Immunohematology Case Studies
2017 - 5

Dr. Divjot Singh Lamba
div834@gmail.com
Assistant Professor,
Dept. of IHBT,
GGSMCH, Faridkot,
Punjab, India.
Clinical History

47 year male donor, who donated in voluntary blood donation camp. Repeat donor who had donated 4 times in the past with last donation 1 year ago.

Sample of the donor received in immunohematology lab for blood group discrepancy – Preliminary testing by technical person by tube method reported as RBCs grouping as O positive and serum grouping as A

<table>
<thead>
<tr>
<th>Anti- A</th>
<th>Anti- B</th>
<th>Anti- D</th>
<th>Anti-AB</th>
<th>A cell</th>
<th>B cell</th>
<th>O cell</th>
<th>Auto control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>4+</td>
<td>0</td>
<td>0</td>
<td>4+</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Serologic History

Blood grouping both CELL and SERUM done by tube method as per departmental SOP.

**ABO/Rh:**

<table>
<thead>
<tr>
<th>Anti- A</th>
<th>Anti- B</th>
<th>Anti- D</th>
<th>Anti-AB</th>
<th>A cell</th>
<th>B cell</th>
<th>O cell</th>
<th>Auto control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak+ (delayed)</td>
<td>0</td>
<td>4+ (delayed)</td>
<td>Weak+ (delayed)</td>
<td>0</td>
<td>4+</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

As cell and serum grouping results don’t match it is a blood group discrepancy
Serologic History (continued)

Extended blood grouping done by tube method

<table>
<thead>
<tr>
<th></th>
<th>Anti-AB</th>
<th>Anti-A1</th>
<th>Anti-H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak+ (delayed)</td>
<td>0</td>
<td>0</td>
<td>4+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serum grouping</th>
<th>Immediate Spin (IS)</th>
<th>Room Temperature (RT)</th>
<th>Anti Human Globulin (AHG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A cell</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B cell</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>O cell</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Auto control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Preliminary Blood group: A\text{\textsubscript{weak}} RhD positive
Current Sample Presentation Data

DAT: Negative by gel technology (Bio-Rad)

Antibody Screen Method: Bio-Rad 3 cell panel
Antibody Screen Results: Negative

<table>
<thead>
<tr>
<th>SCREEN I</th>
<th>SCREEN II</th>
<th>SCREEN III</th>
<th>AUTO</th>
<th>DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Challenge with the Current Presentation

There are a few challenges in this current presentation which need to be solved:

1. Cell and serum blood groupings do not match: Blood group in initial testing is O RhD Positive and in extended tests is $A_{\text{weak}}$ RhD Positive.
   Possibility of $A_{\text{weak}}$ subtype that needs to be confirmed.

2. Group discrepancy detected on detailed testing from tube sample needs to be confirmed from bag sample to rule out any technical error during taking donation from the donor.
Possible Answers and Next Steps

A\textsubscript{weak} RhD positive blood group of donor needs to be confirmed by:

1. Cold adsorption technique using anti-A from pooled serum of B donors followed by heat elution, for confirmation of A\textsubscript{weak} phenotype.

2. Blood group extended testing and cold adsorption to be performed from sample obtained from donor bag (from bag tube segment post stripping) as well for final confirmation of donor blood group.
Further Work

Cold adsorption and Heat Elution techniques to be performed on Donor tube Sample:

Washed Donor Cells

Cold Adsorption of washed cells with anti-A from pooled type B donor serum at 4°C for 90mins
Washed 5 to 6 times

Supernatant of last wash preserved

Packed cells mixed with equal volume of 4% Bovine serum albumin
Further Work (Continued)

Heat elution of the mixture done at 56\(^0\)C for 10 mins in a water bath with intermittent shaking

Centrifuged at 3000 rpm for 3 mins and supernatant (Eluate) obtained

Eluate subjected to further testing with ABO pooled reagent cells as follows:
Result of cold adsorption and heat elution steps: Positive reactivity of A cells with eluate confirms presence of adsorbed anti-A in the eluate obtained after heat elution of cold adsorbed donor cells thus confirming $A_{\text{weak}}$ status of donor cells.

<table>
<thead>
<tr>
<th>Plain Card</th>
<th>Eluate</th>
<th>Last Wash</th>
</tr>
</thead>
<tbody>
<tr>
<td>A cell</td>
<td>3+</td>
<td>0</td>
</tr>
<tr>
<td>B cell</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O cell</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Updated Clinical Information

1. Telephone contact with the donor – saying thank you for blood donation and explaining him the problem being faced in confirmation of his blood group.

2. Apologies for delay in dispatching of his donor card.

3. Take history of any previous donation if any, and any previous documentation of his blood group or any similar difficulty faced in confirmation of his blood group in the past.
Further Testing Results and Interpretations

Final confirmation of donor blood group from donor bag –
Cold adsorption and heat elution steps from bag samples (from bag tube segment post stripping) as done previously with donor tube Sample.
Conclusions

Final Donor Blood group is: $A_{\text{weak}}$ RhD Positive
and donor given final blood group report.

Donor to be considered as A positive and if required to be transfused; to receive O positive RBC units and A positive/AB positive FFP/PC.

Of note, this transfusion recommendation may change depending on countries.
Summary of Case Challenges

1. $A_{\text{weak}}$ ABO typing confirmation.
2. Use of cold adsorption technique.
3. Use of heat elution technique.

Other possibility to further study this case: Molecular investigation of the $ABO$ gene
Lessons Learned by the Case

1. Cell grouping and serum grouping must be performed by Tube method as a Gold standard.

2. Serum grouping step is very important in blood group confirmation and cell and serum grouping results must correlate.

3. Cell washing step is very important in tube testing

4. Final blood group confirmation in case of donor blood group discrepancy must be done by a specialized immunohematology lab with special tests like cold adsorption techniques and heat elution techniques.