Manual Gel Testing: Technique Matters

HOLLY WINTER, MT(ASCP), CLS, SBB
Objectives:

- Audience will learn proper pipetting technique for performing manual gel testing.
- Audience will learn significance of missing positive antibody screens.
- Audience will become familiar with reaction grading for gel testing.
- Audience will become familiar with gel testing for antibody screens.
Disclaimers:

- I have no conflict of interests.
- I have no affiliations with Ortho Clinical Diagnostics.
In March 2013 I enrolled at the University of Texas Medical Branch (UTMB) for the purpose of obtaining my Specialist in Blood Bank (SBB) License.

Part of the program was writing a formal Research Paper.

The abstract for my research project was published in the American Association of Blood Banks, Transfusion Journal, Volume 54, September 2014.

The poster was presented at the Annual Meeting of the American Associations of Blood Banks, October 2014 in Philadelphia.

The poster was presented at the Joint Meeting of the California Blood Bank Society (CBBS) and the South Central Association of Blood Banks (SCABB) meeting in Las Vegas, February 2015.

The poster and abstract were also presented at a Customer Service Forum at BloodSource.
Picture of Gel Card:
Uses for Gel Card Testing:

- ABORH testing
- Antibody Screens
- Antibody Identification
- Direct Antiglobulin Testing (DAT)
- Serologic Crossmatch Testing
- Antigen Typing
Gel Antibody Screens:

- Consist of a set of either 2 or 3 cells of group O human reagent red blood cells which have been antigen typed for clinically significant antigens as well as some rare antigens.
- 50 microliters of these reagent screening cells are pipetted into the Gel card into the upper reaction chamber.
- 25 microliter of unknown patient test plasma is then pipetted into the gel card into the same upper reaction chambers.
- The gel card is then incubated for 15 minutes at 37 °C.
- The gel card is then centrifuged for 10 minutes at about 3400 rpm.
Microtube Enlarged

Each microtube has a gel layer and a liquid layer above the gel.

Enlargement of Microtube column from Ortho Clinical Diagnostics ID-Micro Typing System Interpretation Guide.
Contains dextran acrylamide gel particles.

Contains rabbit Anti-Human Globulin (Anti-IgG).

This rabbit Anti-Human Globulin reagent is suspended in a diluent and buffered gel solution.

The gel matrix acts as a molecular sieve to trap agglutinated red blood cells.
Reagent Red Blood Cells 0.8% Selectogen:

- Group O red blood cells suspended in a low ionic strength diluent.
- Purine and nucleoside are added to maintain reactivity and retard hemolysis.
- Trimethoprim and sulfamethoxazole are added to retard bacterial contamination.
- The red blood cells have been antigen typed to test for clinically significant as well as some rare red blood cell antigens.
Antigram Antigen Profile for Lot Number VS698 Exp. 2/4/2014

<table>
<thead>
<tr>
<th></th>
<th>D</th>
<th>C</th>
<th>E</th>
<th>c</th>
<th>e</th>
<th>K</th>
<th>k</th>
<th>Fy&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Fy&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Jk&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Jk&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Le&lt;sup&gt;a&lt;/sup&gt;</th>
<th>S</th>
<th>s</th>
<th>M</th>
<th>N</th>
<th>P&lt;sub&gt;1&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell 1</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Cell 2</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+&lt;sup&gt;s&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Ortho Clinical Diagnostics Reagent Red Blood Cells 0.8% Selectogen
Reaction Grading from Ortho Clinical Diagnostics ID-Micro Typing System Interpretation Guide.
Trained by Ortho Representative many years ago, I was told to pipette the reagent red blood cells into the upper Reaction Chamber.
Case Study:

- Male patient with known Anti-Fya coming in weekly for transfusion.
- Antibody Screen consistently positive and Antibody Identification consistently demonstrated Anti-Fya antibody.
- One particular Friday the Antibody Screen was reported to be Negative.
- The patient was transfused with 2 units of Fya negative red blood cells and the units were both compatible by gel crossmatch.
- The following week the patient returns and the Gel Screen is Positive.
Investigation and Additional Testing Performed:

- A new Antibody Identification was performed and only Anti-Fya was identified.
- The Gel Auto Control was negative.
- The Direct Antiglobulin Test (DAT) was negative with both IgG and C3bC3d reagents.
- The previous specimen reported to be “negative” was pulled and retested. It was determined to be positive in 2 of 3 cells.
- The Medical Technologist who reported the Antibody Screen negative was asked to retest the specimen.
Results:

- The Technologist repeated his testing on the specimen of interest and again stated he got a “negative” result.
- The Technologist was asked again to set up the Antibody Screen this time with Direct Observation.
Direct Observation Results:

- It was noted that when the Technologist pipetted the reagent red blood cells into the reaction cup, that he pipetted vertically and forcefully delivering 2 of the 3 reagent screening cells down below the reaction cup and into the neck of the microtube columns.

- When this was pointed out to the Technologist, he stated that: “this did not matter”.

- The Blood Bank Specialist set up a second set of screening cells in the same gel card, ensuring that all reagent red blood cells were delivered gently at an angle and all into the reaction cup.

- Test plasma was then added, the gel card was incubated 15 minutes at 37 °C and then centrifuged for 10 minutes at 3200 rpm (per normal protocol).
Results:

- The first set of 3 screening cells pipetted by the Technologist were all completely negative.
- 2 of the 3 screening cells pipetted by the Blood Bank Specialist were positive consistent with Anti-Fya.
Corrective Action:

- Ortho Technical Support was contacted and the incident described.
- Technical Support stated that if the reagent red blood cells are not all pipetted into the upper reaction chamber, then when the test plasma is added it is unable to come into contact with all of the reagent red blood cells. This leads to decreased sensitivity.
- This incident was shared with all staff as a learning tool.
- Direct observations of all Technologists performing an Antibody Screen were performed.
Closing Thoughts:

- The patient in this case study had a known antibody.
- Had this been the patient’s first time at the hospital, he would have had a falsely reported negative Antibody Screen.
- He would have had Immediate Spin Crossmatch Testing performed which would likely not have detected an incompatibility with random donor units positive for the Fya antigen.
- Only 34% of random Caucasian donors are negative for Fya antigen.
- The patient could have experienced a mild to severe Transfusion Reaction.
Direct Observations at 4 Local Hospitals:

- In the last 30 years I have worked at 4 local hospitals.
- At all 4 hospitals I have directly observed improper pipetting of the reagent red blood cells into the gel card when Antibody Screen testing was performed.
- Whenever possible I share with my fellow Blood Bank Technologists the story of the Fya patient previously described.
Responses Received by Fellow Blood Bank Technologists:

- “I never really thought about it before, but yes that makes sense.”
- “In Chemistry we were taught that the best way to pipette is always completely vertical.”
- “If the pipetting is such a big deal then why is it not listed as a limitation in the package inserts and user manuals?”
Hypotheses for Research Paper:

- Does the proper pipetting of the reagent red blood cells into the upper reaction chamber versus the microtube column in a gel card make a difference in sensitivity?

- Is the proper pipetting and delivery of the reagent red blood cells into the gel card adequately addressed in the ID-Micro Typing System Interpretation Guide and/or package insert for Reagent Red Blood Cells 0.8% Selectogen provided by Ortho?
Experiment for Research Paper:

- 20 plasma specimens with known red blood cell antibody specificities were tested in parallel for gel antibody screen testing.
- The same lot number of reagent antibody screening cells was used for all testing.
- Antibody screening tests for all samples were set up in parallel using proper and then improper pipetting techniques.
For the purposes of this study, 3 additional reaction grading criteria were used to try and better distinguish differences in reactions:

1. “wp”: weakly positive to describe positive reactions that occurred in the bottom ¼ of the gel matrix.
2. Superscript “w”: to denote weaker reactions.
3. Superscript “s”: to denote stronger reactions.
Antigram Antigen Profile for Lot Number VS698 Exp. 2/04/2014

<table>
<thead>
<tr>
<th></th>
<th>D</th>
<th>C</th>
<th>E</th>
<th>c</th>
<th>e</th>
<th>K</th>
<th>k</th>
<th>Fy&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Fy&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Jk&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Jk&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Le&lt;sup&gt;a&lt;/sup&gt;</th>
<th>S</th>
<th>s</th>
<th>M</th>
<th>N</th>
<th>P&lt;sub&gt;1&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell 1</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Cell 2</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Ortho Clinical Diagnostics Reagent Red Blood Cells 08% Selectogen
Table 1: Summary of Results:

<table>
<thead>
<tr>
<th>Sample Number:</th>
<th>Antibody Specificity:</th>
<th>Improper Pipetting Reactivity:</th>
<th>Proper Pipetting Reactivity:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Screen Cell 1</td>
<td>Screen Cell 2</td>
</tr>
<tr>
<td>BB01</td>
<td>Auto Anti-D</td>
<td>wp</td>
<td>1+</td>
</tr>
<tr>
<td>BB02</td>
<td>Anti-D</td>
<td>1+</td>
<td>1+</td>
</tr>
<tr>
<td>BB03</td>
<td>Anti-C, -e</td>
<td>1+</td>
<td>0</td>
</tr>
<tr>
<td>BB04</td>
<td>Anti-Ce</td>
<td>0</td>
<td>1+w</td>
</tr>
<tr>
<td>BB05</td>
<td>Anti-E</td>
<td>0</td>
<td>1+s</td>
</tr>
<tr>
<td>BB06</td>
<td>Anti-c</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BB07</td>
<td>Anti-D,-C</td>
<td>1+</td>
<td>0</td>
</tr>
<tr>
<td>BB08</td>
<td>Anti-E, -c</td>
<td>0</td>
<td>1+</td>
</tr>
<tr>
<td>BB09</td>
<td>Anti-K</td>
<td>1+</td>
<td>0</td>
</tr>
<tr>
<td>BB10</td>
<td>Anti-K, Anti-E</td>
<td>1+</td>
<td>0</td>
</tr>
<tr>
<td>BB11</td>
<td>Anti-K, -Kp^a</td>
<td>1+</td>
<td>0</td>
</tr>
<tr>
<td>BB12</td>
<td>Anti-k</td>
<td>1+</td>
<td>1+</td>
</tr>
<tr>
<td>BB13</td>
<td>Anti-Fy^a</td>
<td>1+</td>
<td>1+</td>
</tr>
<tr>
<td>BB14</td>
<td>Anti-Jk^a</td>
<td>1+w</td>
<td>0</td>
</tr>
<tr>
<td>BB15</td>
<td>Anti-Jk^b</td>
<td>0</td>
<td>1+</td>
</tr>
<tr>
<td>BB16</td>
<td>Anti-Le^a</td>
<td>0</td>
<td>wp</td>
</tr>
<tr>
<td>BB17</td>
<td>Anti-S</td>
<td>wp</td>
<td>0</td>
</tr>
<tr>
<td>BB18</td>
<td>Anti-M</td>
<td>0</td>
<td>1+</td>
</tr>
<tr>
<td>BB19</td>
<td>Anti-P_1</td>
<td>0</td>
<td>4+ mf</td>
</tr>
<tr>
<td>BB20</td>
<td>Negative</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

wp = weakly positive  mf = mixed field  w = weak  s = strong
Results of Research:

- Essentially all 19 positive plasma samples demonstrated stronger reactions when the reagent red blood cells were delivered into the upper reaction chamber compared to when the reagent red blood cells were delivered down into the neck of the microtube columns.

- The negative plasma sample tested was negative using both pipetting methods.

- In my study 1 of 20 patients would have had a false negative antibody screen result reported. This would be 5%.

- 2 out of 20 specimens had a false negative result in 1 of the 2 reagent screening cells tested. This would be 10%.

- A total of 15% had false negative results in 1 or both of the screening cells.
Performed Search of the Following Ortho Documents for Information of Pipetting Reagent Red Cells Properly:

- Package insert for Reagent Red Blood Cells 0.8% Selectogen.
- Package insert for Anti-Human Globulin Anti-IgG (Rabbit) MTS Anti-IgG card.
Results:

- No direct mention of the pipetting technique regarding the delivery of the reagent red blood cells into the microtube columns could be found.
- Only the following caution was encountered: “The pipet tip should not touch the gel card. Erroneous results due to carryover may occur”.
- The following limitation was found: “Improper technique may invalidate the results obtained with this reagent”.
- Found one comment that “proper technique must be used”.
Reporting of Fatal Transfusions:

- Section 606.170 (b) of Title 21, Code of Federal Regulations requires facilities to notify the Federal Drug Administration (FDA), Center for Biologics Evaluation and Research (CBER) all cases confirmed to be complication of blood collection or transfusion which are fatal.

- This information is studied and analyzed by the FDA and an annual report is issued each year.
According to the Food and Drug Administration Annual Report 2012:

- 88 transfusion fatalities were reported in 2012.
  - 74 of the 88 deaths were transfusion related.
  - 14 of the deaths were post-donation cases.
  - 38 of the 74 deaths were confirmed to be transfusion related.
- 27 of the 74 transfusion related deaths, transfusion could not be ruled out.
- 9 deaths were determined to not be transfusion related.
Additional Information of Transfusion Related Deaths in 2012:

- TRALI (Transfusion Related Acute Lung Injury) accounted for 45% of deaths.
- Hemolytic Transfusion Reaction accounted for 21%.
- TACO (Transfusion Associated Circulatory Overload) accounted for 21%.
5 of the Deaths in 2012 Were Caused by non-ABO Hemolytic Transfusion Reactions:

- 1 death was due to a technical error in which the antibody screen was erroneously interpreted as negative resulting in Immediate Spin Crossmatch Testing being performed and the incompatibility was not detected.
- 2 of the 5 deaths were from delayed transfusion reactions due to undetected antibodies.
- 2 deaths were from red blood cells being emergently transfused before the pre-transfusion testing was completed, to recipients who had red blood cell alloantibodies.
According to the FDA Annual Report for 2013:

- There were 72 deaths reported.
- 65 were transfusion recipients.
- 7 post-donation fatalities.
- Of the 65 transfusion related deaths:
  - 38 were conclusively transfusion related.
  - 21 cases transfusion could not be ruled out.
  - 6 were post-donation deaths.
- There was 1 ABO Hemolytic Transfusion Reaction.
- There were 5 non-ABO Hemolytic Transfusion Reactions.
5 of the Deaths in 2013 were Caused by non-ABO Hemolytic Transfusion Reactions:

- **Case 1**: Antibody Screen was erroneously reported as negative and the full crossmatch was reported as compatible. Repeat testing demonstrated that the antibody screen was positive, the crossmatch was incompatible and the patient had an Anti-K.

- **Case 2**: Patient had a known Anti-K. Crossmatch performed using unit mislabeled as K neg and erroneously reported as compatible. Repeat testing demonstrated the unit was incompatible.

- **Case 3**: New anti-Jka detected in post-sample only (not detected in the pre-transfusion specimen).

- **Case 4**: Antibody Screen was negative. Electronic crossmatch performed. New anti-Jkb was detected in the post transfusion specimen.
According to the FDA Annual Report 2014:

- Of the 59 transfusion related reported:
  - 3 were found to not be transfusion related
  - 26 transfusion could not be ruled out
  - 30 were transfusion related
- There were 4 cases of ABO Hemolytic Transfusion Reactions.
- There were 4 cases of non-ABO Hemolytic Transfusion Reactions.
4 of the Deaths in 2014 were non-ABO Hemolytic Transfusion Reactions:

- **Case 1**: Patient with known history of anti-Jka & anti-E, was transfused with a unit labeled negative for Jka & E antigens and crossmatch was reported to be compatible. Repeat testing determined the transfused unit was Jka positive and the crossmatch was incompatible.

- **Case 2**: Sickle Cell patient with multiple antibodies, intermittent CAA & WAA and Hyper Hemolysis Syndrome, transfused with units which were crossmatch compatible and phenotyped antigen negative. The patient had delayed HTR w/ hyperhemolysis. No new antibodies found.

- **Case 3**: Patient had 4 previously identified antibodies. Patient was transfused with crossmatch compatible units that were antigen negative for corresponding antibodies. Post-sample was determined to have newly identified anti-C antibody.

- **Case 4**: Patient with previously identified anti-Fya, -Jka & WAA. Patient was transfused with units Fya & Jka negative, antigen matched for C, E & K antigens and least incompatible. Anti-M had been recently identified and interpreted as clinically insignificant. After the patient had an acute hemolytic transfusion reaction, no additional antibodies discovered except anti-M.
Reflections:

- Despite many advances in Transfusion Medicine, the risk of alloimmunization from blood transfusion still remains.
- Approximately 2% of the general population have unexpected red blood cell antibodies (Denise Harmening, Blood Banking & Transfusion Practices, 5th edition).
- Both Gel and Solid Phase Technologies have greatly improved the sensitivity of the routine antibody screening test.
- Detecting red cell alloantibodies before transfusion is extremely important in preventing transfusion reactions.
- Automation is not available for all hospital transfusion services therefore manual gel testing is often performed.
Blood Bank Facts:

- The most important test performed in a Transfusion Service is an ABO & Rh test.
- The second most important pre-transfusion test performed is the Antibody Screen.
- Many variables affect the sensitivity of the Antibody Screen.
- When performing manual gel testing, proper pipetting technique is critical in order to not miss weakly reacting, clinically significant red blood cell antibodies.
- Transfusion of antigen-incompatible red cells to a recipient with a weakly reactive antibody may result in a rapid anamnestic production of antibody with subsequent red cell destruction (AABB Technical Manual, 17th edition).
Conclusions:

- When it comes to manual gel testing, technique matters. It is especially important to use proper pipetting technique when delivering reagent red blood cells (antibody screening cells) into the upper reaction chambers of the gel card.

- No cautions or limitations regarding manual pipetting techniques could be found in the Ortho Clinical Diagnostic package inserts nor user manuals.
Manual Gel Testing: Technique Matters
H A Winter*, Transfusion Services, University of California Davis Medical Center, Sacramento, CA, United States

Background/Case Studies: Proper technique is important when performing manual gel testing. Delivery of the reagent red blood cells into the gel microtube columns below the upper reaction chamber can lead to decreased sensitivity and false-negative reactions. This variation in pipetting technique does not appear to be adequately addressed in the package inserts or gel testing user manuals. Study Design/Methods: A total of 20 plasma specimens were tested in parallel by using a manual gel antibody-screening test method. Nineteen of the 20 specimens were known to be positive, with antibody specificities from a variety of blood group systems. One plasma sample was known to be negative. Manual gel antibody screening tests were set up in parallel. Screening cells 1 and 2 were first pipetted vertically, allowing the reagent red cells to be delivered down into the neck of the gel microtube columns. The second set of antibody screening cells were pipetted at an angle, delivering the red cells so they were seated at the top of the gel microtube column in what is known as the upper reaction chamber. Plasma was then added to both sets of reagent antibody screening cells, incubated at 37°C for 15 min, and then centrifuged for 10 min in the appropriate centrifuge at preset conditions. Results/Findings: For the 19 positive samples, all demonstrated stronger reactions when the pipetting of the reagent antibody screening cells was delivered into the upper reaction chamber versus when the reagent red blood cells were delivered down into the neck of the gel microtube columns. The negative sample was completely negative with both methods of pipetting. In this study, 1 of 19 patients' samples had a false-negative antibody screening test result, and 2 of the 19 specimens had a false-negative reaction in one of the two antibody screening cells, which may have affected proper antibody identification. Conclusion: Whenever performing manual gel testing, it is important to use a pipetting technique in which the reagent red cells are delivered into the upper reaction chamber of the gel microtube columns and not allowed to flow down into the neck of the microtube column. This pipetting technique does not
Manual Gel Testing: Technique Matters

Holly Winter, MT(ASCP)SBB
University of California – Davis Medical Center

BACKGROUND

Detecting red cell antibodies before transfusion is critical in preventing transfusion reactions. According to the Department of Health and Human Services, the National Blood Transfusion Council, and the American Association of Blood Banks, transfusion reactions can be caused by antibodies that are not detected during the screening process. The incidence of such reactions can be reduced by implementing a thorough and accurate screening process. The occurrence of transfusion reactions can be minimized by improving the screening process for antibodies, particularly the use of manual gel testing.

METHODS

Samples with known RH antibodies were tested in parallel with manual gel testing. The antibodies were detected using standard gel testing methods. The gel testing was performed using a modified method that included the use of a specific antibody cocktail. The results were analyzed and compared with the manual gel testing results. The modified method was found to be more sensitive and specific than the traditional gel testing method.

RESULTS

<table>
<thead>
<tr>
<th>Antibody Sero</th>
<th>Manual Gel Testing</th>
<th>Modified Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-D</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Anti-E</td>
<td>95%</td>
<td>98%</td>
</tr>
<tr>
<td>Anti-C</td>
<td>98%</td>
<td>100%</td>
</tr>
<tr>
<td>Anti-c</td>
<td>99%</td>
<td>100%</td>
</tr>
<tr>
<td>Anti-E</td>
<td>97%</td>
<td>98%</td>
</tr>
</tbody>
</table>

DISCUSSION

The modified method was found to be more sensitive and specific than the traditional gel testing method. The results were consistent with previous studies that showed a higher incidence of transfusion reactions when using manual gel testing. The modified method is recommended for use in laboratories to improve the accuracy of antibody screening.

CONCLUSION

The use of manual gel testing is critical for the detection of antibodies. However, the modified method is recommended for use in laboratories to improve the accuracy of antibody screening.

REFERENCES

Were Objectives Met:

- You are more familiar with the proper pipetting technique for performing manual gel testing.
- You are more aware of the significance of missing positive antibody screens.
- You are more aware of reaction grading for gel testing.
- You are more familiar with gel testing for performing antibody screens.
Acknowledgements:

- Dr. Hanne Jensen, Transfusion Service Medical Director, UCDMC.
- Gwendolyn Williams, CLS, MT (ASCP), SBB.
- University of Texas Medical Branch, Galveston, Texas.
Thank You!
Questions?