ABO Anomalies
Ruth Smith
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Landsteiner's Law

- States that:

  "The lack of an A or B Antigen results in the production of the corresponding Antibody"

<table>
<thead>
<tr>
<th>Red blood cell type</th>
<th>Group A</th>
<th>Group B</th>
<th>Group AB</th>
<th>Group O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibodies in Plasma</td>
<td>Anti-B</td>
<td>Anti-A</td>
<td>None</td>
<td>Anti-A and Anti-B</td>
</tr>
<tr>
<td>Antigens in Red Blood Cell</td>
<td>A antigen</td>
<td>B antigen</td>
<td>A and B antigens</td>
<td></td>
</tr>
</tbody>
</table>

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## Expected grouping results

<table>
<thead>
<tr>
<th>ABO Group</th>
<th>Red cells vs Anti A</th>
<th>Red cells vs Anti B</th>
<th>Plasma vs A1 Cells</th>
<th>Plasma vs B Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>AB</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>O</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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Anomalous Results

- Reactions which do not fit into the expected pattern need further investigation. NEVER assign a blood group until all investigations are complete.
- Full grouping MUST be performed on all new patients/donors.
- Repeat test to ensure reactions are genuine.
- If test performed manually rule out any technical errors such as incorrect tube labelling, transposition, SOP not followed etc.
- If discrepancy is genuine additional tests may need to be performed to try and resolve the problem.

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Additional Antigens

- Unexpected positive reactions in the forward group.
- Acquired B Antigen (very unlikely)
- Red cells sensitised with another antibody e.g. Warm or Cold AIHA.
- Chimerism
- Panagglutination (Whartons jelly)
Missing Antigens

- Unexpected negative/weak reactions in the forward group.
- ABO subgroup
- Disease state e.g. leukaemia/cancer
- Foetus/Newborn with poorly developed antigens.
Missing Antibodies.

- Missing/weak reactions in the reverse group.
- Disease state – Hypogammaglobulinemia
- Age of individual (<5 years or >80 years)
- Post ABO incompatible bone marrow/stem cell transplant
- Transfusion of plasma components/plasma exchange
Additional Antibodies

- Unexpected positive reactions with A or B cells in the reverse group.
- Cold reacting alloantibodies e.g. Anti A1, Anti M, Anti P1
- Cold reacting autoantibodies e.g. Anti I
- Transfusion of ABO incompatible plasma products
- Treatment with Intravenous Immunoglobulin (IVIg)
Mixed Field Reactions

- Sample has 2 distinct populations
- ABO mismatched red cell transfusion
- Subgroup e.g. A3
- Chimerism
- BMT of different ABO type

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Investigation of anomalous results (1)

- Before performing any additional tests:
- Repeat group
- Check historical group (if one available)
- Check diagnosis
- Check transfusion history
- Request fresh sample
- These steps may save time!
Investigation of anomalous results (2)

- If discrepancy appears to be genuine:
  - Repeat group using different method
  - Consider extended incubation times at either 4 degrees C or 37 degrees C as appropriate
  - Use extended grouping reagents if available
  - Consider Antibody screen/investigation/auto control
  - Consider using washed red cells

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## MANUAL GROUPING WORKSHEET

<table>
<thead>
<tr>
<th>Patient's Name</th>
<th>Hospital Number</th>
<th>Sample Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Manual tube group

<table>
<thead>
<tr>
<th>Test</th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-D</th>
<th>AB Serum</th>
<th>A1 cells</th>
<th>A2 cells</th>
<th>B cells</th>
<th>O cells</th>
<th>Auto</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 rr control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2 control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B rr control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O R1r</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### A1 subtyping

<table>
<thead>
<tr>
<th>Test</th>
<th>A1 cells</th>
<th>A2 cells</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AB serum</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 3 cell screen

<table>
<thead>
<tr>
<th>Test</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell 1</td>
<td>KK (cell)</td>
</tr>
<tr>
<td>Cell 2</td>
<td>Kk (cell)</td>
</tr>
</tbody>
</table>

### Reagents

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Bombay Phenotype

- **H Allele (HH or Hh)**
  - Produces H Transferase Enzyme
  - Precursor Substance

- **O Allele**
  - No Enzyme
  - No Conversion
  - H Antigen

- **A/B Allele**
  - A/B Transferase Enzyme
  - A/B Substance

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Precursor substance

H antigen

A antigen

B antigen

Key to symbols:
- R = 'remainder' of the chain
- GlcNAc = N-acetyl-D-glucosamine
- Gal = D-galactose
- Fuc = L-fucose
- GalNAC = N-acetyl-D-galactosamine
THE ABO GENETIC PATHWAY

HH or Hh genotype

PRECURSOR SUBSTANCE

h genotype

UNCHANGED PRECURSOR SUBSTANCE

ABO GENES (Enzymes)

A

A

A and B

AB

O (none)

B

UNCHANGED PRECURSOR SUBSTANCE

(no A or B antigens)

H SUBSTANCE

A

B

H

UNCHANGED PRECURSOR SUBSTANCE

(no A or B antigens)

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# Bombay Grouping Results

<table>
<thead>
<tr>
<th></th>
<th>Anti A</th>
<th>Anti B</th>
<th>Anti H</th>
<th>A1 cells</th>
<th>B cells</th>
<th>O cells</th>
<th>Auto</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group O</td>
<td>0</td>
<td>0</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group O (h)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>0</td>
</tr>
</tbody>
</table>

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Example 1

<table>
<thead>
<tr>
<th>Red Cells vs Anti A</th>
<th>Red Cells vs Anti B</th>
<th>Plasma vs A1 Cells</th>
<th>Plasma vs B Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>4+</td>
<td>0</td>
<td>2+</td>
<td>4+</td>
</tr>
</tbody>
</table>

Problem:
Cause:
Resolution:

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Example 1 cont

<table>
<thead>
<tr>
<th>Anti A</th>
<th>Anti B</th>
<th>Anti A1</th>
<th>AB Serum</th>
<th>A1 Cells</th>
<th>A2 Cells</th>
<th>B Cells</th>
<th>O Cells</th>
<th>Auto</th>
</tr>
</thead>
<tbody>
<tr>
<td>4+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2+</td>
<td>0</td>
<td>4+</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
### Example 1 cont

<table>
<thead>
<tr>
<th></th>
<th>Anti A</th>
<th>Anti B</th>
<th>Anti A1</th>
<th>AB Serum</th>
<th>A1 Cells</th>
<th>A2 Cells</th>
<th>B Cells</th>
<th>O Cells</th>
<th>Auto</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4+</td>
<td>0</td>
<td>4+</td>
<td>0</td>
<td>2+</td>
<td>2+</td>
<td>4+</td>
<td>2+</td>
<td>0</td>
</tr>
</tbody>
</table>
Example 2

<table>
<thead>
<tr>
<th>Red Cells vs Anti A</th>
<th>Red Cells vs Anti B</th>
<th>Plasma vs A1 Cells</th>
<th>Plasma vs B Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>3+</td>
<td>1+</td>
<td>0</td>
<td>4+</td>
</tr>
</tbody>
</table>

Problem:
Cause:
Resolution:

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Example 3

<table>
<thead>
<tr>
<th>Red cells vs Anti A</th>
<th>Red cells vs Anti B</th>
<th>Plasma vs A1 cells</th>
<th>Plasma vs B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3+</td>
</tr>
</tbody>
</table>

Problem:
Cause:
Resolution:

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## Example 3 cont.

<table>
<thead>
<tr>
<th></th>
<th>Red cells vs Anti A</th>
<th>Red cells vs Anti B</th>
<th>Red cells vs Anti AB</th>
<th>Plasma vs A1 cells</th>
<th>Plasma vs B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>1+</td>
<td>0</td>
<td>3+</td>
</tr>
</tbody>
</table>

Indicates weak subgroup of A
- e.g. Ax

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### Example 4

<table>
<thead>
<tr>
<th>Red cells vs Anti A</th>
<th>Red cells vs Anti B</th>
<th>Plasma vs A1 cells</th>
<th>Plasma vs B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>MF</td>
<td>0</td>
<td>1+</td>
<td>2+</td>
</tr>
</tbody>
</table>

**Problem:**

**Cause:**

**Resolution:**

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Example 5

<table>
<thead>
<tr>
<th>Red cells vs Anti A</th>
<th>Red cells vs Anti B</th>
<th>Plasma vs A1 cells</th>
<th>Plasma vs B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Problem:
Cause:
Resolution:

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### Example 6

<table>
<thead>
<tr>
<th></th>
<th>Red cells vs Anti A</th>
<th>Red cells vs Anti B</th>
<th>Control</th>
<th>Plasma vs A1 cells</th>
<th>Plasma vs B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

**Problem:**

**Cause:**

**Resolution:**

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### Example 7

<table>
<thead>
<tr>
<th>Red cells vs Anti A</th>
<th>Red cells vs Anti B</th>
<th>Plasma vs A1 cells</th>
<th>Plasma vs B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>4+</td>
<td>4+</td>
<td>0</td>
<td>1+</td>
</tr>
</tbody>
</table>

**Problem:**

**Cause:**

**Resolution:**

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Example 7 cont.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Antibody Screen</th>
<th>Auto control</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>Positive</td>
<td>Negative</td>
<td>Cold alloantibody</td>
</tr>
<tr>
<td>Patient 2</td>
<td>Positive</td>
<td>Positive</td>
<td>Cold autoantibody</td>
</tr>
</tbody>
</table>

Cold alloantibody – perform identification panel
Cold autoantibody - Use pre warming technique

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### Example 8 (Patient JJ)

<table>
<thead>
<tr>
<th></th>
<th>Red cells vs Anti A</th>
<th>Red cells vs Anti B</th>
<th>Plasma vs A1 cells</th>
<th>Plasma vs B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>3+</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Problem:**

**Cause:**

**Resolution:**

---

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**Example 9**

<table>
<thead>
<tr>
<th>Red cells Vs Anti A</th>
<th>Red cells Vs Anti B</th>
<th>Plasma Vs A1 cells</th>
<th>Plasma Vs B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>MF</td>
<td>2+</td>
<td>+/-</td>
</tr>
</tbody>
</table>

**Cause:**

**Problem:**

**Resolution:**

*Innovation and excellence in health and care*
Remember:

- NEVER report a group until all investigations are complete.
- Only issue group O red cells until discrepancy has been resolved.
- Do NOT assume that the forward group is correct.
Thank you for listening.
Any Questions???