HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER
MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank		
Document:	Internal Policy and Procedure		
Title:	ABO Blood Grouping and Rh Typing		
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.

2. DEFINITONS:

N/A

3. POLICY:

3.1 Correct ABO grouping is the essential step for the compatibility testing.
3.2 It is based on the principle of direct agglutination. It should be done by the direct (forward) and indirect (reverse) method.
3.3 All blood donor samples are subjected initial immune-hematological testing.
3.4 ABO/Rh-D of donated blood must be confirmed using a segment from RBC components.
3.5 Any discrepancies are solved before releasing any blood/blood components.
3.6 If a discrepancy is encountered in cell and serum grouping, all tests should be repeated by the same technician.
3.7 If the discrepancy persists, the sample should be handed over to be repeated by another staff.
3.8 All reagents shall be used and controlled according to the supplier's recommendations and procedures.

4. PROCEDURE:

4.1 **Principle:**
- 4.1.1 The ABO and Rh blood groups are the most significant in transfusion practice.
- 4.1.2 The ABO system contains four major ABO phenotypes: A, B, O, and AB. They are determined by:
  - 4.1.2.1 Forward grouping; to detect the presence or absence of A and B antigens on the red blood cells by testing the red cells with anti A and anti B.
  - 4.1.2.2 Reverse grouping; to detect the presence or absence of the corresponding antibodies in the serum/ plasma with known A1 and B cells.
- 4.1.3 Rh (D) positive or Rh (D) negative red blood cells are classified by the presence or absence of D antigen on the red blood cells which can be detected by anti D (monoclonal-polyclonal blend).
- 4.1.4 Red cells that react weakly or not at all in direct agglutination tests with anti-D may react with anti-D by the indirect antiglobulin test (IAT).

4.2 Before starting grouping, check the previous grouping result (s).
4.3 **Slide method: (**Follow the reagent manufacturer's instructions**.)**
- 4.3.1 This is a preliminary ABO blood grouping and Rh typing, to be confirmed by tube method and reverse serum typing.
- 4.3.2 Sample:
  - 4.3.2.1 By finger puncture (for blood donor, use the same puncture site done for Hb estimation).

4.3.2.1.1 With free blood flow, discard the first drop of blood and use second drop.
4.3.2.1.2 Collect the blood in anticoagulant capillary tube or plastic transfer pipettes.
4.3.2.2 Blood samples on EDTA.
4.3.2.3 Tube segments of blood bag.

4.3.3 Reagents and materials:
4.3.3.1 Anti A grouping serum (Monoclonal)
4.3.3.2 Anti B grouping serum (Monoclonal)
4.3.3.3 Anti D grouping serum (Polyclonal)
4.3.3.4 Rh Control
4.3.3.5 Glass slides
4.3.3.6 Wooden applicator sticks
4.3.3.7 Sterile cotton/gauze
4.3.3.8 Alcohol swab
4.3.3.9 Protective medical gloves
4.3.3.10 Capillary tubes coated with anticoagulant and dropper bulb
4.3.3.11 Plastic transfer pipettes
4.3.3.12 Sharps disposal container and biohazard container
4.3.3.13 Rh View Box
4.3.3.14 Stop Watch
4.3.3.15 Lancet
4.3.3.16 Marking pen

4.3.4 ABO Typing:
4.3.4.1 At room temperature, Place one drop of Anti A and Anti B reagent on the opposite ends of a clean properly labelled slide (by marker pin).
4.3.4.2 Using collected blood from the donor by finger puncture, by anticoagulated capillary tube or plastic transfer pipette, place one drop of whole blood to each drop of Anti-A and Anti-B reagent on the slide.
4.3.4.3 Mix thoroughly using separate applicator sticks over a circular area 20mm in diameter, within 2 minutes (> 2 minutes, gives false positive results).
4.3.4.4 Read macroscopically for agglutination and record the test results on the form.

4.3.5 Rh (D) Typing:
4.3.5.1 Check Rh view box temperature (45°C-47°C).
4.3.5.2 Place on drop of Anti D and Rh-control reagent on the opposite ends of a clean properly labelled slide on top of lighted Rh view box.
4.3.5.3 Add one drop of whole blood to each drop of Anti D and Rh-control reagents.
4.3.5.4 Mix using separate applicator sticks, within 2 minutes. (>2 minutes gives false positive results).
4.3.5.5 Read macroscopically for agglutination and record test results on the form.

4.3.6 Interpretation of results:
4.3.6.1 Agglutination is positive reaction.
4.3.6.2 No agglutination is negative reaction.
4.3.6.3 If Rh-control show agglutination or samples give weak or doubtful reactions, do not interpret blood group results and retest using tube or gel method.

4.3.6.3.1

Anti D	Rh Control	Result
+	-	Rh – positive
-	-	Rh – negative
-	+	Invalid
+	+	Invalid

4.3.6.3.2

Anti B	Anti A	Result
-	+	A
+	-	B

+	+	AB
-	-	O

4.3.7 Limitations of procedure: False test results may occur due to:
- 4.3.7.1 Improper incubation time or temperature.
- 4.3.7.2 Too light or too heavy a cell suspension.

4.3.8 Procedure notes:
- 4.3.8.1 Slide testing is not suitable for detection of ABO antibodies in serum or plasma.
- 4.3.8.2 Evaporation of the reaction mixture can cause the red cells to aggregate and be misinterpreted as agglutination. Drying around the edges of the mixture must not be confused with agglutination.
- 4.3.8.3 The reagent anti-D must specifically indicate that it is suitable for slide tests.
- 4.3.8.4 The manufacturer's instructions will indicate the type of reagent control to use.

4.3.9 Safety: Slide testing imposes a greater risk of exposure to infectious samples. Blood bank staff must follow the proper safety measures.
- 4.3.9.1 Wear protective gloves.
- 4.3.9.2 Dispose all materials used in biohazard and sharp containers.
- 4.3.9.3 Avoid any blood contamination.
- 4.3.9.4 No attempt to pick up broken glass with fingers.
- 4.3.9.5 All blood and blood products must be treated as potentially infectious as per laboratory safety manual.

4.4 **Tube method:** (Follow the reagent manufacturer's instructions)

4.4.1 Specimen:
- 4.4.1.1 Anticoagulated blood drawn into CPD, EDTA or heparin; test the specimen as soon as possible: store at 2 - 8°C if delayed.
- 4.4.1.2 Test the donor blood within the expiration date of the donor unit. Forward and reverse ABO grouping for blood donors are done from EDTA samples extracted from blood bags during donation. Use RBCs from segment originally attached to the unit being typed for confirmation.
- 4.4.1.3 Avoid haemolysed specimens.

4.4.2 Reagents, Supplies, Equipment:
- 4.4.2.1 Anti-A antisera (monoclonal)
- 4.4.2.2 Anti-B antisera (monoclonal)
- 4.4.2.3 Anti-D (monoclonal/polyclonal blend)
- 4.4.2.4 A1 cells
- 4.4.2.5 B cells
- 4.4.2.6 Antihuman globulin serum
- 4.4.2.7 Coombs control cells
- 4.4.2.8 Isotonic saline. (0.9% Na Cl)
- 4.4.2.9 Rh control
- 4.4.2.10 12 mm x 75mm disposable glass test tubes
- 4.4.2.11 Centrifuge
- 4.4.2.12 Disposable pipettes
- 4.4.2.13 Marking pen
- 4.4.2.14 Cell washer (If not available, manual washing can be done)

4.4.3 Safety Precautions:
- 4.4.3.1 Do not open the centrifuge while the rotor is running.
- 4.4.3.2 All blood and blood products must be treated as potentially infectious.
- 4.4.3.3 Dispose glass fragments in sharp disposal container and do not pick up broken glass with fingers.

4.4.4 Procedure:
- 4.4.4.1 Forward grouping:
  - 4.4.4.1.1 Prepare 2-5 % red cells suspension in isotonic saline. Wash cord blood 4 times.
  - 4.4.4.1.2 Label four test tubes with patient/ donor number and with antisera to be added (anti A, anti B, anti D, Rh control).

4.4.4.1.3 Add one drop each of antisera to the first three tubes and one drop of Rh control to the 4th tube.

4.4.4.1.4 Add one drop of the prepared cell suspension to each test tube using a transfer pipette.

4.4.4.1.5 Mix well and centrifuge at 900-1000 g for 20 seconds (the speed specified by the manufacture/ the centrifuge program specified for ABO and Rh (D) grouping).

4.4.4.1.6 Gently resuspend the cell button and examine for agglutination (see the table of grading of agglutination in the previous chapter).

4.4.4.1.7 Read, interpret and record the test result, and compare with results of reverse grouping and the previous grouping results.

4.4.4.1.8 In case of a negative, weak or unclear result in Rh (D) tube:

4.4.4.1.8.1 Incubate the tube for 15-30 minutes at room temperature (follow manufacturer's instructions).

4.4.4.1.8.2 Centrifuge at 900-1000 g for 20 seconds (the speed specified by the manufacture/ the centrifuge program specified for ABO and Rh (D) grouping).

4.4.4.1.8.3 Gently resuspend the cell button and examine for agglutination (see the table of agglutination reaction in the previous chapter)

4.4.4.1.8.4. Read and interpret.

4.4.4.1.8.5 Record test results.

4.4.4.2 Reverse Grouping for ABO grouping:

4.4.4.2.1 Label two test tubes with patient ID number and the cell type to be added (A1 and B cells)

4.4.4.2.2 Add 2 or 3 drops each of serum or plasma to two clean, labeled test tubes.

4.4.4.2.3 Add 1 drop of A1 reagent red cells to the tube labelled A1.

4.4.4.2.4 Add 1 drop of B reagent red cells to the tube labelled B.

4.4.4.2.5 Gently mix the contents of the tubes; then centrifuge at 900-1000 g for 20 seconds (the speed specified by the manufacture/ the centrifuge program specified for grouping).

4.4.4.2.6 Examine the serum overlying the red cell buttons for evidence of hemolysis (if using serum). Gently resuspend the cell buttons, and examine them for agglutination.

4.4.4.2.7 Read, interpret, and compare the result with forward grouping and the previous grouping results.

4.4.4.2.8 Record test results.

4.4.4.3 Weak D (DU) procedure:

4.4.4.3.1 It is done for the donor's blood bags and the infants typed as Rh-negative (if their mothers are Rh-negative). It is also done When weak or 1+ reactions are found or when Rh typing discrepancies are found between current and previous results.

4.4.4.3.2 If no agglutination is observed with anti D after immediate spin, add one drop anti-D, incubate the test and Rh control tubes for 15-30 minutes at 37° C.

4.4.4.3.3 Mix, centrifuge both tubes at 900-1000 g for 20 seconds (the speed specified by the manufacture/ the centrifuge program specified for grouping), resuspend the cells examine for agglutination. If the test red cells are agglutinated in the anti D tube but not in the control tube, record the test sample as D-positive and does not proceed with antiglobulin phase test.

4.4.4.3.4 Wash the cells four times with isotonic saline, and decant the supernatant completely and add 2 drops of anti-human globulin. Mix and centrifuge at 900-1000 g for 20 seconds (the speed specified by the manufacture/ the

centrifuge program specified for AHG step). Washing is to remove unbound antibody (IgG anti-D).

4.4.4.3.5 Resuspend the cells and examine for agglutination. Record graded reaction.

4.4.4.3.6 Add coombs control cells (CCC) to all negative AHG tests. Mix, centrifuge and check for the presence of agglutination. If agglutination is not present, repeat weak D testing.

4.4.4.3.7 Read, interpret, and compare the result with the previous grouping results.

4.4.4.3.8 Record test results.

4.4.5 Interpretation:

4.4.5.1 Interpret ABO results as follows:

Reaction of Red Cells with Antisera (Red Cell Grouping)		Reaction of Serum with Reagent Red Cells (Serum Grouping)		Interpretation
Anti-A	**Anti-B**	**A1 cells**	**B cells**	
-	-	+	+	O
+	-	-	+	A
-	+	+	-	B
+	+	-	-	AB
+/-	+/-	+/-	+/-	Invalid

+ = Agglutination **-** = No agglutination

4.4.5.1.1 Resolve weak reactions or discrepancies before recording results and/or issuing components.

4.4.5.2 Interpret Rh results as follows:

Reaction of Red Cells with			Interpretation
Anti-D	**Du test (AHG)**	**Rh control**	
+	N/A	-	Rh positive
-	+	-	Rh positive
-	-	-	Rh negative
-	+	+	Invalid test
+	N/A	+	Invalid test

+ = Agglutination **-** = No agglutination N/A = Not applicable

4.4.5.2.1 Report any agglutination of tested cells with anti-D, even in Du test, as Rh-positive (Not Rh-negative Du-positive).

4.4.5.2.2 No agglutination of tested cells with anti-D and AHG must be reported as Rh-negative.

4.4.5.2.3 Agglutination in the control tube invalidates the test and no interpretation can be made

4.4.5.2.4 If test and control are positive, perform DAT. If positive, do not test for weak D (Du) by indirect antiglobulin test.

4.4.6 Procedure notes:

4.4.6.1 Both red cell testing and serum testing serve as a quality control measure to verify each other.

4.4.6.2 Agglutination of the red cells and either hemolysis or agglutination in tests with serum constitute positive test results.

4.4.6.3 Positive reactions characteristically show 3+ to 4+ agglutination by reagent ABO antibodies; reactions between the test serum/ plasma and the reagent red cells are often weaker.

4.4.6.4 If the reaction of Anti A with the red cells gives less than +4 degree, retest the cells with Anti A1 reagent (if available) and interpret.

4.4.6.5 A smooth cell suspension after resuspension of the cell button is a negative test result.

4.4.6.6 The serum/ plasma tests may be incubated at room temperature for 15 to 30 minutes to enhance weak reactions. Also, it may be incubated at 4 °C.

4.4.6.7 If correct Rh type of the patient is in question, consider the recipient as Rh negative.

4.4.6.8 Urgently notify the patient's physician (panic value measures) if you are unable to result Rh of newborn.

4.4.6.9 Perform forward and reverse typing at room temperature (20° – 24° C).

4.4.6.10 Bacterial contamination of the specimen may cause false results.

4.4.6.11 The addition of the reagent to the tube before the addition of the red cell suspension acts as a visual check for the presence of the anti-D to eliminate false-negative reactions due to failure to add the reagent.

4.4.6.12 Test results must be recorded at the time done in order to reduce the risk of transcription errors from delayed recording.

4.4.7 Considerations in neonates:

4.4.7.1 Reverse grouping is not performed on cord blood or fetal samples age less than 4 months.

4.4.7.2 If the reaction of Anti A with the red cells gives less than +4 degree, retest the cells with Anti A1 reagent (if available) and interpret.

4.4.7.3 If Rh (D) result is negative, infants of D-ve mothers are tested further for the presence of weak D antigen (or use gel card which detect D weak) but this is not required for pre-transfusion testing of patients' samples.

4.5 **Using gel microtyping system** (Refer to the chapter "Column Technology & the Gel Microtyping System").

5. MATERIALS AND EQUIPMENT:

5.1 **Forms and Records:**

5.1.1 Donor blood group register.

5.2 **Reagents, supplies, equipment:**

5.2.1 As previously mentioned with each test.

6. RESPONSIBILITIES:

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

6.2 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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