ABO discrepancies
Recognition and Resolution

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Recognition and resolution of ABO discrepancies

- Discrepancy: results of Forward do not agree with Reverse grouping

- Discrepancies may be indicated when following observations are noted:
  1. Agglutination strengths of reactions ☞ are weaker than expected
     - ✓ agglutination reactions in Forward grouping → are 3+ to 4+
     - ✓ agglutination reactions in Reverse grouping → are 2+ to 4+
  2. Expected reactions in Forward & Reverse grouping ☞ are missing
     - ✓ e.g., group O individual → missed one or both reactions in serum testing with reagent A_1 and B cells
  3. Extra reactions ☞ are noted in either Forward or Reverse grouping

- The first step in the resolution → identify the source of problem: .
  1. technical problems
  2. sample-related problems

Technical errors in ABO typing

- technical errors can be classified into several categories:
  - ① identification and documentation errors,
  - ② reagent and equipment problems
  - ③ SOP errors

- Sources of error can be eliminated more readily:
  - ✓ a new sample can be obtained → to eliminate possible contamination or identification problems
  - ✓ RBC suspensions prepared from patient samples can be washed 3 times before repeated testing
- When a technical error is discovered and corrected → ABO discrepancy can be quickly resolved with repeated testing
  - ✓ if discrepancy still exists ☞ possibility of a problem related to sample itself (related to patient or donor) should be considered.

SOP: standard operating procedure

Technical errors in ABO typing

Practical Application: Guidelines for Investigating ABO Technical Errors
Identification or Documentation Errors
Correct sample identification on all tubes Results are properly recorded Interpretations are accurate and properly recorded
Reagent or Equipment Errors
Daily quality control on ABO typing reagents is satisfactory Inspect reagents for contamination and hemolysis Centrifugation time and calibration are confirmed
Standard Operating Procedure Errors
Procedure follows manufacturer's directions Correct reagents were used and added to testing Red blood cell suspensions are at the correct concentration Cell buttons are completely suspended before grading the reaction

Sample-related ABO discrepancies

- can be divided into 2 groups:
  1. discrepancies that affect RBC testing
  2. discrepancies that affect serum (plasma) testing
  - ✓ a logical approach to solving these problems ☞ determine the side of ABO test (RBC testing or serum testing)

- strengths of agglutination reaction (in both Forward & Reverse grouping) → is a key point

- ❖ the most commonly encountered ABO discrepancies in Lab ☞ relating to weak or missing ABO Abs in serum/plasma testing

Sample-related ABO discrepancies

Overviews of ABO Discrepancies

PROBLEMS WITH RED CELL TESTING	PROBLEMS WITH SERUM/PLASMA TESTING
Extra antigens Group A with acquired B antigen B(A) phenotype Polyagglutination Rouleaux Hematopoietic progenitor cell transplants	Extra antibodies A subgroups with anti-A_1 Cold alloantibodies Cold autoantibodies Rouleaux IVIG
Missing or weak antigens ABO subgroup Pathologic etiology Transplantation	Missing or weak antibodies Newborn Elderly Pathologic etiology Immunosuppressive therapy for transplantation
Mixed-field reactions Transfusion of group O to group A, B, or AB Hematopoietic progenitor stem cell transplants A_3 phenotype	

Sample-related ABO discrepancies

- ABO Discrepancies Associated with Red Cell Testing (forward grouping):
  1. extra antigens present
  2. missing or weak antigens
  3. mixed-field reactions

Extra antigens Group A with acquired B antigen B(A) phenotype Polyagglutination Rouleaux Hematopoietic progenitor cell transplants
Missing or weak antigens ABO subgroup Pathologic etiology Transplantation
Mixed-field reactions Transfusion of group O to group A, B, or AB Hematopoietic progenitor stem cell transplants A_3 phenotype

Extra antigens present

– Acquired B Antigen

Group A with Acquired B Antigen			
ABO Testing Results			
Patient Red Cells with		Patient Serum with Reagent Red Cells	
Anti-A	Anti-B	A_1	B
4+	1+	0	4+

- EVALUATION OF ABO TESTING RESULTS
  1. agglutination of patient's RBCs with anti-A is strong (4+).
  2. agglutination of patient's RBCs with anti-B is 1+ ☞ weaker than usually expected (3+ to 4+).
  3. The ABO RBC testing result → group AB
  4. The results of serum testing reactions → group A.
- CONCLUSION
  - ✓ group A with acquired B → notice weaker agglutination with anti-B reagents
  - ✓ Serum testing reactions ☞ are typical for a group A individual.

Extra antigens present

– Acquired B Antigen

- **Background information**
  - ✓ only in group A_1 individuals with diseases of lower GI tract → cancers of colon and rectum, intestinal obstruction, or gram-negative septicemia
  - ✓ the most common mechanism:
    - ❖ a bacterial deacetylating enzyme → alters A immunodominant sugar (N-acetylgalactosamine) by removing acetyl group → resulting sugar (galactosamine) resembles B immunodominant sugar (D-galactose) ☞ cross-reacts with many anti-B reagents
    - ❖ the observation was linked to → use of ES-4 mAb anti-B clone at pH 6.5-7.0
      - if formulation of clone was acidified to pH 6.0 → the acquired B antigen was not observed
  - ✓ RBCs agglutinated strongly by anti-A and weakly by anti-B in combination with a serum containing anti-B ☞ suggest acquired B antigen
- ❖ for transfusion purposes ☞ patients should receive RBCs of group A

Extra antigens present

– Acquired B Antigen

- RESOLUTION OF ABO DISCREPANCY

1. Determine the patient's diagnosis and transfusion history.
   - ✓ the first step in resolution of any ABO discrepancy → obtain more information about patient ☞ may provide additional clues about cause of ABO discrepancy
2. Test patient's serum against autologous RBCs.
   - ✓ Anti-B in patient's serum → does not agglutinate autologous RBCs with acquired B antigen.
3. Test RBCs with:
   - ✓ additional monoclonal anti-B reagents from other manufacturers ☞ that not to react with acquired B antigen
   - ✓ a source of human polyclonal anti-B

Extra antigens present

– B(A) Phenotype

B(A) Phenotype			
ABO Testing Results			
Patient Red Cells with		Patient Serum with Reagent Red Cells	
Anti-A	Anti-B	A_1	B
1+	4+	4+	0

- EVALUATION OF ABO TESTING RESULTS
  1. agglutination of patient's RBCs with anti-A is weak (1+).
  2. agglutination of patient's RBCs with anti-B is strong (4+).
  3. results of serum testing → are typical of a group B individual.
- CONCLUSION
  - ✓ a possible B(A) phenotype
    - ✓ a group B ☞ with an extra reaction with anti-A in RBC testing

Extra antigens present

– B(A) Phenotype

- BACKGROUND INFORMATION
- B(A) phenotype → observed as a result of ↑sensitivity of mAb reagents
  - ✓ These reagents ☞ can detect trace amounts of A or B antigens that are nonspecifically transferred by glycosyltransferase enzymes
- the B gene enzyme → transfers trace amounts of N-acetylgalactosamine (immunodominant sugar for A-Ag) + D-galactose (immunodominant sugar for B-Ag) to H-Ag
  - ✓ trace amounts of A-Ags ☞ are detected with certain mAb reagents
  - ❖ A similar mechanism → can cause A(B) phenotype
- RESOLUTION OF ABO DISCREPANCY
  1. Determine patient's diagnosis and transfusion history.
  2. Test RBCs with:
     - ✓ additional mAb anti-A reagents from other manufacturers or
     - ✓ a source of human polyclonal anti-A

Extra antigens present

- Other cases for extra antigens in ABO RBC testing:

  a) Polyagglutination of RBCs by most human sera → as a result of exposure of a hidden Ag on RBC membrane ☞ because of bacterial infection or genetic mutation.

  - ✓ polyagglutination is rarely detected → because of the routine use of mAb reagents (have replaced human-derived ABO antisera)

  b) Wharton's jelly → nonspecific aggregation of serum-suspended RBCs ☞ because of abnormal concentrations of serum proteins or Wharton's jelly in cord blood samples (false-positive agglutination)

Missing or Weakly Expressed Antigens

- In this category of ABO discrepancies → RBCs demonstrate weaker or no reactions with reagent anti-A and anti-B

- Phenomena associated with this category include:
  1. ABO subgroups
  2. Weakened A and B antigen expression → in leukemia or Hodgkin's disease

Missing or Weakly Expressed Antigens

– Subgroup of A

Subgroup of A			
ABO Testing Results			
Patient Red Cells with		Patient Serum with Reagent Red Cells	
Anti-A	Anti-B	A_1	B
0	0	0	3+

- EVALUATION OF ABO TESTING RESULTS
  1. No agglutination of patient's RBCs with both anti-A and anti-B reagents ⇒ patient appears to be a group O phenotype.
  2. The results of serum testing are typical of a group A individual ☞ agglutination of serum with reagent B RBCs is strong (3+)
- CONCLUSION
  - ❖ reactions are characteristic of a missing antigen in RBC testing:
    - ✓ Serum testing results → are those expected in a group A individual.
    - ✓ Anti-A (found in group O individuals) → is absent in serum testing.

Missing or Weakly Expressed Antigens

– Subgroup of A

- BACKGROUND INFORMATION
  - ✓ weak or missing reactions with anti-A and anti-B reagents → correlate with subgroups of A and B.
  - ✓ Subgroups of A <1% of group A population (subgroups of B are even rarer)

- RESOLUTION OF ABO DISCREPANCY
  1. Determine the patient's diagnosis and transfusion history.
  2. Repeat RBC testing with:
     - ✓ extended incubation times ☞ may enhance Ag-Ab reaction
     - ✓ human polyclonal anti-A,B or monoclonal blend anti-A,B

Additional Testing Results	
	Anti-A,B
Patient red cells	1+
Conclusion: Probable subgroup of A	

Additional Testing Results	
	Anti-A,B
Patient red cells	0
Next Step: Perform adsorption and elution studies with anti-A; these studies assist in determining the presence of A antigens on the patient's red cells	

Mixed-Field Reactions

- Mixed-field (MF) reactions → can occur in RBC testing with either anti-A or anti-B reagents.
  - ✓ a MF-reaction → contains agglutinates with a mass of un-agglutinated RBCs
  - ✓ MF-reaction → is due to presence of 2 distinct cell populations

- MF-reactions can occur in:
  1. transfusion of group O RBCs to group A, B, or AB individuals,
  2. recipients of hematopoietic progenitor transplants,
  3. individuals with A3 phenotype,
  4. patients with Tn-polyagglutinable RBCs

- Example:
  - ✓ testing RBCs from a patient recently transfused with non–ABO-identical RBCs (group O donor RBCs to a group AB recipient) → can yield MF-observations

Mixed-Field Reactions

Group B Patient Transfused with Group O RBCs			
ABO Testing Results			
Patient Red Cells with		Patient Serum with Reagent Red Cells	
Anti-A	Anti-B	A_1	B
0	2+mf	4+	0

- EVALUATION OF ABO TESTING RESULTS
  1. 1. The strength of agglutination reaction with anti-B → is weaker than expected for group B individuals.
  2. 2. The anti-B mixed-field grading of reactivity is a 2+ reaction with a sufficient number of un-agglutinated cells
  3. 3. The results of serum testing → are typical of a group B individual.
- CONCLUSION
  - ✓ a group B individual possibly transfused with group O RBCs

Mixed-Field Reactions

- BACKGROUND INFORMATION
  - ✓ In certain situations, ABO-identical RBC products might not be available for transfusion → group O RBC products are transfused
    - ❖ If many group O RBC units are transfused ☞ MF- reactions may appear in ABO red cell testing.

- RESOLUTION OF ABO DISCREPANCY
  1) Determine the patient's diagnosis and recent transfusion history.
  2) Determine whether the patient is a recent HPC recipient.
  3) Investigate pre-transfusion ABO phenotype history, if possible.

HPC: hematopoietic progenitor cell

ABO Discrepancies Associated with Reverse grouping

- ABO discrepancies that affect serum/plasma testing include:
  1. presence of additional Abs other than anti-A and anti-B
  2. absence of expected ABO Ab reactions
- The most commonly encountered ABO discrepancies → absence of expected ABO Ab reactions.

PROBLEMS WITH SERUM/PLASMA TESTING
Extra antibodies A subgroups with anti-A_1 Cold alloantibodies Cold autoantibodies Rouleaux IVIG
Missing or weak antibodies Newborn Elderly Pathologic etiology Immunosuppressive therapy for transplantation

Additional Antibodies in Reverse grouping

– detection of anti-A_1

Group A_2 with Anti-A_1			
ABO Testing Results			
Patient Red Cells with		Patient Serum with Reagent Red Cells	
Anti-A	Anti-B	A_1	B
4+	0	2+	4+

- EVALUATION OF ABO TESTING RESULTS
  1. agglutination pattern with anti-A and anti-B reagents → typical of a group A individual.
  2. results of serum testing with A_1 and B red cells → indicate a group O individual
- CONCLUSION
  - ✓ an extra reaction in serum testing with reagent A_1 RBCs (2+) ☞ possible explanations include: anti-A1, cold allo-Ab, cold auto-Ab, or rouleaux.
    - ❖ This example: ABO discrepancy resulting from group A_2 with anti-A_1.

Additional Antibodies in Reverse grouping

– detection of anti-A_1

- RESOLUTION OF ABO DISCREPANCY

1. Determine the patient's diagnosis and transfusion history.
2. Test the patient's RBCs with anti- A_1 lectin

Additional Testing Results	
Patient Red Cells Tested with Anti-A_1 Lectin	Conclusion
0	Subgroup of A; suspect anti-A_1 antibody

3. Test the patient's serum with 3 examples of A_1 and A_2 reagent RBCs ☞ to confirm presence of anti- A_1 antibody

Additional Testing Results					
Patient Serum Tested with					
A_1 Cells	A_1 Cells	A_1 Cells	A_2 Cells	A_2 Cells	A_2 Cells
2+	2+	2+	0	0	0

- CONCLUSION

✓ Agglutination is observed with A_1 RBCs → providing evidence for anti-A_1. Anti- A_1 may be present in 1-8% of group A_2 phenotype.

Additional Antibodies in Reverse grouping

– Cold Ab

Cold Autoantibody and Cold Alloantibody in Serum/Plasma Testing			
ABO Testing Results			
Patient Red Cells with		Patient Serum with Reagent Red Cells	
Anti-A	Anti-B	A_1	B
4+	4+	0	1+

- EVALUATION OF ABO TESTING RESULTS
  1. Strong agglutination reactions in RBC testing → consistent with a group AB individual.
  2. The results of serum testing with reagent B red cells demonstrate a weaker extra reaction (1+).
     - ❖ This serum testing → appears to be consistent with a group A individual.
- CONCLUSION
  - ✓ a possible extra reaction in serum testing with reagent B red cells.

Additional Antibodies in Reverse grouping

– Cold Ab

- BACKGROUND INFORMATION
- Patients may possess Allo-Abs to other BG system (+ ABO) ☞ anti-P1, anti-M, anti-N, anti-Le^a, and anti-Le^b.
  - ✓ they react at ≤ RT ⇒ these Abs are cold.
  - ✓ screening cells → are valuable in distinguishing between ABO Abs and allo-Abs
- Patients may also possess Auto-Abs (directed toward own RBC antigens)
  - ✓ If auto-Abs are reactive at ≤ RT → are cold.
    - ❖ Cold auto-Abs usually are anti-I or anti-IH → react against all adult RBCs ☞ including: screening cells, A_1 and B cells, autologous cells.
- ➢ Autocontrol (autologous control) → to differentiate a cold auto-Ab from a cold allo-Ab.
  - ✓ If autocontrol is positive ☞ reactions observed with A1 & B cells and screening cells are probably result of auto-Abs.

Additional Antibodies in Reverse grouping

– Cold Ab

- RESOLUTION OF ABO DISCREPANCY

1. Determine patient's diagnosis and transfusion history.
2. Test patient's serum → with screening cells and an autocontrol at RT.

✓ This strategy helps distinguish whether cold allo-Ab or cold auto-Ab is present.

Interpretation of Testing Results			
	Screening Cells	Autologous Red Cells	Conclusion
Patient serum	Pos*	Neg	Cold alloantibody
Patient serum	Pos	Pos	Cold autoantibody

*Positive reaction if the corresponding antigen is present on the screening cell.

3. If an allo-Ab is detected → Ab- identification techniques can be performed.
4. If an auto-Ab is detected → special techniques to identify the Ab (a mini-cold panel) and remove Ab reactivity (prewarming techniques) can be used.

Additional Antibodies in Reverse grouping

– Rouleaux

Rouleaux			
ABO Testing Results			
Patient Red Cells with		Patient Serum with Reagent Red Cells	
Anti-A	Anti-B	A_1	B
4+	4+	2+	2+

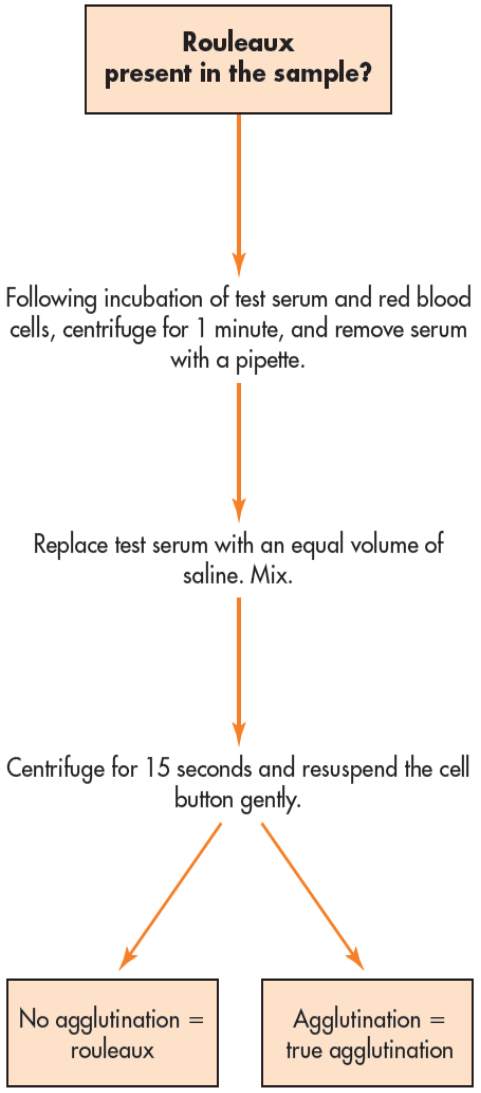
- EVALUATION OF ABO TESTING RESULTS
  1. Strong agglutination reactions are observed in RBC testing → consistent with expected results of group AB.
  2. Serum testing results → consistent with those of a group O.
- CONCLUSION
  - ❖ possibility of extra reactions in serum testing → because of all–Ab, auto–Ab, or rouleaux.
    - ✓ The phenomenon of rouleaux is demonstrated in this example.

Additional Antibodies in Reverse grouping

– Rouleaux

- BACKGROUND INFORMATION
  - Rouleaux can produce false-positive agglutination:
    - ✓ RBCs resemble stacked coins under microscopic examination.
    - ✓ ↑ concentrations of serum proteins → can spontaneous agglutination of RBCs.
    - ✓ Diseases associated with rouleaux → MM and WM
  - problems with rouleaux:
    1) extra reactions in serum testing in ABO phenotyping
    2) extra reactions in ABO RBC typing (if unwashed RBC suspensions are used)

- RESOLUTION OF ABO DISCREPANCY
  1. Determine the patient's diagnosis and transfusion history.
  2. Wash RBC suspension and repeat the phenotyping.
  3. Perform saline replacement technique ☞ for distinguish true agglutination from rouleaux



MM: multiple myeloma / WM: Waldenstrom's macroglobulinemia

Missing or Weak ABO Antibodies in Serum/Plasma Testing

- ABO Abs may be missing or weakened in certain patient-related situations.

Missing or Weak ABO Antibodies in Serum or Plasma Testing			
ABO Testing Results			
Patient Red Cells with		Patient Serum with Reagent Red Cells	
Anti-A	Anti-B	A_1	B
0	0	0	0

- EVALUATION OF ABO TESTING RESULTS
  1. agglutination pattern with anti-A and anti-B reagents → group O.
  2. results of serum testing with reagent A1 and B red cells → group AB.
- CONCLUSION
  - ✓ missing serum reactions with reagent A1 or B cells.

Missing or Weak ABO Antibodies in Serum or Plasma Testing

- BACKGROUND INFORMATION
  - investigation of patient's history (including age, diagnosis, Ig levels) ☞ provides clues to missing reactions in serum testing.
    - ✓ patient's age is an important factor ☞ ↓ concentrations of ABO Abs in newborns and elderly adults.
    - ✓ patient's diagnosis is essential ☞ ↓ Ig levels are associated with several pathologic states.
  - patient's diagnosis, Ig levels and serum protein electrophoretic patterns → are helpful data in identification & resolution of cause for this ABO discrepancy.

Missing or Weak ABO Antibodies in Serum or Plasma Testing

RESOLUTION OF ABO DISCREPANCY

1. Determine patient's diagnosis, age, and Ig levels, if available.
2. Incubate serum testing for 15 minutes at RT → then centrifuge and examine for agglutination ☞ incubation step often solves the problem.

✓ If the results are still negative → place serum testing at 4° C for 5 minutes with an autologous control.

✓ The autologous control validates the test by ensuring that positive reactions are not attributable to a cold autoantibody.

Interpretation of Additional Testing Results

4° C	A_1 Red Cells	B Red Cells	Autologous Red Cells	Conclusion
Patient serum	Pos	Pos	Neg	Group O
Patient serum	Pos	Pos	Pos	Cold autoantibody

Thank You