Introduction to ABO and Rh Grouping Errors

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ABO Discrepancies

- **Definition:** ABO discrepancies happen when there is no match in results between forward and reverse ABO grouping.

- the ABO system is the most important blood group system in relation to transfusions.

- Misinterpreting ABO discrepancies could be life threatening to patients

- If a real discrepancy is encountered, the results must be recorded

- However, the interpretation is delayed until the discrepancy is resolved
How this event is happened?

I. Reaction strengths could be weaker than expected

II. Some reactions may be missing in the reverse or forward typing

III. Extra reactions may occur
What is next step?

1) Identify the problem

2) Most of the time, the problem is technical
   a) Mislabeled tube
   b) Failure to add reagent
   c) Either repeat test on same sample, request a new sample, or wash cells

3) Other times, there is a real discrepancy due to problems with the patient’s red cells or serum
Technical Errors

A. **Clerical errors**
   1. Mislabeled tubes
   2. Patient misidentification
   3. Inaccurate interpretations recorded
   4. Transcription error
   5. Computer entry error

B. **Reagent or equipment problems**
   1. Using expired reagents
   2. Using an un-calibrated centrifuge
   3. Contaminated or hemolyzed reagents
   4. Incorrect storage temperatures

C. **Procedural errors**
   1. Reagents not added
   2. Manufacturer’s directions not followed
   3. RBCs suspensions incorrect concentration
   4. Cell buttons not re-suspended before grading agglutination
Clotting deficiencies

- Serum that does not clot completely before testing is prone to developing fibrin clots that may mimic agglutination.

- Serum that does not clot may be due to:
  - Low platelet counts
  - Anticoagulant therapy (Heparin, Aspirin, etc)
  - Factor deficiencies

- Thrombin can be added to serum to activate clot formation.

- Tubes containing EDTA can be used.
Contamination

1. Sample contamination
   - Microbial growth in tube

2. Reagent contamination
   - Bacterial growth causes cloudy or discolored appearance (those reagents should do be used)
   - Reagents contaminated with other reagents (don’t touch side of tube when dispensing)
   - Saline should be changed regularly
Equipment problems

- Routine maintenance should be performed on a regular basis (daily, weekly, etc)

- Keep instruments like centrifuges, thermometers, and timers calibrated
  - Un-calibrated sera-fuges can cause false results
Hemolysis

- Detected in serum after centrifugation (red)

- Important if not documented

- Can result from:
  - Complement binding
    - Anti-A, anti-B, anti-H, and anti-Le\(^a\)
  - Bacterial contamination

Red supernatant
Red Blood Cells (affect the forward grouping result)

1) Extra antigens
   a) Acquired B: is a false B-like antigen (Lower GI tract disease, Cancer of colon/rectum, Intestinal obstruction, Gram negative septicemia)
   b) B(A) phenotype: Patient is Group B with an apparent extra A antigen
   b) Rouleaux: extra serum proteins
   c) Polyagglutination: Due to bacterial infections, Expression of hidden T antigens react with antisera
   d) Wharton’s Jelly: gelatinous substance derived from connective tissue that is found in cord blood gelatinous substance derived from connective tissue that is found in cord blood

2) Missing or weak A/B antigen
   a) ABO Subgroups
   b) Disease (leukemia, Hodgkin’s disease)

   Resolution: test with Anti-A₁, Anti-H, and anti-A,B for A subgroups

3) Mixed field reactions
   a) Ab-coated RBC: Post-transfusion incompatibility; autoimmune hemolytic anemia, Maternal-fetal agglutination
   b) Bone marrow/stem cell recipient
   c) A₃ phenotype
Serum (affect the reverse grouping result)

1) Missing or weak antibodies:
   a) Newborns
   b) Elderly people
   c) Hypogammaglobulinemia
   d) Immunosuppression

2) Extra antibodies
   a) Cold antibodies (allo- or auto-)
      ▪ Cold antibodies may include anti-I, H, M, N, P, Lewis
   b) Anti-A₁ in an A₂ or A₂B individual
   c) Presence of plasma expanders
      ▪ Hydroxyethyl starch (HES), Dextran, etc
   d) Monoclonal gamma globulins
   e) Unexpected alloantibodies
   f) Rouleaux formation:
      ▪ Multiple Meloma
      ▪ Waldenstrom’s Macroglobulinemia
Rh false-results

- Causing positive or negative false results contributing to develop allo-antibody (anti-D)

- If the host or recipient recognizes the donor RBC surface antigens as foreign, the host will mount an immune response to the donor RBC’s.

- Unexpected antibodies are a result of red cell stimulation (e.g., transfusion, HDN, pregnancy, ...
Rh Typing: slide or test tube method

- False (+) results:
  1. Drying
  2. Roleaux formation
  3. Auto-agglutination
  4. Patient’s red cells heavily coated with Ab’s
  5. Presence of cold agglutinins
Rh Typing: slide or test tube method

• False (-) results:
  1. Use of old cells
  2. Wrong cell concentration
  3. Hemolysis
  4. Inadequate mixing of cells
  5. Inactive typing sera
  6. Incorrect temperature
  7. Existence of D weak variants
  8. High concentration of blocking antibodies
Conclusion:

1. Educating and training program for those people in charge of blood banking in hospital or Medical Diagnostic Lab.

2. There is still concern problem in blood grouping, indirect Coombs test and Cross-match.

3. The purpose of pre-transfusion compatibility testing including ABO-Rh Grouping is to prevent hemolytic transfusion reaction technical and clerical against blood components.

4. Allo-Ab screening is positive in between 0.3-38% of samples depending on the population study (blood donors or patients group) and the test method sensitivity
“Education/Training and Research Never End”