Phenotype

There are two options when a patient has been recently transfused – continued

- Molecular genotype (predicted phenotype)
  - Patient DNA extracted
  - DNA analyzed for single nucleotide polymorphisms (SNPS) that code for blood group antigens
  - Common assays provide information on >30 antigens
  - Is not restricted by recent transfusion
  - Limitations: has an increased turn around time ~ 72 hours

Genotype Example



PreciseType Human Erythrocyte Antigen (HEA)
Phenotype by DNA Analysis Report

Sample Information

Patient/Donor Name:
Patient/Donor ID:
Chip ID:
Lot #:

Legend

Approval

Performed by:
Signature:
Date:
Reviewed by:
Signature:
Date:

IMMUCOR

Blood Group	Antigen	Result	Comments
Rh	c	+	
	C	0	
	e	+	
	E	0	
	V	0	
	VS	0	
Kell	K	0	
	k	+	
	Kp^a	0	
	Kp^b	+	
	Js^a	0	
	Js^b	+	
Duffy	Fy^a	+	
	Fy^b	(0)*	Not at risk for anti-Fy^a
Kidd	Jk^a	+	
	Jk^b	+	
MNS	M	0	
	N	+	
	S	+	
	s	+	
	U	+	
Lutheran	Lu^a	0	
	Lu^b	+	
Diego	Di^a	0	
	Di^b	+	
Colton	Co^a	+	
	Co^b	0	
Dombrock	Do^a	+	
	Do^b	0	
	Hy	+	
	Jo^a	+	
Landsteiner-Wiener	LW^a	+	
	LW^b	0	
Scianna	Sc1	+	
	Sc2	+	

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