Community Blood Center

Titration of Antibodies

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.

Objectives

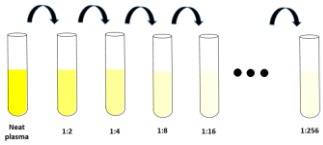
- Explain what antibody titration is.
- Interpret titer results.
- Discuss applications of antibody titration in the blood bank.

What is antibody titration?

- Semi-quantitative method to determine strength of an antibody
- Begins with serial dilution
  - Test each dilution against antigen-positive red cells
  - Observe which is the last dilution with 1+ reactivity

Antibody Titration:

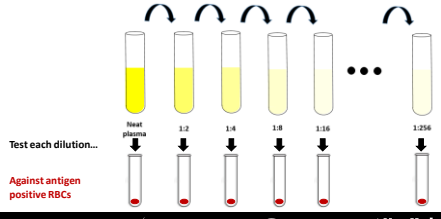
First step: serial dilution



Titration

Second Step: test each dilution against RBCs expressing corresponding antigen

- Utilize method/phase where antibody can be detected



Titration

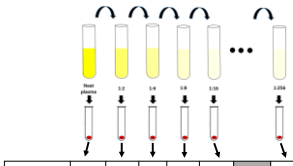
Second Step: test each dilution against RBCs expressing corresponding antigen

- Utilize method/phase where antibody can be detected

Patient's plasma contains anti-D

Test each dilution against the same D+ RBC

Record results



	Neat	1:2	1:4	1:8	1:16		1:256
D+ RBC, IAT	3+	2+	1+	0V	0V		0V

Titration

Third Step: endpoint of titer is last dilution with 1+ reactivity

- Reported as reciprocal of dilution

Patient's plasma contains anti-D

Test each dilution against the same D+ RBC

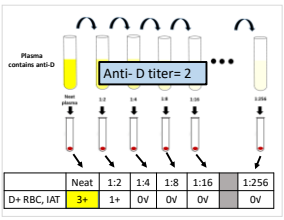
Record results

	Neat	1:2	1:4	1:8	1:16		1:25
D+ RBC, IAT	3+	2+	1+	0V	0V		0V

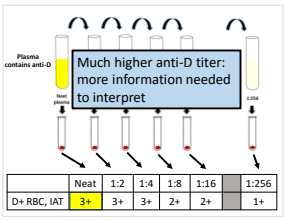
Last dilution with 1+ reactivity is 1:4 dilution

Anti-D titer = 4

What does a titer tell us?



	Neat	1:2	1:4	1:8	1:16		1:256
D+ RBC, IAT	3+	1+	0V	0V	0V		0V



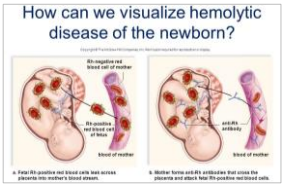
	Neat	1:2	1:4	1:8	1:16		1:256
D+ RBC, IAT	3+	3+	3+	2+	2+		1+

Applications of Titrations

- Monitoring at-risk pregnancies in alloimmunized mothers
- Isohemagglutinin (anti-A/anti-B titrations) titrations
  - Donors
  - Patients
- "HTLA" reactivity

Monitoring at-risk pregnancies

- Pregnant women with clinically significant antibodies at risk for Hemolytic Disease of the Fetus/ Newborn (HDFN)
  - IgG antibody crosses placenta and causes destruction of fetal RBCs
  - Anti-D or other clinically significant antibodies



What can titer results tell us?

- Is maternal antibody titer increasing over time?

Gestation	Plasma tested against	Neat	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
20 Weeks	c+ RBC, IAT	2+	1+	0V	0V	0V	0V	0V	0V	0V

What do we know?
- Anti-c clinically significant
- Anti-c can cause HDFN

What don't we know?
- Are fetal cells c+?
- What is the risk for HDFN?

Scenario 1: Is this pregnancy at risk for HDFN?

- Is maternal antibody titer increasing over time?

Gestation	Plasma tested against	Neat	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
20 Weeks	c+ RBC, IAT	2+	1+	0V	0V	0V	0V	0V	0V	0V
24 Weeks	c+ RBC, IAT	3+	2+	2+	1+	0V	0V	0V	0V	0V

Scenario 1: Is this pregnancy at risk for HDFN?

- Is maternal antibody titer increasing over time?

Gestation	Plasma tested against	Neat	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
20 Weeks	c+ RBC, IAT	2+	1+	0V	0V	0V	0V	0V	0V	0V
24 Weeks	c+ RBC, IAT	3+	2+	2+	1+	0V	0V	0V	0V	0V
28 Weeks	c+ RBC, IAT	3+	3+	2+	2+	2+	1+	1+	0V	0V

- Anti-c titer increased from 2 at 20 weeks to 64 at 28 weeks….
- Increasing titer indicates fetal cells are most likely c+
- Pregnancy at risk for HDFN

Switch to monitoring pregnancy with Doppler ultrasound

Scenario 2: Is this pregnancy at risk for HDFN?

- Is maternal antibody titer increasing over time?

Gestation	Plasma tested against	Neat	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
20 Weeks	c+ RBC, IAT	2+	1+	0V	0V	0V	0V	0V	0V	0V
24 Weeks	c+ RBC, IAT	2+	1+	0V	0V	0V	0V	0V	0V	0V
28 Weeks	c+ RBC, IAT	2+	1+	0V	0V	0V	0V	0V	0V	0V

- Anti-c titer steady over time at 2
- May indicate fetal cells are not c+

Continue monitoring pregnancy by antibody titration

Why is maternal antibody titration problematic?

- Must use consistent methodology
  - Often tube testing in saline IAT
- Serial dilution carries risk of error
  - Human error in pipetting
  - Inherent error in pipetting
- Subjectivity of grading hemagglutination reactions
  - What I call a 2+, you might call a 1+
- Antigen expression on RBCs varies
  - Homozygous vs heterozygous expression of antigen
- Variation of antigen sites per RBC
  - Example: Convention D antigen 10,000-33,000/RBC

Titration results: Problems with reproducibility

Mitigating the problems of reproducibility

- Current sample tested in parallel with previous sample
  - **Example:** previous sample anti-D titer of 4

Appears that current titer has increased to 8! Is this clinically significant?

	Plasma tested against	Neat	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
Current sample	D+ RBC, IAT	3+	2+	1+	1+	0V	0V	0V	0V	0V

Mitigating the problems of reproducibility

- **Current sample tested in parallel with previous sample**
  - Previous sample frozen for future testing
  - **Example:** previous sample anti-D titer of 4

	Plasma tested against	Neat	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
Current sample	D+ RBC, IAT	3+	2+	1+	1+	0V	0V	0V	0V	0V
Previous sample	D+ RBC, IAT	3++	2+	1+	1+	0V	0V			

However, re-testing of previous sample also shows a titer of 8

- **Institutions define what a "critical titer" is**
  - May be an increase in titer > 2 tubes
  - Some antibodies may have fixed critical titer (example: anti-D critical titer of 16)
- **Once "critical titer" is reached, monitor pregnancy by more sensitive method**
  - Doppler ultrasound

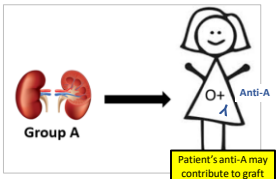
Applications of Titrations

- Monitoring at-risk pregnancies in alloimmunized mothers
- Isohemagglutinin (anti-A/anti-B) titrations
  - Donors
  - Patients
- "HTLA" reactivity

When do we need to know titers of Anti-A/Anti-B?

Patients receiving non-ABO identical solid organ transplants

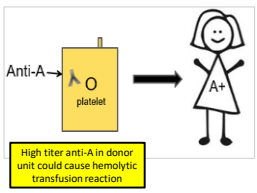
- Isohemagglutinin titers of transplant patients may be monitored to determine eligibility to receive a non-ABO identical organ.
- Titration of isohemagglutinins on ***PATIENT*** plasma



When do we need to know titers of Anti-A/Anti-B?

Donor plasma-containing products transfused to non-ABO identical recipient

- Examples:
  - Group O platelets transfused to group A patients
  - Group O whole blood to be used in trauma cases (recipient type unknown)
- Only "low titer" anti-A acceptable in these products
- Titration of isohemagglutinins on ***DONOR*** plasma (usually performed by blood center)



Low Titer O Whole Blood

- Used in trauma setting, potentially before recipient type is known
- Anti-A is titered in donor plasma
- Each institution defines what "low titer" is

Why only anti-A?

- Anti-A titers are higher than anti-B titers
- ~40% of population is group A, ~9% group B

Example of isohemagglutinin titration on donor products

Group O Platelets	1:100 dilution tested against A_1 cells at immediate spin
Unit 1	2+
Unit 2	1+
Unit 3	3+
Unit 4	0
Unit 5	0

Labeled: "Anti-A titer <100"

Isohemagglutinin titers

- Methods vary
  - Tube, gel, solid phase (automation)
  - IS phase appropriate, may include IAT
- No standard for performing isohemagglutinin titers
  - Each institution may use different method
  - Each institution may use different cut-offs for determining "low" or "high" titer

Applications of Titrations

- Monitoring at-risk pregnancies in alloimmunized mothers
- Isohemagglutinin (anti-A/anti-B titrations) titrations
  - Donors
  - Patients
- "HTLA" reactivity

"High Titer, Low Avidity"

"HTLA": this *characteristic reactivity* may help a reference lab identify some antibodies

- NOT A BLOOD GROUP SYSTEM!

Here are some blood group systems that have corresponding antibodies that demonstrate "HTLA" reactivity:

Knops, Ch/Rg, Cost, JMH antibodies

What is "HTLA" reactivity?

Usually, an antibody decreases in strength with every dilution

Titer (tested at IAT)	Neat plasma	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
Normal, strong antibody	4+	4+	3+	3+	2+	2+	1+	1+	0√
High titer, high avidity									

What is "HTLA" reactivity?

Titer (tested at IAT)	Neat plasma	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
Normal, strong antibody High titer, high avidity	4+	4+	3+	3+	2+	2+	1+	1+	0√
Normal, weak antibody Low titer, low avidity	1+	1+w	0√	0√	0√	0√	0√	0√	0√

Usually, weak antibodies have very low titer

What is "HTLA" reactivity?

Titer (tested at IAT)	Neat plasma	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
Normal, strong antibody High titer, high avidity	4+	4+	3+	3+	2+	2+	1+	1+	0√
Normal, weak antibody Low titer, low avidity	1+	1+w	0√	0√	0√	0√	0√	0√	0√
"High titer, low avidity"	1+	1+	1+	1+w	1+w	1+w	+/-	+/-	0√

**when performing titers for investigating "HTLA" reactivity, reactions are read microscopically until no reactivity is observed (rather than stopping at 1+).

In "HTLA" reactivity, weak reactions persist over multiple dilutions

Why is "HTLA" reactivity important

- May aid in identification of antibody
  - If unknown reactivity has "HTLA" characteristics, may point to an antibody in one of those blood groups **Knops, Ch/Rg, Cost, JMH antibodies**

"HTLA" is not a blood group system!

- **It is never appropriate to say a patient has "anti-HTLA" or "HTLA antibody"**
  - "HTLA" characteristic reactivity may aid in antibody identification
  - The antibody must be identified

Objectives

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