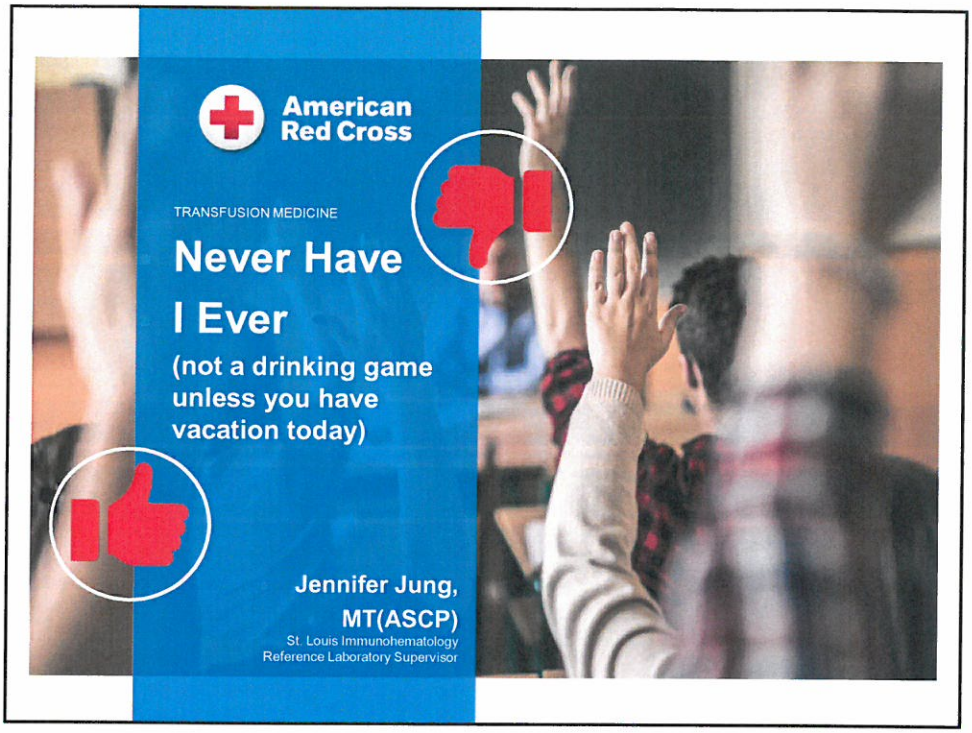

Never Have I Ever
How to Play

A Transfusion Medicine Never Have I Ever statement will be presented.

If the statement is something that you have ever experienced, then you should select "I Have" in the poll.

If the statement is something that you have never experienced, then you should select "Never" in the poll.

We are all winners for sharing our experiences. ☺

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Poll time

In the top right of your Demio screen you will see Chat, Polls, and Handouts.

Please, click on the Polls tab.

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Poll time

If you do not see chat, polls, and handouts, you may need to click the far-right circle to open chat.

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3

Poll time

Each poll will have the statement followed by two options:

- I Have
- Never

Please select your response.

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1. Never Have I Ever…
Seen an ABO Discrepancy

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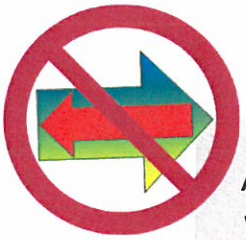
ABO Discrepancies



An ABO discrepancy exists when the results of the red cell tests do not agree with the serum tests;

forward and reverse disagree.

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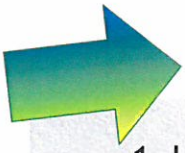
ABO Discrepancies
Four Main Categories



1- Unexpected reaction in red cell testing

2- Missing reaction in red cell testing

3- Unexpected reaction in serum testing

4- Missing reaction in serum testing

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ABO Discrepancies
Key points

- Recognize the discrepancy exists
- Do not interpret the ABO testing until the discrepancy is investigated and resolved.
- Provide group O red cells if transfusion can't be delayed.
- Always start by repeating all testing. It may help to use a new washed cell suspension.
- Decide which of the four categories you are dealing with by suspecting that the strongest reactions are most likely correct.

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ABO Discrepancies
Investigation

Anti-A	Anti-B	Anti-A,B	A1 cells	A2 cells	B cells	Discrepancy category	Possible causes and next steps
3+	0	3+	0	0	0	most likely an A; **missing reactions in reverse**	Age related, extend incubation, lower temp, enzyme treat cells
4+	4+	4+	1+	1+	1+	most likely an AB; **extra reactions in reverse**	Rouleaux, examine using microscope, saline replacement Cold reacting allo or autoantibody – examine antibody screen, antibody identification
2+	4+	4+	4+	4+	2+	most likely a B; **extra reactions in forward and reverse**	Possible cold reactive auto antibody; warm wash cells, adsorb serum

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ABO Discrepancies Investigation

Anti-A	Anti-B	Anti-A,B	A1 cells	A2 cells	B cells	Discrepancy category	Possible causes and next steps
3+	0	4+	1+	0	4+	most likely an A; **extra reaction in reverse**	Possible A subgroup with an anti-A1, antibody ID
4+	4+	4+	0	0	2+	most likely an AB; **extra reaction in reverse**	Cold reactive alloantibody, such as anti-M, perform Aby ID
4+	2+	4+	0	0	4+	most likely an A; **extra reaction in forward**	Acquired B

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2. Never Have I Ever…

Had a DAT with a Positive Saline Control

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DAT
Direct Antiglobulin Test

- Detects in vivo immunoglobulin coating of patient cells
- Used for investigation of hemolytic transfusion reactions, HDFN, AIHA, and DIHA.
- When antibody is made, it coats antigen positive cells first. When cells are saturated or destroyed (no more antigen sites), the antibody will be detected in the serum.
- Most labs test with polyspecific antiglobulin first and if positive reflex to anti-IgG and anti-C3 tests.
- If positive, saline/inert control must be nonreactive or the test is invalid.

DAT
Positive Saline Control

Causes

- Spontaneous agglutination
  - IgM cold autoagglutinins
  - Heavy coating of IgG or rare warm reactive IgM

Resolution

- Wash patient cells with warm saline
- Treat cells with 0.01M Dithiothreitol (DTT)
  - DTT will disperse in vitro bound antibody, but in vivo bound antibody will remain

Case #1

- 41 yo male patient with Sickle Cell, Liver Disease
- Customer reports history of anti-E, anti-Fy^a, anti-Jk^b
- Patient transfused 5 days prior with O Negative units
- Our initial testing

ABO/Rh	Anti-						Cells			DAT				
	A	B	A,B	A_1	D	Cont	A_1	A_2	B		PS	IgG	C3	Control
IS	4+^{mf}	w+	4+^{mf}		2+^{mf}	w+	0	3+	4+	IS	1+	w+		w+

- E-Fy(a-)Jk(b-) panels cells are all 2-4+ positive at IS and PEG/IAT and microscopically to 3+ at LISS/IAT. Auto control is positive at all phases.

Case #1

- Patient cells are incubated for 15 minutes at 37^{o}C and then washed 6x with warm saline. All cell testing is repeated using the warm suspension:

ABO/Rh	Anti-						Cells			DAT				
	A	B	A,B	A_1	D	Cont	A_1	A_2	B		PS	IgG	C3	Control
15' 37C warm wash x6	4+^{mf}	0	4+^{mf}		1+^{mf}	0				IS	w+	0/+m	NA	0 / 0
Allo Ads 2x 4C							0	0	4+	RT	w+	NA	w+	0 / 0
ABO/Rh Interpretation: A Positive, patient received O Neg unit										DAT Interpretation: micro IgG, weak C3				

- Eluate Studies: Warm autoantibody, no underlying alloantibodies
- Serum Studies: Cold autoantibody with underlying anti-E, anti-Fy^a, anti-Jk^b, anti-K

Case #2

- 71 yo female patient with anemia, AML
- Customer reports positive screen cell I
- Patient was transfused 10 days prior

- <u>IRL testing:</u>
- Patient is B positive
- All panel cells and auto control weakly positive at PEG/IAT and 1+ at 15' 4C = Cold autoantibody
- Initial DAT testing:

	DAT			
	PS	IgG	C3	Control
IS	0 / +^{m}	0 / +^{m}		0 / +^{m}

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Case #2

- Patient cell suspension is incubated for 15' at 37°C. Following incubation, patient cells are washed x6 with warm saline. DAT is repeated:

	DAT			
	PS	IgG	C3	Control
IS	0 / +^{m}	0 / +^{m}		0 / +^{m}

- Patient cells are treated with 0.01M DTT and DAT is repeated:

	DAT			
	PS	IgG	C3	Control
IS	0 / 0			0 / 0
RT	0 / 0✓			0 / 0
DAT Interpretation: Negative				

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3. Never Have I Ever…
Seen Mixed Field in a Patient Antigen Typing

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Mixed Field

- Defined as a pattern of agglutination in which small clumps of cells exist amid a sea of free cells.
- Indicates more than one cell population is present.
- Has been present in
  - Recently transfused patients
  - Bone marrow transplants patients
  - Women whose circulation contains fetal red cells
  - Patients with T or Tn transformed red cells
  - Mosaics, Chimeras

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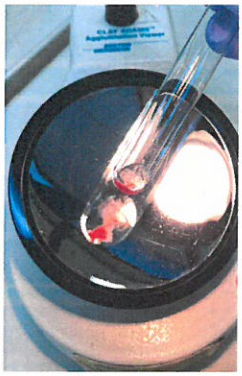
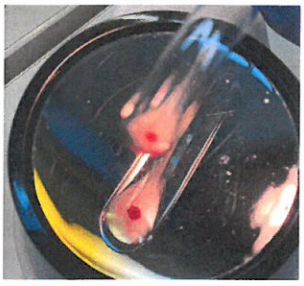
Mixed Field

Negative trail of cells slowly flowing through the supernatant, button remains on wall of tube



Agglutinated cell button coming away from tube wall

Large clump of agglutinated cells in a sea of free nonreactive cells



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Mixed Field Investigation

- Review transfusion history for recent transfusion event.
- Review medical history for allogeneic bone marrow or stem cell transplant.

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Mixed Field Resolution

Mixed field in a recently transfused patient can be resolved by separating transfused cells from donor cells. Two techniques are possible based on patient diagnosis:

- If the patient does not have Sickle Cell Disease, separation using cell density
- If the patient has Sickle Cell Disease, separation using hypotonic wash

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4. Never Have I Ever…
Performed a Cell Separation

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Cell Separation using Cell Density

Autologous immature RBCs (reticulocytes) can be separated from the transfused RBC population because they have a lower specific gravity.

The transfused sample is washed several times with saline and then hard spun. Multiple microhematocrit tubes are filled with approximately 60mm of packed red cells and then spun for 15 minutes.

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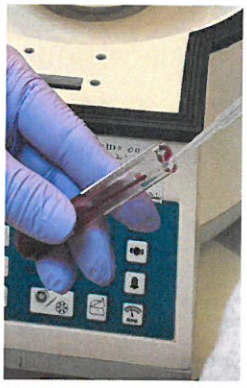
Cell Separation using Cell Density



Filling a microhematocrit tube using a transfer pipette

24 filled microhematocrit tubes ready for centrifugation

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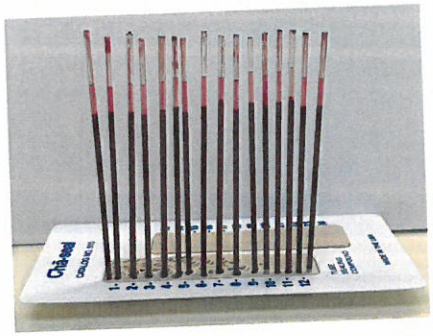
Cell Separation using Cell Density



The autologous reticulocytes concentrate at the top of the RBC layer in the microhematocrit tube.

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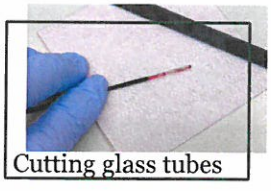
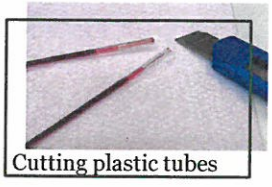
Cell Separation using Cell Density

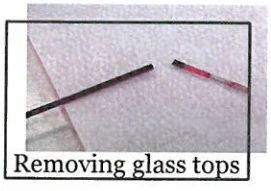
- To harvest the autologous cells, the top 2-5mm of the red cells in the microhematocrit tubes are cut off.



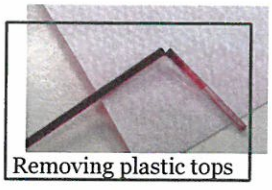
Cutting glass tubes



Cutting plastic tubes



Removing glass tops



Removing plastic tops

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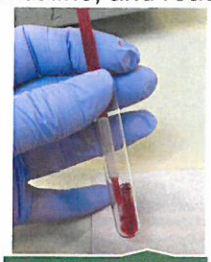
Cell Separation using Cell Density

- The cut off red cell tops are placed in a new test tube and contents flushed out with saline. The harvested cells are washed, suspended in saline, and ready for testing.

Cut off red cell tops



Flushing out the red cells

Autologous cells ready for testing

- The harvested autologous RBCs may be used for phenotyping, a direct antiglobulin test (DAT), or auto adsorption.

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Cell Separation using Cell Density Limitations

- Separation is best accomplished when blood samples are obtained 3 days or more after transfusion.
- If possible, the separation should be performed within 24 hours of sample collection.
- Because of the density of RBCs with abnormal hemoglobin (HgbS), separation by the microhematocrit centrifugation method is not effective. Another method must be used for these patients.
- If the patient is not producing adequate reticulocytes, the separation will be unsuccessful.

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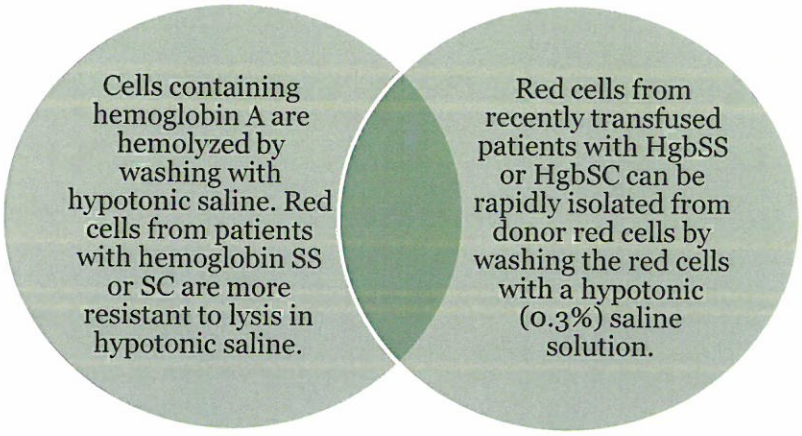
Cell Separation using Hypotonic Wash



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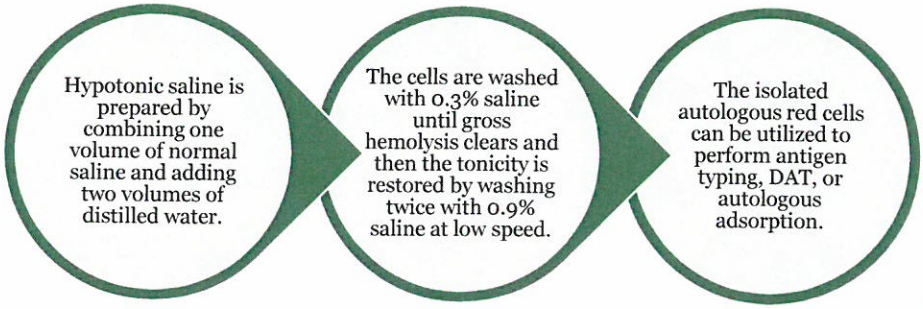
Cell Separation using Hypotonic Wash



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Cell Separation using Hypotonic Wash Limitations / Advantages

May need to start with a large sample volume if the patient has recently received an exchange transfusion. There will be a lot of hemolysis because of the many cells containing normal hemoglobin.

Advantages over cell density is that this method is not dependent on the presence of reticulocytes, takes a short amount of time, and reagents are readily available in most clinical laboratories.

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5. Never Have I Ever…
Had an Incompatible Crossmatch with a Negative Antibody Screen

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Incompatible Crossmatch, Negative Screen

- The negative screen tells us common alloantibody specificities are excluded from the patient specimen.
- What else could be causing the reactivity detected in the crossmatch?

AN ANTIGEN THAT IS NOT EXPRESSED ON THE SCREEN CELLS – LOW PREVALENCE ANTIGEN

THE DONOR CELLS ARE COATED WITH IMMUNOGLOBULIN.

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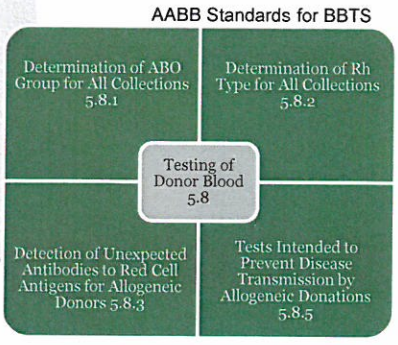
Incompatible Crossmatch, Negative Screen - Donor

- Performing a DAT is not a required test for blood donations by any regulatory organization.



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Incompatible Crossmatch, Negative Screen - Donor

- All healthy individuals have some amount of IgG on their red cells, but very few have enough to be detectable.

- DAT-positive blood units do not predispose the recipient to any adverse outcomes.
- Crossmatch testing may be complicated by the donor's positive DAT

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Incompatible Crossmatch, Negative Screen - Donor

To investigate if the incompatible crossmatch is caused by the donor, simply perform a DAT on the donor's cells in the same method as the incompatible crossmatch.

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Incompatible Crossmatch, Negative Screen - Antigen

- Options for investigating antibodies to low prevalence antigens include:
  - Testing reagent cells known to be positive for various low prevalence antigens with the patient's serum.
  - Treating the incompatible unit with enzymes or chemicals to help determine the specificity.
  - Typing the red cells from the incompatible unit with known examples of antibodies directed against low prevalence antigens.

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Incompatible Crossmatch, Negative Screen Case Study

- Patient presented to ER with 3-day hx of weakness & lightheadedness with the addition of nausea/vomiting the previous day
- 77 yo male with MDS, GERD, and gastritis who has received approximately 3 txns per month for past year
- Hgb 5.2 g/dl upon admission, two units ordered
  - The first unit was started at 17:35 and completed at 19:00
  - Prior to starting the second unit, the patient complained of back pain and chills.
  - A transfusion reaction work-up was ordered.

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Incompatible Crossmatch, Negative Screen Case Study

- Transfusion reaction investigation results:

Test		Pre-transfusion	Post-transfusion
Temperature		99°F	101°F
HGB		5.2 g/dl	6.3 g/dl (decreasing to 6.0 g/dl)
Urinalysis	Color	Clear	Clear
	Blood	Negative	Small
	RBC	None	<1
Serum	Bilirubin	1.08	3.67
	Creatinine	0.75	0.78
	Haptoglobin	<40	<40
	LDH	<209	265
Blood Bank Testing	ABO/Rh	O Positive	O Positive
	Antibody Screen	Negative	Negative
	DAT	Negative	W+ IgG
	Elution	NT	Negative
	AHG Crossmatch	Positive	Positive
	Gram Stain on Unit	Negative	

- Based on the clinical and laboratory data, the conclusion of Hemolytic Transfusion Reaction was made. Pre- and Post-transfusion samples were sent to the IRL for additional testing.

Incompatible Crossmatch, Negative Screen Case Study

- The IRL confirmed the testing noted in the hospital Blood Bank.
  - Pre-transfusion DAT = negative
    Post-transfusion DAT = weakly positive due to IgG
  - Pre- and Post-transfusion Antibody screen = nonreactive at IS, LISS/37°C, LISS/IgG, PEG/IgG, and Polybrene IS and IgG
  - Pre- and Post-transfusion crossmatch = 1+ incompatible at PEG/IAT.
- Due to the HTR, a positive DAT on the unit was NOT a suspected cause and the IRL began the antibody investigation by searching for an antibody to a low prevalence antigen.

Incompatible Crossmatch, Negative Screen Case Study

- We tested red cells from the incompatible unit with several sources of antibodies and got lucky:

American Red Cross Washington, D.C. 20006 — **Preliminary Donor Antigen Testing Worksheet** — American Red Cross

Batch Information

Batch # 081814-2010-6 | Pages in batch: | Page 2 of 2

Position #	DIN/WBN Preliminary Type	Anti-Kp^a	Anti-Js^a	Anti-V	Anti-Cw	Anti-Wr^a	Anti-Di^a	Anti-Co^b	Anti-Yt^b	Anti-Lu^a	Anti-
		Test Results									
1	W2011141113408	0	0	0	0	2+	0	0	0	0	

- The incompatible unit tested positive for the Wr^a antigen.

Incompatible Crossmatch, Negative Screen Case Study

- Wr(a+) reagent red blood cells were tested with the pre- and post-transfusion serum as well as the eluate
- The patient's red cells tested negative Wr^a.

	Supplier Lot #	Donor/ Vial#	RhHr D	C	E	c	e	f	V	Cw	MN M	N	S	s	P Pi	Lew Leb	Lut Lua	Lub	Kell K	k	Kpa	Kpb	Jsa	Jsb	Duf Fya	Fyb	Kid Jka	Jkb	Xg Xga	Additional Antigens	Pre serum	Post serum	Post Eluate	LW
1 OOD	Imm-10F 42727	B2849 TC	+	+	0	0	+		0	0	0	+	0	+	+	+	0	0	+	0	+	0	+	0	+	+	0	+	+	+	Wr(a+)	LISS 4+	LISS 1+	4+	4+
2 OOD	Immucor 38680	B5269 2	+	+	0	0	+		0	+	+	+	+	+	+	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	Wr(a+)	1+	1+	2+	0
3 OOD	Imm-16 35638	H1484 8	0	0	0	+	+		0	0	+	0	+	0	0	0	0	0	+	0	+	0	+	0	+	+	0	+	+	+	Wr(a+)	1+	1+	2+	0
																																1+	1+	1+	0

- Anti-Wr^a was identified

Incompatible Crossmatch, Negative Screen Case Study

- AABB Standards for Blood Banks and Transfusion Services state "If no clinically significant antibodies were detected…and there is no record of previous detection of such antibodies, at a minimum, detection of ABO incompatibility shall be performed." 5.16.1.1.
- Tests for ABO incompatibility could include an Immediate Spin crossmatch (as was performed in this case) or a computer crossmatch. These techniques may not detect an incompatible crossmatch caused by an antibody to a low prevalence antigen.
- Antibodies to low prevalence antigens provide a special challenge to the Blood Bank. Routine testing may not detect the presence of these antibodies.
- It is important to remember that even though all testing is performed and acceptable according to standard Blood Banking practices, the possibility still exists for an incompatibility due to red cell alloimmunization.

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References

- Cohn C, Delaney M, Johnson S, Katz L. Technical Manual. 20th ed. Bethesda, Maryland: AABB; 2020.
- Harmening D. Modern Blood Banking & Transfusion Practices. 7th ed. Philadelphia, Pennsylvania: F. A. Davis Company; 2019.
- Puri V, Chhikara A, Sharma G, Sehgal S, Sharma S. Critical evaluation of donor direct antiglobulin test positivity: Implications in cross-matching and lessons learnt. *Asian J Transfus Sci*. 2019;13(1):70-72. doi:10.4103/ajts.AJTS_125_17

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