HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank		
Document:	Internal Policy and Procedure		
Title:	ABO Group and Rh Typing Problem Solving		
Applies To:	All Blood Bank Staff		
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1. PURPOSE:

1.1 To determine the correct ABO group of an individual and ensure the reliability of the result.
1.2 To resolve Rh typing problems.

2. DEFINITONS:

N/A

3. POLICY:

3.1 To resolve an ABO grouping discrepancy when the reactions in the forward grouping do not match the reactions in the reverse grouping.
3.2 Where expected positive reaction grading are weak or 1+ in strength .
3.3 To resolve a discrepancy between historical results and current test results.
3.4 Rh typing should be investigated when:
  3.4.1 The Rh control test is positive.
  3.4.2 Results are weak or 1+ positive with commercial anti-D reagent. Microscopic readings should only be done if mixed field agglutination is suspected.
  3.4.3 Rh typing discrepancies are found between current and previous results.
3.5 Previous transfusion records shall be reviewed. Previous results shall be compared with current results.
3.6 All reagents shall be used and controlled according to the supplier's recommendations and procedures.
3.7 If a Rh typing problem is detected and transfusion is necessary before resolution. Rh negative blood products should be issued until the problem is resolved, especially for children and women of child-bearing age.
3.8 False positive reactions with anti-D (control negative) may be caused by the presence of antibodies to antigens of low frequency in antisera of human origin. Repeating the Rh typing with monoclonal reagents (if available) should resolve the problem. Monoclonal reagents do not have contaminating antibodies.
3.9 Any discrepancy shall be investigated and resolved with appropriate documentation before reporting the ABO group and issuing red cells.
3.10 The same principles of resolving ABO discrepancy are applied for both tube and gel card methods.

4. PROCEDURE:

4.1 **Principle:**
  4.1.1 Discrepancies occur due to technical errors, absence of expected antigens on RBC's , unexpected reactions with Anti-A and Anti-B, or due to unexpected serum reactions.
  4.1.2 History of pregnancy or previous transfusion should be questioned.
4.2 **Specimens:**
  4.2.1 Freshly drawn clotted whole blood
  4.2.2 Anticoagulated (EDTA) specimen
  4.2.3 Segment from discrepant blood unit

4.3 **Materials** (as per availability):
- 4.3.1 Anti-A. Anti-B
- 4.3.2 A1, A2 and B cells
- 4.3.3 Anti-Human Globulin (AHG)
- 4.3.4 Screening cells
- 4.3.5 ABO compatible cord serum or plasma
- 4.3.6 Anti-A1 (lectin)
- 4.3.7 Other Antisera as indicated

4.4 **Quality control:**
- 4.4.1 Routine daily quality control of Antisera and cells to be tested against known cells and antisera positive and negative for the corresponding antigen and antibody.
- 4.4.2 If a different antiserum is used (i.e. with a lot number different than the one in use), a positive and negative control should be tested.

4.5 **Initial investigations:**
- 4.5.1 Repeat ABO typing from the same sample.
- 4.5.2 If initial tests were performed on red cells suspended in serum or plasma, testing should be repeated after washing red cells several times with saline. This retest will eliminate many of the problems associated with plasma proteins or autoantibody.
- 4.5.3 Test a new sample. A new sample for testing should be requested when the ABO discrepancy reflects a disagreement between the current test results and a previous test result or when specimen contamination is suspected.
- 4.5.4 Review the patient's medical history for medical conditions that could alter or interfere with ABO typing. This review can include the following:
  - 4.5.4.1 Medical diagnosis.
  - 4.5.4.2 Historical blood group.
  - 4.5.4.3 Transfusion history.
  - 4.5.4.4 Transplantation history.
  - 4.5.4.5 Current medications.
- 4.5.5 Review the results of plasma testing against autologous red cells and group O red cells in the antibody screen to evaluate potential interference by autoantibodies or alloantibodies. A direct antiglobulin test may be helpful.

4.6 **Causes of abo discrepancies:**
- 4.6.1 Causes in cell typing:
  - 4.6.1.1 Mixed-field agglutination: as in;
    - 4.6.1.1.1 Blood transfusion; for the life of transfused cells.
    - 4.6.1.1.2 B.M. transplantation; until the production of patient's own cells stops.
    - 4.6.1.1.3 Chimerism: remains for life.
    - 4.6.1.1.4 Group A subgroup red cells with anti-A. (refer to reagent manufacture instructions).
    - 4.6.1.1.5 Resolution: Obtain the patient history of diagnosis, blood transfusion, bone marrow transplantation and Medication.
  - 4.6.1.2 Weak expression of A and B antigens: as in leukaemia and neonates;
    - 4.6.1.2.1 Resolution:
      - 4.6.1.2.1.1 Incubate patient washed cells with Anti A, Anti B, and Anti- AB at room temperature (or 4 °C) for 30 minutes.
      - 4.6.1.2.1.2 Treat patient RBCs with ficin, papain or bromelin to enhance the reaction.
      - 4.6.1.2.1.3 For neonates, repeat ABO testing after the age of 2-4 years. This is because the adult levels of ABO expression are generally present by age 2 to 4 years.
    - 4.6.1.2.2 Detecting Weak A And B Antigens and Antibodies by Cold Temperature Enhancement:
      - 4.6.1.2.2.1 Principle: Prolonged incubation at low temperatures can enhance antibody binding and detection of weak ABO antigens and antibodies. Because it is often unclear whether

an ABO discrepancy is the consequence of weak antigens or of antibodies, testing both red cells and serum in parallel is recommended.

4.6.1.2.2.2 Specimen:

4.6.1.2.2.2.1 Washed red cells to investigate missing red cell antigens.

4.6.1.2.2.2.2 Serum or plasma to investigate missing iso-agglutinins.

4.6.1.2.2.3 Reagents:

4.6.1.2.2.3.1 Monoclonal or polyclonal anti-A, anti-B, and anti-A, B.

4.6.1.2.2.3.2 A1, A2, B, and O reagent red cells (serum investigations).

4.6.1.2.2.3.3 6% albumin.

4.6.1.2.2.4 Procedure:

4.6.1.2.2.4.1 Set up tube test for red cell grouping (Note: When testing patients' red cells, it is recommended to also incubate cells with 6% albumin as a control to detect spontaneous or autoagglutination. During testing of patients' plasma, group O reagent red cells should be included to detect cold autoantibody or alloantibody.)

4.6.1.2.2.4.2 Incubate all tubes for 30 minutes at room temperature.

4.6.1.2.2.4.3 Centrifuge tubes.

4.6.1.2.2.4.4 Gently resuspend cell buttons and examine for agglutination.

4.6.1.2.2.4.5 If no agglutination is observed, incubate tubes for 15 to 30 minutes at 4 °C.

4.6.1.2.2.4.6 Centrifuge and examine for agglutination.

4.6.1.2.2.5 Interpretation:

4.6.1.2.2.5.1 No interpretation can be made if the 6% albumin control for spontaneous agglutination is positive or if cold autoantibody or alloantibody is detected.

4.6.1.3 Polyagglutinable state: to inherited or acquired abnormalities of red cell membrane

4.6.1.3.1 Resolution: Use monoclonal Anti A or Anti B reagents. (refer to reagent manufacture instructions).

4.6.1.4 Non-specific aggregation of red blood cells, which simulates agglutination due to presence of concentrated serum proteins as in Wharton's Jelly contaminated cord blood sample.

4.6.1.4.1 Resolution: Wash patient cells and make 2-5% suspension with isotonic saline.

4.6.1.5 Rouleaux formation: which simulates agglutination.

4.6.1.5.1 Resolution: Wash cells and dilute the serum with saline.

4.6.1.6 False negative reaction with Anti A or Anti B reagents: due to the presence of high concentration of A and B blood group substances in the serum/ plasma which will neutralize Anti A or Anti B before getting to RBCs membrane.

4.6.1.6.1 Resolution: Wash patient cells and make 2-5% cell suspension with isotonic saline.

4.6.1.7 Spontaneous auto-agglutination: in the presence of diluents, due to cold reactive autoagglutinins.

4.6.1.7.1 Resolution: Wash patient cells with saline, then incubate at 37°C for 30 min.

4.6.1.7.2 Removing autoantibody by warm saline washes:
4.6.1.7.2.1 Principle:
4.6.1.7.2.1.1 Red cells heavily coated with autoantibodies can spontaneously agglutinate or autoagglutinate and lead to false-positive reactions with anti-A, -B, and -D. Washing red cells with warm saline will often remove sufficient autoantibody to allow determination of ABO and Rh type.
4.6.1.7.2.2 Specimen:
4.6.1.7.2.2.1 Red cells with spontaneous agglutination or autoagglutination interfering with red cell antigen typing.
4.6.1.7.2.3 Reagents:
4.6.1.7.2.3.1 Warm isotonic saline.
4.6.1.7.2.3.2 Monoclonal or polyclonal anti-A and anti- B.
4.6.1.7.2.3.3 Control reagent such as 6% albumin.
4.6.1.7.2.4 Procedure:
4.6.1.7.2.4.1 Warm red cell suspension to 37°C for 15 minutes to 1 hour.
4.6.1.7.2.4.2 Wash cells with warm (37°C) saline several times to remove autoantibody.
4.6.1.7.2.4.3 Type the washed red cells with anti-A, anti-B, and anti-D and 6% albumin. If control is still positive, using sulfhydryl reagents to disperse autoagglutination.

4.6.1.8 False positive reaction due to presence of antibodies in patient serum/ plasma against colouring dyes: used to color Anti A and Anti B reagents.
4.6.1.8.1 Resolution: Wash patient cells with saline.

4.6.1.9 Acquired B phenotype:
4.6.1.9.1 Check patient diagnosis: as in colon infection.
4.6.1.9.2 Monoclonal anti B will not react with acquired B antigen. (refer to reagent manufacture instructions)

4.6.1.10 Acquired A like antigens:
4.6.1.10.1 Resolution: Use enzyme treated cells to abolish reactivity with anti A.

4.6.1.11 False positive results due to presence of small clots:
4.6.1.11.1 Resolution: Obtain a new blood specimen on EDTA.

4.6.2 Causes in serum/ plasma typing:

4.6.2.1 False positive results due to red cell aggregation in the presence of high molecular weight plasma expanders (colloids):
4.6.2.1.1 Resolution: Dilute the serum/ plasma and take history.

4.6.2.2 False positive results due to presence of antibodies other than Anti A or Anti B (e.g. Anti M, N):
4.6.2.2.1 Resolution: Perform antibody screening.
4.6.2.2.2 Resolving abo discrepancies caused by unexpected alloantibodies:
4.6.2.2.2.1 Principle:
4.6.2.2.2.1.1 Some alloantibodies (e.g. anti-P1 and anti-M) react at room temperature. Unexpected positive reactions resulting in an ABO discrepancy can occur if A1 reagent red cells, B reagent red cells, or both, used for serum grouping, are positive for the antigen.
4.6.2.2.2.2 Procedure:
4.6.2.2.2.2.1 Test the patient's serum or plasma with antibody detection red cells at room

temperature. If a cold-reactive alloantibody is identified, phenotype reagent A1 and B red cells for the presence of the antigen, if the information is not available from the manufacturer.

4.6.2.2.2.2 Test serum or plasma against A1 and B red cells lacking the specific antigen of interest.

4.6.2.2.2.3 If the antibody detection test is negative at room temperature, the patient may possess an alloantibody to a low-incidence antigen present on A1 or B reagent red cells. Retest the serum or plasma with other randomly selected A1 and B red cell samples.

4.6.2.3 False negative results due to absence of ABO antibodies: in presence of immunodeficiency state or B.M transplants (group A patient receive group O B.M. will have O cells but produce only anti- B).

4.6.2.3.1 Resolution: obtain the patient history.

4.6.2.4 False positive/ negative results due to other group transfusion of FFP, cryoprecipitate or platelet:

4.6.2.4.1 Resolution: obtain the patient history.

4.6.2.5 False positive results due to presence of small fibrin clots

4.6.2.5.1 Resolution: Obtain a new blood specimen on EDTA.

4.6.2.6 False positive results due to presence of antibodies against diluent preservatives of reagent A and B cells:

4.6.2.6.1 Resolution: Wash reagent cells with isotonic saline and make 2-5% cell suspension with saline.

4.6.2.7 False negative result in case of high ABO antibody titre:

4.6.2.7.1 Resolution: Dilute the serum to avoid the prozone.

4.6.2.8 Unexpected reaction with A1 cells:

4.6.2.8.1 Resolution: Examine the patient cells with anti-A1 to confirm A subgroup.

4.6.2.8.2 Confirming anti-a1 in an a2 or weak a subgroup:

4.6.2.8.2.1 Principle:

4.6.2.8.2.1.1 A2 and other A subgroup can possess an anti-A1 in serum or plasma, which will react with A1 reagent cells during reverse or serum grouping. Anti-A1 is a common cause of ABO discrepancies in A subgroup.

4.6.2.8.2.2 Specimen:

4.6.2.8.2.2.1 Red cells and serum or plasma to be evaluated.

4.6.2.8.2.3 Reagents:

4.6.2.8.2.3.1 Dolichos biflorus lectin (anti-A1).

4.6.2.8.2.3.2 Group A1, A2, and O control red cells.

4.6.2.8.2.4 Procedure:

4.6.2.8.2.4.1 Testing Red Cells:

4.6.2.8.2.4.1.1 Add 1 drop of A1 lectin to each test and control tubes.

4.6.2.8.2.4.1.2 Add 1 drop of 2% to 5% saline suspension of red cells to the appropriate tubes (tested, A1 and A2 cells).

4.6.2.8.2.4.1.3 Centrifuge, examine and record agglutination.

4.6.2.8.2.4.2 Testing Serum/Plasma:

4.6.2.8.2.4.2.1 Serum should be tested against several examples (e.g. two each) of A1, A2, and O red cells using established serum grouping methods.

4.6.2.8.2.5 Interpretation:

4.6.2.8.2.5.1 The lectin should strongly agglutinate A1 red cells (3+ to 4+) but should not agglutinate A2 or O red cells. Group A red cells that fail to agglutinate with the lectin can be considered A2 or another A subgroup.

4.6.2.8.2.5.2 Anti-A1 in the patient's serum will agglutinate all A1 samples. Anti-A1 will not agglutinate autologous, A2, or group O red cells. If the patient's serum agglutinates group A2 or group O red cells, another cause for unexpected reactivity should be investigated.

4.6.2.8.2.5.3 If commercial lectin preparations are used, the manufacturer's directions should be followed for appropriate testing method and controls.

4.6.2.9 False positive results due to presence of cold autoagglutinins: as anti I and anti IH.

4.6.2.9.1 Resolution: Warm the serum and reagent cells to 37°C

4.6.2.9.2 Determining serum group without centrifugation (settled reading):

4.6.2.9.2.1 Principle:

4.6.2.9.2.1.1 Strongly reactive cold autoantibodies, such as anti-I and anti-IH, can agglutinate adult red cells, including reagent red cells, at room temperature. With few exceptions, agglutination by these cold agglutinins is weaker than that caused by anti-A and anti-B. One method to identify anti-A and anti-B in the presence of cold antibodies is by using a "settled reading."

4.6.2.9.2.2 Specimen:

4.6.2.9.2.2.1 Serum or plasma to be evaluated.

4.6.2.9.2.3 Reagents:

4.6.2.9.2.3.1 A1, B, and O reagent red cells.

4.6.2.9.2.4 Procedure:

4.6.2.9.2.4.1 Warm serum/plasma and reagent red cells to 37°C.

4.6.2.9.2.4.2 Add 2 to 3 drops serum to pre-labelled (A1, B, O) clean test tubes.

4.6.2.9.2.4.3 Add 1 drop of the appropriate reagent red cells to each of the labelled tubes.

4.6.2.9.2.4.4 Mix contents and incubate at 37°C for 1 hour.

4.6.2.9.2.4.5 Remove and examine for agglutination. Do not centrifuge samples (settled reading).

4.6.2.9.2.5 Notes:

4.6.2.9.2.5.1 Weak examples of anti-A and anti-B may not be detected by this method.

4.6.2.9.2.5.2 If the group O red cell control shows agglutination, no valid conclusion can be made regarding ABO type.

4.6.2.10 False positive result in the presence of rouleaux:

4.6.2.10.1 Detecting antibodies in the presence of rouleaux—saline replacement:

4.6.2.10.1.1 Principle:

4.6.2.10.1.1.1 Patients' samples with abnormal concentrations of serum proteins, altered serum protein ratios, or high-molecular-weight volume expanders can aggregate reagent red cells and can mimic agglutination. Rouleaux are red cell aggregates that adhere along their flat surfaces, giving a "stacked coin" appearance microscopically.

4.6.2.10.1.2 Specimen:

4.6.2.10.1.2.1 Plasma to be evaluated.

4.6.2.10.1.3 Procedure: After routine incubation and resuspension, proceed with the following steps if the appearance of the resuspended red cells suggests rouleaux formation. The saline replacement technique is best performed by the test tube method.

4.6.2.10.1.3.1 Re-centrifuge the cell mixture.

4.6.2.10.1.3.2 Remove the serum, leaving the red cell button.

4.6.2.10.1.3.3 Replace the serum with an equal volume of saline (2 drops).

4.6.2.10.1.3.4 Resuspend the red cell button gently, and observe for agglutination. Rouleaux will disperse when suspended in saline. True agglutination is stable in the presence of saline.

4.6.2.10.1.4 Notes:

4.6.2.10.1.4.1 In some instances, simple dilution of serum 1:3 with saline is sufficient to prevent rouleaux and to detect ABO isoagglutinins.

4.6.2.10.1.4.2 Review of the patient's recent medical history and other laboratory results may be helpful (eg, history of multiple myeloma).

5. MATERIALS AND EQUIPMENT:

5.1 **Forms and Records:**

N/A

5.2 **Materials:**

5.2.1 As specified in each procedure.

6. RESPONSIBILITIES:

6.1 It is the responsibility of the technician/specialist in the pre transfusion area to perform the ABO grouping of donors and patients

6.2 If a discrepancy is encountered in cell and serum grouping, all tests should be repeated by the same technician (Upon availability, anti-A1 and anti H lectins may be used if required) .
If the discrepancy persists, the sample should be handed over to be repeated by another staff.
It is the responsibility of all staff performing the ABO grouping to ensure that quality-controlled reagents and proper cell concentrations are used.

7. APPENDICES:

N/A

8. REFERENCES:

8.1 The Unified Practical Procedure Manual for Blood Banks in The Arab Countries, 1434-2013.
8.2 The Standard Policy for Blood Banks in The Kingdom of Saudi Arabia, 1st edition, 1435-2014.
8.3 National Standards for Clinical laboratories and Blood Banks, 1st edition, 2015.
8.4 AABB Technical manual, 18th edition, 2014.
8.5 AABB Standards for Blood Banks and Transfusion Services, 30th edition, 2016.
8.6 Mollison's Blood Transfusion in Clinical Medicine; 12th edition, 2014.
8.7 Modern Blood Banking & Transfusion Practices, 6th edition, 2012.

9. APPROVALS:

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