## 2019 Advanced Track Webinars

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<tbody>
<tr>
<td>9 January 2019</td>
<td>ADAMTS-13 Testing</td>
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<tr>
<td>6 March 2019</td>
<td>Advanced Antibody Work-Up Techniques: MMA &amp; Molecular Testing</td>
</tr>
<tr>
<td>15 May 2019</td>
<td>Monoclonal Therapy</td>
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<td>Sickle Cell: In the Transfusion Service and In Real Life!</td>
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**Link to register:**

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BLOOD BANKING

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<td>17 April 2019</td>
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<td>30 October 2019</td>
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RISE OF THE SUPERHEROES OF SEROLOGY
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• PACE, Florida and California DHS
• 1.0 Contact Hours
• Each attendee must register to receive CE at: https://www.surveymonkey.com/r/AntibodyBodyDetectionTest
• Registration deadline is May 3, 2019
• Certificates will be sent via email only to those who have registered May 17, 2019
Questions?

- You are all muted
- Q&A following session - Type in questions
• Course content is for information and illustration purposes only. Immucor makes no representation or warranties about the accuracy or reliability of the information presented, and this information is not to be used for clinical or maintenance evaluations.

• The opinions contained in this presentation are those of the presenter and do not necessarily reflect those of Immucor.
It’s an “Antibody Detection Test”, my Dear Watson

Dawn M Rumsey, ART, BB(ASCP)CM
Global Support Engineer, Reagents
Immucor Inc
“Detection is, or ought to be, an exact science, and should be treated in the same cold and unemotional manner.”

The Sign of Four

“There is nothing like first-hand evidence.”

A Study in Scarlet

“Before we start to investigate, let us try to realize what we do know, so as to make the most of it, and to separate the essential from the accidental.”

The Adventure of the Priory School

Sherlock Holmes
Antibodies

There are 5 classes of immunoglobulin. IgG has 4 subclasses.

<table>
<thead>
<tr>
<th>Name</th>
<th>Properties</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>Found in mucous, saliva, tears, and breast milk. Protects against pathogens.</td>
<td><img src="image1" alt="IgA structure" /></td>
</tr>
<tr>
<td>IgD</td>
<td>Part of the B cell receptor. Activates basophils and mast cells.</td>
<td><img src="image2" alt="IgD structure" /></td>
</tr>
<tr>
<td>IgE</td>
<td>Protects against parasitic worms. Responsible for allergic reactions.</td>
<td><img src="image3" alt="IgE structure" /></td>
</tr>
<tr>
<td>IgG</td>
<td>Secreted by plasma cells in the blood. Able to cross the placenta into the fetus.</td>
<td><img src="image4" alt="IgG structure" /></td>
</tr>
<tr>
<td>IgM</td>
<td>May be attached to the surface of a B cell or secreted into the blood. Responsible for early stages of immunity.</td>
<td><img src="image5" alt="IgM structure" /></td>
</tr>
</tbody>
</table>
IgG

- **Fab**: Fragment antigen-binding
- **Fc**: Constant fragment
- **V:** Variable domain
- **C:** Constant domain
- **H, L:** Heavy, light chains

Antigen-binding site

Papain cleavage sites

**C = constant domain**

**V = variable domain**

**H, L = heavy, light chains**
IgM

Structure
- Pentamer (19S)
- Extra domain (C_H4)
- J chain

J Chain

C_H4
Direct versus Indirect

Direct agglutination caused by IgM

Sensitized by IgG + bridging antibody = Indirect agglutination
Hemolysis

When the RBC membrane ruptures, the hemoglobin in solution is clear.

Intact RBCs make a suspension look cloudy.
Why do we do it?

After determining the patient or donor ABO/Rh, we want to know:

- if there is already a defense mechanism in place to attack any foreign invader? ie. Does the patient have a history of an antibody?
- If there is a means of protecting part of our “self” should it end up in a foreign situation? ie. Does the donor have an antibody that could harm the recipient?

To solve both questions in the Transfusion Medicine arena, we need to perform an Antibody Detection Test (ADT).
Antigen Antibody Reactions

- Ag/Ab reactions must be visualized to be interpreted properly

- Can be done by:
  - Agglutination (tube, or gel micro columns)
  - Adherence (solid phase)
  - Hemolysis
  - Precipitation
  - Color change (Absorbance, Florescence)

- The most common methods used in Blood Banking are:
  - Direct or indirect agglutination using an antiglobulin reagent
  - Hemolysis
How Does an Antibody Detection Work?

The ADT provides the correct conditions to visualize a reaction between plasma and red blood cells (RBCs).

Agglutination is the result of two distinct phases:
   I. Antibody uptake (Sensitization)
   II. RBC binding (Agglutination).
Phases of Agglutination

Phase I

Antibody uptake or “Sensitization”
Factors Affecting Sensitization¹

Knowing what can help or hurt antibody uptake can help us troubleshoot an unexpected result:

- **Incubation time**
  - Allows time to reach equilibrium

- **Temperature**
  - IgM - below 37°C
  - IgG - 37°C

- **pH**
  - Neutral range (6.7 - 7.3)

- ** Ionic strength**
  - Lower ionic strength aids antibody uptake

- **Antigen distribution**
  - Numbers of Ag per RBC is predetermined

- **Ag: Ab ratio**
  - Increase antibody to enhance reaction.
Phases of Agglutination

Phase II

Can be either IgG or IgM

Visible agglutination as RBCs bind into clumps
Factors Affecting Binding

Knowing what pushes the reaction to completion helps increase detection of small amounts of antibody on the RBC:

- Positively charged molecules
- Location/position of antigen
- Zeta potential
Zeta Potential

Figure 5 – Schematic representation of zeta potential. Erythrocytes (negative charges) in suspension causing a rearrangement of charges through the formation of two ionic layers that generate a electric potential difference between them, called the Zeta potential (Modified from Pollack & Reckel, 1977 and Rouger & Salmon, 1981).
The Antiglobulin Test

♦ Coombs, Race and Mourant in 1945

♦ Can be performed by 2 methods
  ♦ Direct Antiglobulin (DAT)
  ♦ Indirect Antiglobulin (IAT)

♦ The IAT is used to detect antibodies in vitro
Different Types of Antiglobulin Reagents

- The first reagent was a broad spectrum sera that could bind with any human globulin found on the RBC.

- Reagents made in rabbits were the first licensed.

- Eventually separated into anti-IgG and anti-C3.
Different Types of Antiglobulin Reagents

- The very first m/c antibody was anti-C3d
- Now we have several different m/c reagents for both C3b & C3d
- M/C anti-IgG
The monoclonal Anti-IgG reagent in use contains an IgM antibody directed at IgG. This assistance enables the coated RBC to bind into agglutinates large enough for us to see.
Antibody Screen Cells

- Traditional tube testing
- Can be transitioned to automated platforms
- FDA-licensed and application is determined by who is being tested.
  - per AABB Standards, only donors may be screened using a pooled cell product.
  - Patients must be tested using the 2, 3 or 4 cell configurations
Are We Talking About Genes or Antigens? Zygosity or Dosage?

The number of antigens on an RBC is genetically determined and a function of the characteristics of its Blood Group System.

- Genes can be either Homozygous or Heterozygous
  - ie. $Fy^A/Fy^A$ or $FY^A/FY^B$

- Antigens show Dosage:
  - Double dose $Fy^{(a+b-)}$ or Single dose $Fy^{(a+b+)}$

Homozygous genes produce double dose antigens. Heterozygous genes produce one dose of antigen for each gene present.
The ongoing debate about screen cells

- The choice of screen cell format needs to be fine-tuned to fit your methods and patient population.

- Automation has minimized some of the differences.

- Below we have the original case based on tube methods.

<table>
<thead>
<tr>
<th></th>
<th>Pooled Cell</th>
<th>2 - Cell</th>
<th>3 - Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contains a D- cell</td>
<td>no</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Faster TAT</td>
<td>Fastest</td>
<td>Average</td>
<td>Slowest</td>
</tr>
<tr>
<td>Sensitivity by ↑↑ Ag sites</td>
<td>&lt; sensitive</td>
<td>&gt;pooled, &lt;3-cell</td>
<td>Best</td>
</tr>
<tr>
<td>Guaranteed JK &amp; FY double dose</td>
<td>no</td>
<td>maybe</td>
<td>yes</td>
</tr>
</tbody>
</table>
Group O Reagent Red Blood Cells used in the detection or identification of unexpected antibodies shall include at least the following common antigens in each lot of the product: D, C, E, c, e, K, k, Fy^a, Fy^b, Jk^a, Jk^b, Le^a, Le^b, P_1, M, N, S, and Ss.
# Two-cell Screen Cells

## PANOSCREEN

### Master List

**IMMUCOR, INC. Norcross, GA 30071 USA**  
**US LICENSE NO: 886**  
**LOT NO: 06538**  
**EXPIRES: 2019/04/19**

<table>
<thead>
<tr>
<th>VIAL</th>
<th>Donor</th>
<th>Rh - Hr</th>
<th>Kell</th>
<th>Duffy</th>
<th>Kidd</th>
<th>Lewis</th>
<th>P</th>
<th>MN</th>
<th>Lutheran</th>
<th>Xg</th>
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<tbody>
<tr>
<td>I</td>
<td>R1R1 B9673</td>
<td>+ + 0 0 + 0 + 0 + 0 + 0 + 0 + 0 + 0 + 0</td>
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<td></td>
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</tr>
</tbody>
</table>

* Indicates those antigens whose presence or absence may have been determined using only a single example of a specific antibody.  
An antigen designated with a 'w' represents a weakened expression of the antigen that may or may not react with all examples of the corresponding antibody.
# Three-cell Screen Cells

## PANOSCREEN Master List

**IMMUCOR, INC. Norcross, GA 30071 USA**

**US LICENSE NO:** 886  
**LOT NO:** 07549  
**EXPIRES:** 2019/04/26

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<th>Kidd</th>
<th>Lewis</th>
<th>P</th>
<th>MN</th>
<th>Lutheran</th>
<th>Xg</th>
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<td>rr N2078</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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An antigen designated with a ‘w’ represents a weakened expression of the antigen that may or may not react with all examples of the corresponding antibody.
This patient, whose red cells show extreme anisopoikilocytosis, must be suffering from a bizarre anemia that defies classification.

It is obviously hemolytic, the plasma hemoglobin level being 150 mg%; 30% of red cells show markedly increased fragility and hemolysis;

he is obviously diabetic, blood sugar being 450 mp%,

also probably in renal failure with elevated plasma potassium and inorganic phosphate, as well as high BUN,

also severe acidosis, the pH being 6.65.

How long has this patient been dead?
Effects of Storage on Screen Cells

- As any stored RBC ages, it loses membrane integrity, and level of glucose available to sustain its metabolism.

- Most research in this area focuses on RBC for transfusion and the clinical outcomes.
Effects of Storage on Screen Cells

- For reagent RBCs it is most important to maintain the antigenicity of the RBC to provide consistent testing outcomes during the dating period of the product.

- Antigens known to lose activity faster than most: Le\(^a\), c, Fy\(^a\).
We know there are exceptions to the exact science of antibody detection. (but it’s still science)

By understanding the nature of this test, we can exploit those factors which affect the test to improve reactivity. These results contribute to our decisions on how to move forward to identify the antibody.

We know that specific characteristics of current products offer us choices to maximize the efficiency of service to our patients and donors.

We understand the importance of clear communication of scientific facts.

We know that aging isn’t kind to anyone 😊


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We like you!

Like us on social media!
Thank you!