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

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**Antibody Titration:**

**First step:** serial dilution

![Serial dilution diagram](image)

**Second Step:** test each dilution against RBCs expressing corresponding antigen
- Utilize method/phase where antibody can be detected

![Titration diagram](image)
Second Step: test each dilution against RBCs expressing corresponding antigen
• Utilize method/phase where antibody can be detected

Third Step: endpoint of titer is last dilution with 1+ reactivity
• Reported as reciprocal of dilution

What does a titer tell us?
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?

<table>
<thead>
<tr>
<th>Gestation</th>
<th>Plasma tested against</th>
<th>neat</th>
<th>1:2</th>
<th>1:4</th>
<th>1:8</th>
<th>1:16</th>
<th>1:32</th>
<th>1:64</th>
<th>1:128</th>
<th>1:256</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 Weeks</td>
<td>c RBC, MAT</td>
<td>2+</td>
<td>1+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
</tbody>
</table>

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?

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<tr>
<td>20 Weeks</td>
<td>c RBC, IAT</td>
<td>2+</td>
<td>1+</td>
<td>DV</td>
<td>DV</td>
<td>DV</td>
<td>DV</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25 Weeks</td>
<td>c RBC, IAT</td>
<td>3+</td>
<td>2+</td>
<td>1+</td>
<td>DV</td>
<td>DV</td>
<td>DV</td>
<td>0</td>
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</table>

• 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

Scenario 2: Is this pregnancy at risk for HDFN?

• Is maternal antibody titer increasing over time?

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<td>DV</td>
<td>DV</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24 Weeks</td>
<td>c RBC, IAT</td>
<td>2+</td>
<td>1+</td>
<td>DV</td>
<td>DV</td>
<td>DV</td>
<td>DV</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>28 Weeks</td>
<td>c RBC, IAT</td>
<td>2+</td>
<td>2+</td>
<td>1+</td>
<td>DV</td>
<td>DV</td>
<td>DV</td>
<td>0</td>
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• Anti-c titer steady over time at 2
• May indicate fetal cells are not c+
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
- Subjectivity of grading hemagglutination reactions
  - What I call a 2+, you might call a 1+
- Antigen expression on RBCs varies
  - Hemoglobin vs heterozygous expression of antigen
- Variation of antigen sites per RBC
  - Example: Convention D antigen 10,000-33,000/RBC

Mitigating the problems of reproducibility

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

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<th>1:256</th>
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<tbody>
<tr>
<td>In RBC, IAT</td>
<td>3+</td>
<td>2+</td>
<td>1+</td>
<td>1+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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Appears that current titer has increased to 8! Is this clinically significant?

- 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

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<td>1+</td>
<td>1+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</table>

However, retesting 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

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
- Example:
  - 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)

High titer anti-A in donor unit could cause hemolytic transfusion reaction
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

<table>
<thead>
<tr>
<th>Group O Platelets</th>
<th>1:100 dilution tested against A cells at immediate spin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit 1</td>
<td>2+</td>
</tr>
<tr>
<td>Unit 2</td>
<td>1+</td>
</tr>
<tr>
<td>Unit 3</td>
<td>3+</td>
</tr>
<tr>
<td>Unit 4</td>
<td>0</td>
</tr>
<tr>
<td>Unit 5</td>
<td>0</td>
</tr>
</tbody>
</table>

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?

<table>
<thead>
<tr>
<th>Titer (Sample Dilution)</th>
<th>Neat Plasma</th>
<th>1:2</th>
<th>1:4</th>
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</thead>
<tbody>
<tr>
<td>Normal, strong antibody</td>
<td>4+</td>
<td>4+</td>
<td>3+</td>
<td>2+</td>
<td>2+</td>
<td>1+</td>
<td>1+</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>High titer, high avidity</td>
<td>4+</td>
<td>4+</td>
<td>3+</td>
<td>2+</td>
<td>2+</td>
<td>1+</td>
<td>1+</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Usually, an antibody decreases in strength with every dilution.
What is “HTLA” reactivity?

<table>
<thead>
<tr>
<th>Titer (tested at IAT)</th>
<th>Neat Plasma</th>
<th>1:2</th>
<th>1:4</th>
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<td>3+</td>
<td>2+</td>
<td>2+</td>
<td>1+</td>
<td>1+</td>
<td>0√</td>
</tr>
<tr>
<td>High titer, high avidity</td>
<td>4+</td>
<td>4+</td>
<td>3+</td>
<td>3+</td>
<td>2+</td>
<td>2+</td>
<td>1+</td>
<td>1+</td>
<td>0√</td>
</tr>
<tr>
<td>Normal, weak antibody</td>
<td>1+</td>
<td>1+w</td>
<td>0√</td>
<td>0√</td>
<td>0√</td>
<td>0√</td>
<td>0√</td>
<td>0√</td>
<td>0√</td>
</tr>
<tr>
<td>Low titer, low avidity</td>
<td>1+</td>
<td>1+w</td>
<td>0√</td>
<td>0√</td>
<td>0√</td>
<td>0√</td>
<td>0√</td>
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</tr>
</tbody>
</table>

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

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

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