Blood Bank---

*Reviewing the basics*

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Objectives:

- Define the most commonly performed Blood Bank tests and related terminology.

- Discuss sample collection and routine pre-transfusion testing.

- Evaluate unexpected results and options for transfusing patients in urgent situations.
Sample Collection & Patient ID

Patient Misidentification----Leading cause of transfusion fatalities

From CAP Website: “Mistransfusion occurs from

– misidentification of the intended recipient at the time of collection of the pretransfusion testing sample,
– during laboratory testing and preparation of units to be issued, and
– at the time of transfusion.

– Misidentification at sample collection occurs approximately once in every 1,000 samples, and
– in one in every 12,000 transfusions the recipient receives a unit not intended for or not properly selected for him/her.”
Dr. Richard Friedberg stated:

“If the label’s wrong from the get-go, everything else we do about accuracy and reliability and precision goes out the window.”

From the Q-Probes study: one in 2,500 samples has the WRONG BLOOD in TUBE.
AABB Requirements for patient sample identification and labeling in Stds. 25th ed.

Std. 5.11: Samples and Requests
- “Identifying information for the patient and the blood sample shall correspond and be confirmed at the time of collection using two identifiers.”
AABB Std. 5.11.2

▪ “Blood samples should be identified with an affixed label bearing sufficient information for unique identification of the patient, including two independent identifiers.”

▪ 5.11.2.1: “The completed label shall be affixed to the tubes before the person who drew the specimen leaves the side of the patient.”
CAP-TRM.30575: Does the facility have a plan to implement a system to reduce the risk of mistransfusion for non-emergent red cell transfusions?

Options:

– Collect and test second sample
– Mechanical barrier
– Electronic ID system
– Another System to reduce risk
Sample Age: AABB Std 5.13.3.2

“If the patient has been transfused in the preceding 3 months with blood or a component containing allogeneic red cells, has been pregnant within the preceding 3 months, or the history is uncertain or unavailable, a sample shall be obtained from the patient within 3 days of the scheduled transfusion. Day 0 is the day of draw.”
Sample Age–Continued

- Extended time limited by the type of sample collected.

- Refer to manufacturers instruction for reagents used for compatibility testing.
  - Time period for use of EDTA varies from 7 to 14 days.
Patient History: AABB 5.13.5.

“There shall be a process to ensure that the historical records listed below have been reviewed and compared to current records and that discrepancies have been investigated and appropriate action taken before a unit is issued for transfusion.”
Patient History, *continued*

- Evaluation of patient information
- Diagnosis
- Race
- Medication
- Transfusion history
- Transplant
- Pregnancy
ABO Typing Reagents

- Monoclonal—only commercial source
- Check manufacturers instructions for clone information when performing investigations
- Anti-A,B vs. Anti-A + -B
- Weak A Ag expression readily detectable by routine monoclonal Anti-A reagents.
- New clone of anti-B does not routinely detect Acquired B.
Reagent Red cells for reverse grouping

- Requirement to be Rh negative (D-)

- No requirements for other red cells antigens; they could be M+ or Le(a+)

- Pool of at least 2 donors
Common causes of ABO discrepancies

- Most discrepancies due unexpected antibody are due to Anti-A$_1$
  - 2$^{nd}$ most common is due to other cold reactive antibodies (anti-M, Cold autoantibody)

- Most subgroups of A are routinely detectable
  - Seldom noticed unless there is a serum grouping issue.
Case 1

- Where is the discrepancy observed?
- Which is the most probable cause of the results observed?
- What can be done to resolve it?
- What blood type should be issued in an emergency if discrepancy not resolved?

<table>
<thead>
<tr>
<th>CELL TYPING ANTI</th>
<th>SERUM GROUPING</th>
</tr>
</thead>
<tbody>
<tr>
<td>-A</td>
<td>A₁ cell</td>
</tr>
<tr>
<td>-B</td>
<td>B cell</td>
</tr>
<tr>
<td>3+</td>
<td>2+</td>
</tr>
<tr>
<td>0</td>
<td>4+</td>
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</tbody>
</table>
Case 1-continued

<table>
<thead>
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<th></th>
<th>SERUM GROUPING</th>
</tr>
</thead>
<tbody>
<tr>
<td>-A</td>
<td>-B</td>
<td>A₁ cell</td>
</tr>
<tr>
<td>3+</td>
<td>0</td>
<td>2+</td>
</tr>
</tbody>
</table>

Weaker that expected reaction with A₁ cell
- Subgroup of A?
- Testing of A₂, O red cell and Auto Ctrl provide more info.

- Until resolved, transfuse
  Red Cells: group O
  Plasma: group AB
The need is constant.
The gratification is instant.
Give blood.

Case 2

<table>
<thead>
<tr>
<th>CELL TYING ANTI</th>
<th>A</th>
<th>B</th>
<th>A₁ CELL</th>
<th>B CELL</th>
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<tr>
<td>-A</td>
<td>3+</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-B</td>
<td></td>
<td></td>
<td>2+</td>
<td>4+</td>
</tr>
</tbody>
</table>

-Where is the discrepancy observed?
-Which is the most probable cause of the results observed?
-What can be done to resolve it?
-What blood type should be issued in an emergency if discrepancy not resolved?
Case 2-continued

-Weaker than expected rxn. with A₁ cell
-Subgroup of A or?
-Testing of A₂, O red cell and AC suggest the presence of cold reactive antibody
-Red Cells: group A
Plasma: group O

<table>
<thead>
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<tr>
<td>-A</td>
<td>A₁ cell</td>
</tr>
<tr>
<td>-B</td>
<td>A₂ cell</td>
</tr>
<tr>
<td></td>
<td>B cell</td>
</tr>
<tr>
<td></td>
<td>O cell</td>
</tr>
<tr>
<td></td>
<td>AC</td>
</tr>
<tr>
<td>3+</td>
<td>2+</td>
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<td>3+</td>
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<td>4+</td>
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<tr>
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<td>3+</td>
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<tr>
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<td>0</td>
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Case 3

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<td>ANTI</td>
<td>A₁ cell</td>
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<td>-A</td>
<td>0</td>
</tr>
<tr>
<td>-B</td>
<td>0</td>
</tr>
</tbody>
</table>

- Where is the discrepancy observed?
- Which is the most probable cause of the results observed?
- What can be done to resolve it?
- What blood type should be issued in an emergency if discrepancy not resolved?
**Case 3-continued**

Weaker than expected rxn. with A1 cell; A2 negative

Subgroup of A?

Testing of A2, O red cell and AC suggest possible A sub group.

Red Cells: group O
Plasma: group AB

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<thead>
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</tr>
</thead>
<tbody>
<tr>
<td>-A, -B</td>
<td>A₁ cell</td>
</tr>
<tr>
<td>0, 0</td>
<td>2+</td>
</tr>
</tbody>
</table>
Routine Rh (D) typing testing requirements

AABB STD 5.13.2:

“Rh type shall be determined with anti-D reagent. The test for weak D is unnecessary when testing the patient.”
Anti-D (Rh₀) Antisera

- Monoclonal reagents widely used
- Human polyclonal reagents still available
- Check manufacturers instructions for clone information when performing investigations.
  - Very useful information when resolving discrepancies.
Anti-D: Unusual findings

- Individuals previously typed D-; now type D+

- I.S. reactivity that may not persist at 37°C or IAT
  - i.e. Crawford, Rh²\text{Har}
The need is constant.
The gratification is instant.
Give blood.™

# Clones in Commercial Monoclonal Anti-D Reagents

<table>
<thead>
<tr>
<th>Anti-D</th>
<th>Immucor</th>
<th>Ortho</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gamma-clone</td>
<td>BioClone</td>
<td></td>
</tr>
<tr>
<td>Gama401</td>
<td>MAD2</td>
<td>HUMAN</td>
</tr>
<tr>
<td>F8D8</td>
<td>Gel Card</td>
<td></td>
</tr>
<tr>
<td>Immucor Series 4</td>
<td>MS201</td>
<td>MS201</td>
</tr>
<tr>
<td>MS26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immucor Series 5</td>
<td>TH28</td>
<td></td>
</tr>
<tr>
<td>MS26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

American Red Cross
D typing and Pregnancy

AABB STD 5.20.2: “Women who are pregnant or have been pregnant recently shall be considered for Rh Immune Globulin administration when all the following apply:

1) The woman’s test for D Antigen is negative. A test for weak D is not required.

2) The woman is not actively immunize to the D antigen.”
### Case # 4

Patient typed as Rh negative during 2 pregnancies between 1985 and 1990.

<table>
<thead>
<tr>
<th>Now:</th>
<th>Anti-D</th>
<th>Rh Ctrl</th>
</tr>
</thead>
<tbody>
<tr>
<td>–</td>
<td>2+</td>
<td>0</td>
</tr>
</tbody>
</table>

*Is additional needed?*
Case #4- *Continued*

**Was the correct patient collected?**
- Recollect and repeat typing.

**Could the Rh typing be a false positive?**
- Results are valid. Testing performed at I.S. and Rh Control is negative.

- Monoclonal anti-D is specific for D antigen.
  - If testing performed with polyclonal anti-D, the presence of antibody to a low frequency antigen is possible. Perform testing with a 2nd source of anti-D.
Direct Antiglobulin Test-DAT

Definition: Test used to demonstrate in-vivo coating of red cells with antibodies or complement, in particular IgG and C3d.

Antiglobulin reagents

- Monoclonal
- Blends (rabbit IgG and monoclonal C3)
- Gel card contains Rabbit IgG
- Solid phase indicator cell is coated with monoclonal Anti-IgG
DAT- Testing reminders

*Strict adherence to critical test steps needed!*
- Wash RBCs at least 3 times without delay
- Spin immediately after adding AHG
- Resuspend and read immediately after spin.

*When DAT is positive, what next?*
- Perform testing with anti-IgG and anti-C3 reagents to determine the sensitizing protein.
Elution

**Definition:** Method to free and recover antibody from sensitized red cells.

**When to perform an eluate:**
- DAT is positive with IgG
- DAT is negative- in certain instances when trying to explain decrease red cell survival

**Time period to perform?**
- “Recently transfused” patients, which is commonly defined as transfused within the last 14 days. In other labs, this period is extended to 21 days.
Albumin

- Not considered to be an antibody enhancement reagent

- Does not provide commonly accepted level of sensitivity

- Due to the molecular size, Rh antibodies may react stronger in albumin compared to saline methods.
LISS-additive for tube testing

Advantages:

- Detects most potentially clinically significant blood group antibodies.

- Less sensitive than PEG for detection of warm reactive autoantibodies.

- May facilitate identification of antibodies demonstrating reactivity at 37°C phase of testing. Anti-Le\(^a\), -Le\(^b\), -M and -N and other direct agglutinins may be identified using this method.
LISS-additive for tube testing

Disadvantages:

- Less sensitive than gel or PeG/IAT in detection of some clinically significant blood group antibodies and warm reactive autoantibodies.

- Enhanced detection of most cold reactive autoantibodies.
GEL: Column agglutination technology

Advantages:

- Detects most potentially clinically significant blood group antibodies.

- Uses smaller volumes of serum and reagents.

- Reactions are stable for up to 24 hours.

- Standardized procedure for improved consistency.
GEL: Column agglutination technology

Disadvantages:

- Somewhat less sensitive for detection of some alloantibodies.
- Enhanced detection of warm reactive autoantibodies.
- Requires special equipment and reagent preparation.
Solid Phase: *Advantages*

- Provides stable, well-defined endpoints of the reaction.

- Enhanced sensitivity makes the detection of weak alloantibodies easier.

- Ease of use.

- No predilution of reagents is required.

- Standardized procedure for improved consistency when manufacturer’s panels are tested.
Solid Phase: Disadvantages

- Requires a centrifuge that can spin microplates.

- Requires a 37°C incubator for microplates.

- Requires a light source for reading the final results (non-automated method).

- Increased sensitivity may detect weak autoantibodies that other systems do not.
Case # 5

18 y.o. male with sickle cell disease
- Admitted with acute chest syndrome
- No history of transfusion at your facility

Results of antibody screen with LISS:

<table>
<thead>
<tr>
<th>Antibody</th>
<th>37°C</th>
<th>IAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC I</td>
<td>0</td>
<td>2+</td>
</tr>
<tr>
<td>SC II</td>
<td>2+</td>
<td>4+</td>
</tr>
<tr>
<td>SC III</td>
<td>0</td>
<td>3+</td>
</tr>
</tbody>
</table>

The need is constant.
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Comparison of antibody screening results: LISS vs. PEG:

<table>
<thead>
<tr>
<th></th>
<th>LISS</th>
<th>37C</th>
<th>IAT</th>
<th>PEG/IAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC I</td>
<td></td>
<td>0</td>
<td>2+</td>
<td>3+</td>
</tr>
<tr>
<td>SC II</td>
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<td>SC III</td>
<td></td>
<td>0</td>
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<td>4+</td>
</tr>
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</table>

*Which method is most informative?*
Case # 5-continued

Comparison of antibody screening results: LISS vs. PEG:

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<th>LISS</th>
<th>37C</th>
<th>IAT</th>
<th>PEG/IAT</th>
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</thead>
<tbody>
<tr>
<td>SC I</td>
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<td>0</td>
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</table>

More about this case later.
Antibody identification (Ab-ID)

- Method to identify specificity (ies) of antibodies detected by antibody screen.

- Approaches vary based on
  - staff expertise,
  - available resources, and
  - urgency of patient’s transfusion need.
Ab-ID: Standard Panel vs. Selected panel

- **Standard panel (full):**
  - Designed to identify commonly encountered specificities.
  - Resolves most routine antibody problems.
  - Provides limited information for complex problems or when multiple specificities have been previously identified.

- **Extended panels:** 10 vs. 16 cell panels designed to help separate multiple specificities.
Ab-ID: Selected cell panel

- Panel of cells selected using available information:
  - Ab screen results, pt. history, pt. phenotype

- Requires use of multiple panels and some experience and/or software to create

- Maximizes information obtained with the least amount of sample and testing.
Case # 5 continued......

• LISS
  – indicates the presence of >1 antibody
  – will probably identify one antibody specificity

• Additional testing: auto control? DAT?
  – Phenotype the patient’s red cells?
  – Routine or selected cell panel?
    ▪ Anti-E + -Fya are identified
  – Rule out of additional alloantibodies: PEG?
Automation

- Available instruments capable of performing most BB tests.

- Routinely used for basic testing: ABO-Rh, antibody screening. Ab–ID also available.

- Methodologies used:
  - Gel
  - Solid phase
  - Hemagglutination
Automation

Advantages:

- Minimizes hands-on time
- Positive sample identification
- Standardizes testing
- Records easily archived

Disadvantages:

- TAT can be greater than manual testing
- Flexibility for STAT testing varies
  - May detect unwanted antibody or antibody of undetermined specificity
In closing….

Be observant and inquisitive.

Use available resources to aid in problem resolution:

– patient, family, clinical staff, literature, manufacturers, colleagues, internet……..

Advancements occur everyday in Transfusion Medicine…..

Be an active participant!
If you have questions or comments, feel free to contact me at

harev@usa.redcross.org

Phone: 770-852-4376