



Method Validation of Automated Titration

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Agenda

- Transfusion Basics
- Isohemagglutinin Titration
- Pilot Study
- Results
- Discussion



Transfusion Basics

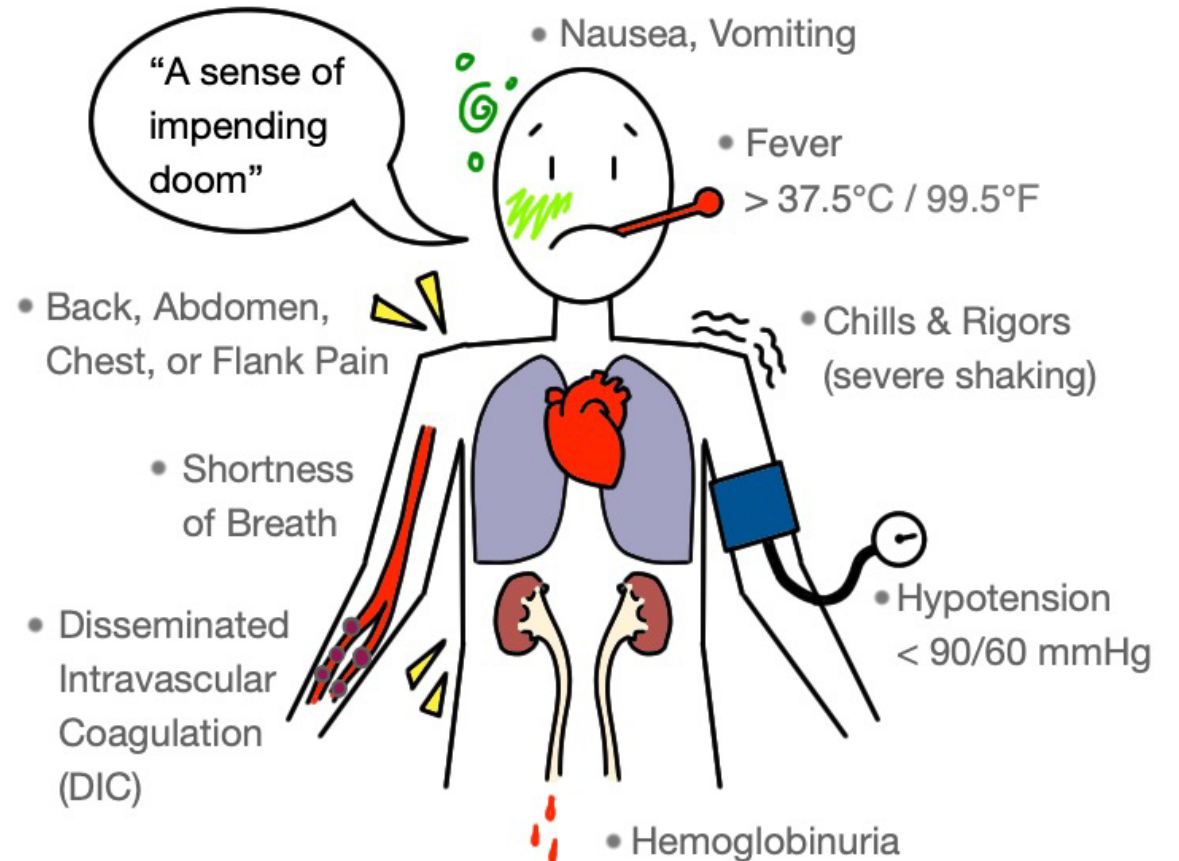
Transfusion Reactions, ABO System, & Blood Donation



Acute Hemolytic Transfusion Reaction

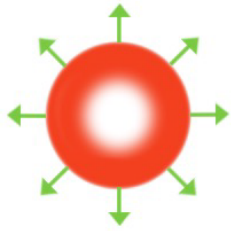
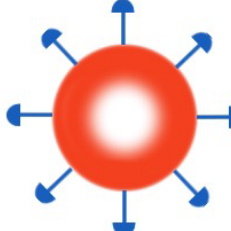
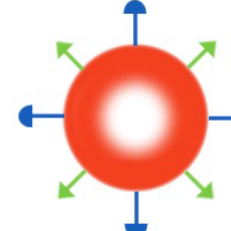





- ABO-incompatible
- Patient antibody → Donor cells
- Donor antibody → Patient cells
- Preventable!

Symptoms of AHTR



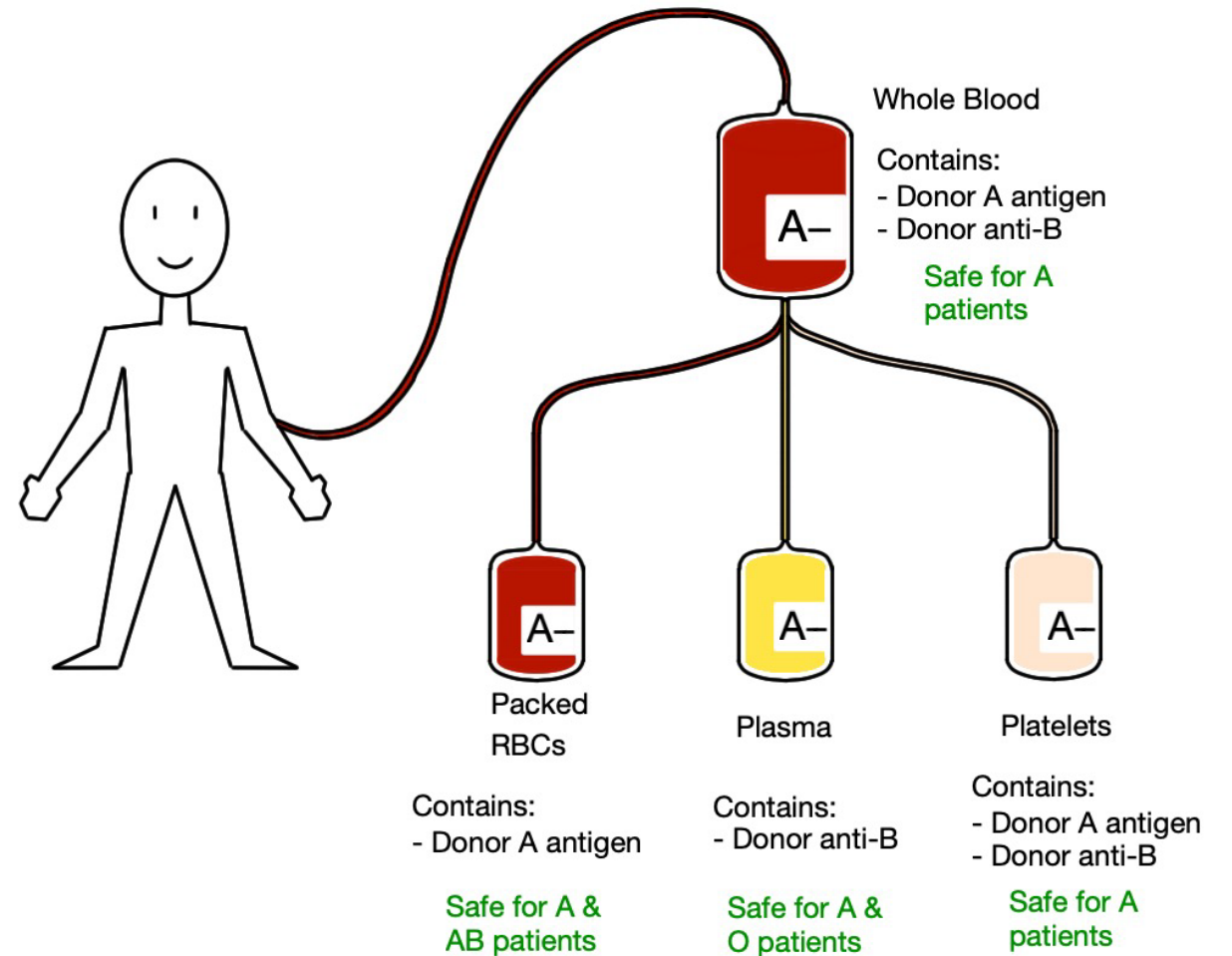
ABO System

- Landsteiner's Law (see table)
- ABO Antibodies = *Isohemagglutinins*
- AHTR

		Blood Type			
		A	B	AB	O
RBC surface antigen					
Plasma antibodies	 anti-B	 anti-A	No ABO antibody	  Both	

Overview of Donation

- Donors give blood
- Separation into components
- Compatibility by component contents



Platelets

- Short supply (5-day shelf life)
- Used for thrombocytopenias, active bleeding
- O platelets implicated in AHTR in A patients, a few B patients



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Image: <https://www.shutterstock.com/image-photo/giving-single-donor-platelet-during-open-297030623>

Whole Blood

- Oldest product (in modern transfusion)
- Components in '60s-'70s
- Renewed interest in whole blood
- How to give when patient type is unknown?



Image: <https://www.istockphoto.com/photo/blood-transfusion-bags-qml47057283-11963764>



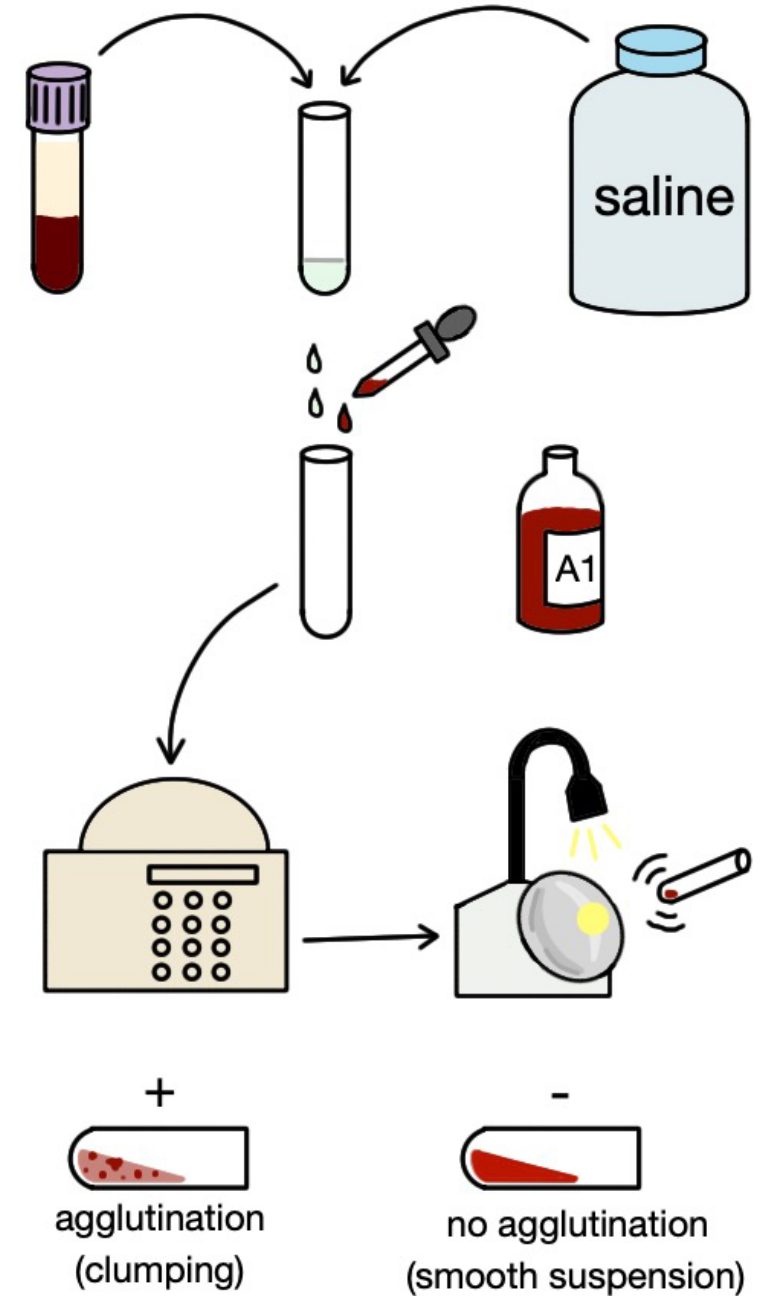
Isohemagglutinin Titration

Assessing Antibody Levels



Process

- Dilute donor plasma
- Mix with RBCs
- Serofuge (centrifuge)
- Check for agglutination (clumping)



Issues

- Labor-intensive
- Time-consuming
- Ergonomics?
- Technique varies
- United States: no official FDA standard for “high titer” threshold
- Other nations
 - Switzerland- 1:16
 - Czech Republic- 1:64
 - Norway- 1:250
 - Japan- 1:512

Automation

- Uses existing devices/platforms
- Removes physical stress and time constraints from human staff
- More precise, less variable
- Proven track record (UK, Europe, etc.)



Image: https://www.immucor.com/global/Products/PublishingImages/NEOiris_Small.png



Pilot Study

Titration Automation at Memorial Blood Centers, St. Paul, MN



Memorial Blood Centers

- Part of Innovative Blood Resources; New York Blood Center Enterprises (NYBCE)
- Long Term Goal: begin system-wide shift from manual titration to standardized automated titration



 **New York** *Blood Center*

Methods

■ 3 Titration Methods

- Manual 1:250 (official)
- Automated 1:1-1:128 (NEO Iris[®], Immucor; Norcross, GA)
- Manual 1:128 (me)

■ Samples

- N = 151
- Type O donations
- Whole blood (56) and platelet units (95)
- 2 weeks of testing, daily samples

■ Comparisons

- Threshold 1:250 vs. 1:128
- Manual vs. Automated
- Single-point vs. Serial doubling
- Anti-A vs. Anti-B isohemagglutinins

Automated Grading

- Visual analysis software
- Last dilution with agglutination is titer
- Titer ≥ 128 : high-titer
- Titer < 128 : low-titer

1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
4+	4+	4+	3+	1+	0	0	0

Example: the most diluted well with agglutination here is 1:16. The titer is 16. This would be a low-titer unit.



Results



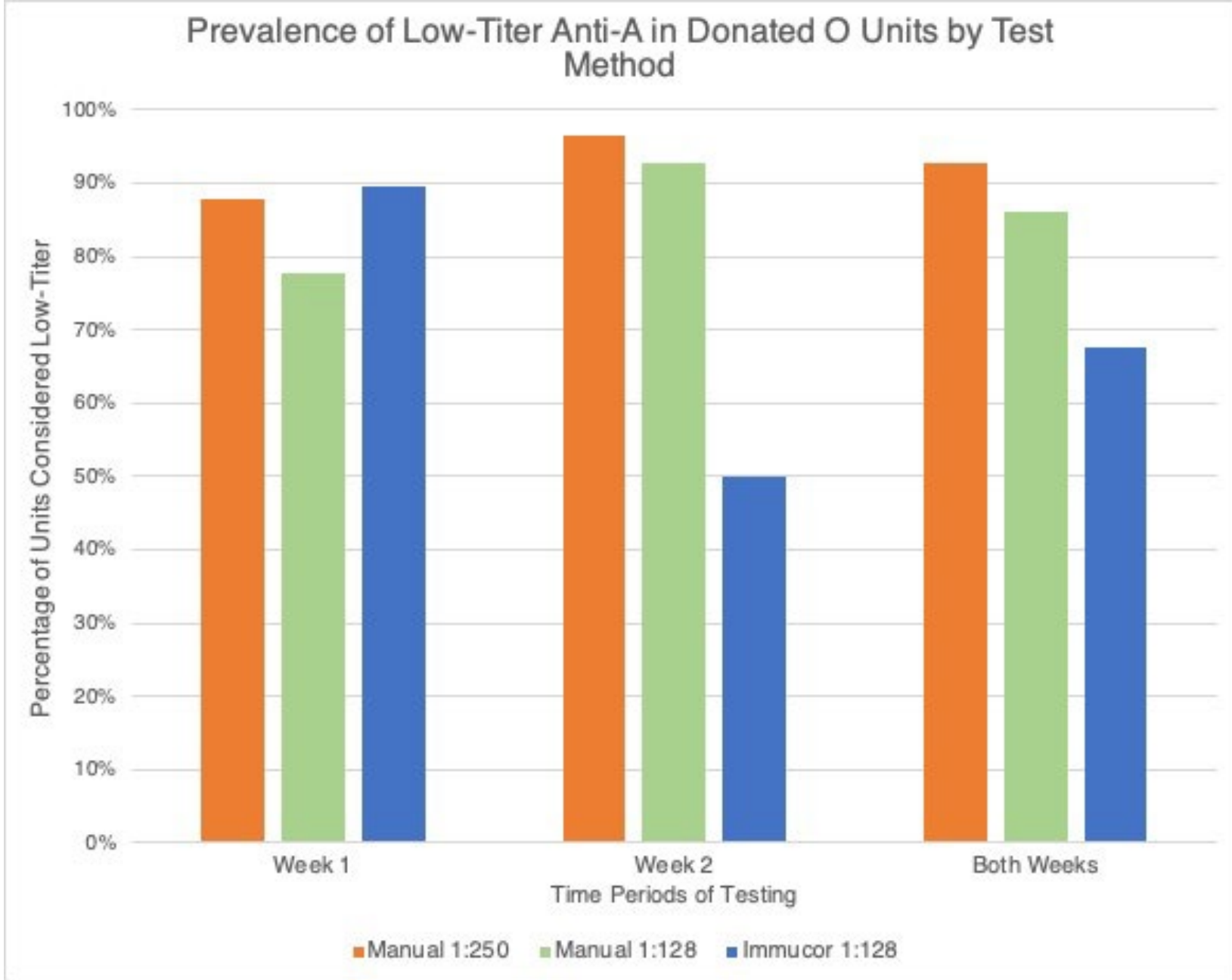


Figure 1: Percentage of units considered low titer for anti-A by each titration method. 67 units were tested in week 1, and 84 units were tested in week 2, for a total of 151 units across both weeks.

Immucor Week 2

- Low low-titer rate mostly due to high “indeterminate grade” rate
- Indeterminate grade
 - Camera checks wells
 - Decreasing agglutination is normal
 - If agglutination goes up after going down, result is invalid and not given a titer value

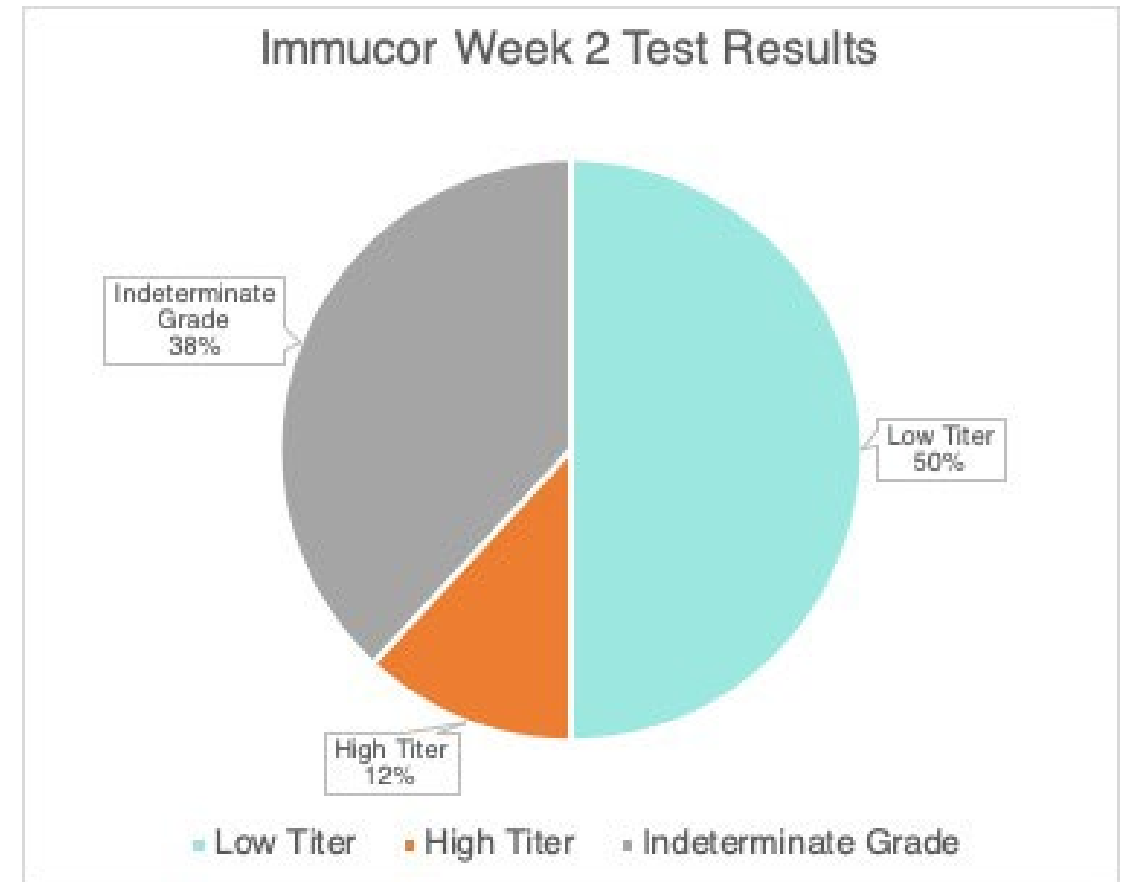


Figure 2: Outcomes of unit testing for anti-A in week 2. “Indeterminate Grade” results are invalid and cannot be given a titer value.

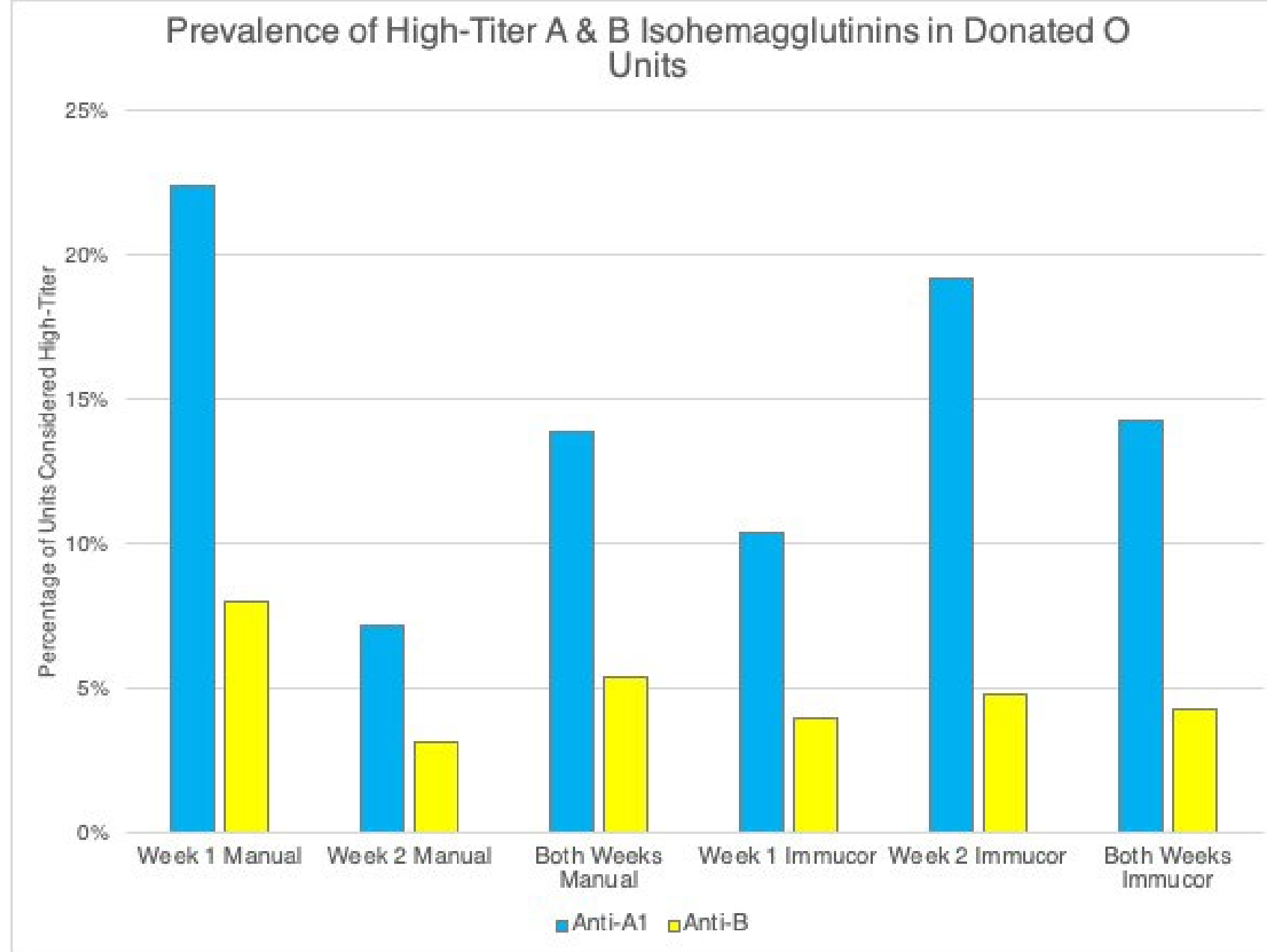


Figure 3: Percentage of whole blood units considered high-titer for either anti-A or anti-B. For week 2 Immucor results, "indeterminate grade" results were not included in analysis. In manual testing, all units which had high anti-B titer also had high anti-A titer.



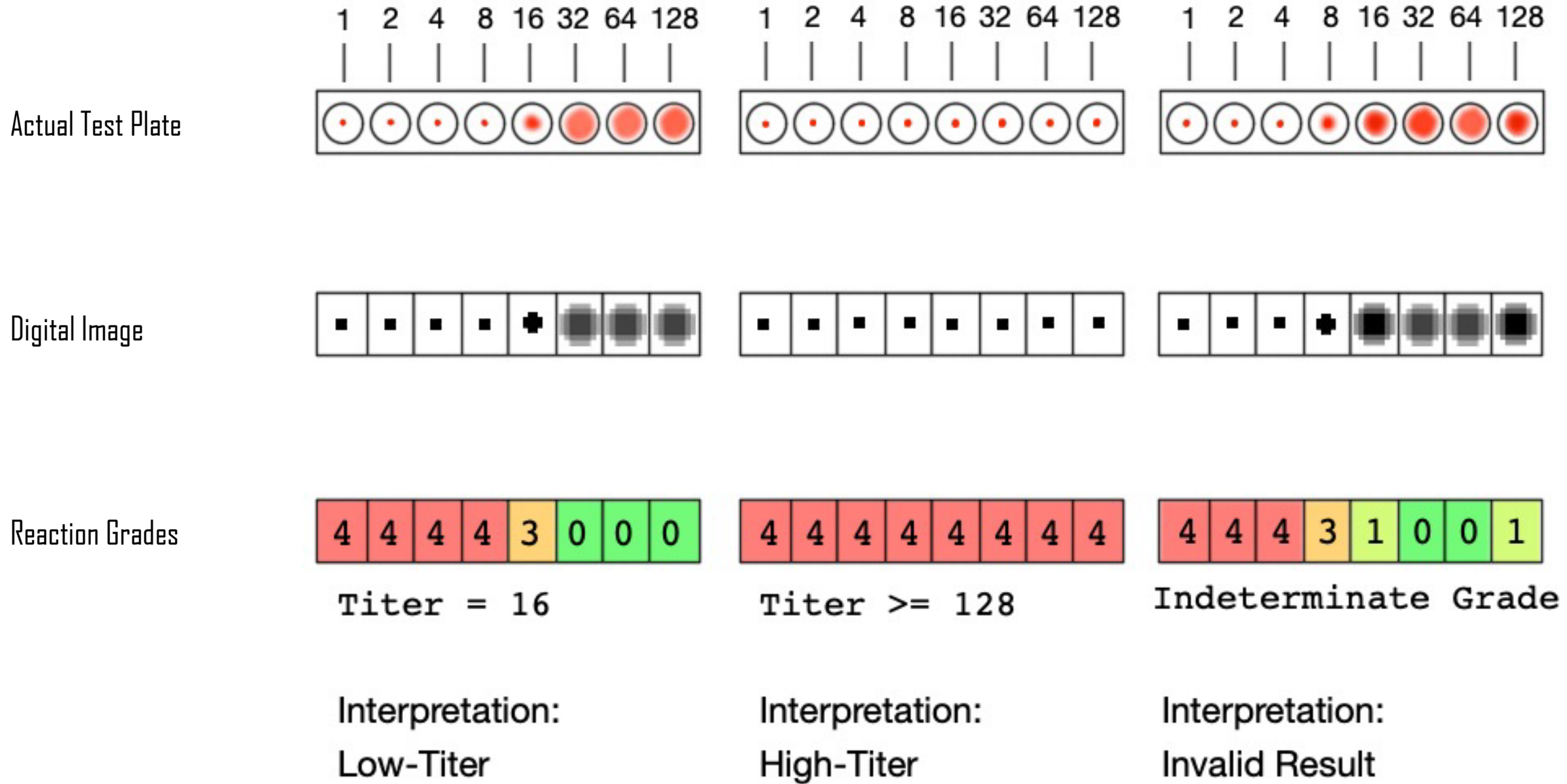
Discussion

Errors & Next Steps



NEO Iris[®]: What Happened?

- New machine, not yet validated
 - Is there a defective component?
- No maintenance prior to pilot study
 - Issue resolved?
 - Ongoing maintenance?
- Staff ran validation tests over weekend
 - Could they have changed certain settings?
- Week 1 samples tested daily, week 2 samples tested at end of week
 - Did the samples deteriorate?



Anti-B: When and Why to Test?

- All high-titer anti-B units were also high-titer anti-A (manual testing)
- Most high-titer anti-A units were low-titer anti-B
- Cost to perform vs. value of knowing unit is safe for B patients?

Possible Next Steps

- Study civilian patient outcomes
- Study titer demographics
- Motivate donors
- Study other methods for reducing titers (e.g., PAS)

Conclusions

- Automation has promise
- More work needed on this particular platform at this site
- Anti-B titration may have limited utility
- Useful for blood production, could factor into future studies

Special Thanks

- Memorial Blood Centers
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