HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER
MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank		
Document:	Internal Policy and Procedure		
Title:	Antibody Screening, Identification, and Titration		
Applies To:	All Blood Bank Staff		
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1. PURPOSE:

1.1 Detection of unexpected antibodies to red cell antigens.
1.2 Identification and titration of unexpected antibodies to red cell antigens.

2. DEFINITONS:

N/A

3. POLICY:

3.1 Antibody screening is performed on:
  3.1.1 Obstetric patients: to identify women with alloantibodies that might cause haemolytic disease of new born.
  3.1.2 Potential candidates for blood transfusion: to detect alloantibodies that might cause a haemolytic transfusion reaction.
  3.1.3 All blood donors.
3.2 Pooled group O antibody-detection cells may be used only for donor testing. Testing of patients' samples must be performed with un-pooled cells.
3.3 Positive test should be followed by antibody identification testing and antibody titration (as per availability).
3.4 The use of antiglobulin reagent that contain only anti IgG is acceptable when performing an antibody screen.
3.5 The cells used for the detection of unexpected antibodies in pre-transfusion testing should be checked daily with weak examples of antibody (as per availability).
3.6 A full reagent red cell panel is performed upon request for patients who have a positive antibody screen.
3.7 For Previously identified antibody, select panels which are antigen-negative to the previously identified antibody but positive for all remaining clinically significant antigens.
3.8 Use a reagent red cell panel that must make it possible to identify with confidence those clinically significant alloantibodies that are most commonly encountered.
3.9 The use of antiglobulin reagent that contain only anti IgG is acceptable when performing antibody screen and identification.
3.10 An autocontrol is run with all serum panels.
3.11 Blood bank should maintain records of all patients in whom clinically significant antibodies have been previously identified.

4. PROCEDURE:

4.1 **Principle:**
  4.1.1 Immunization to foreign red cell antigens may occur through pregnancy, blood transfusion or injection of immunization materials.

4.1.2 Antibody screening tests allow serum/ plasma to react with selected red blood cells under conditions that demonstrate antibody activity either in immediate spin (I.S.), or 37°C or antiglobulin phase (IAT, ICT).

4.1.3 The indirect antiglobulin test "IAT" (= Indirect Coomb's Test "ICT") detects bound red cell antibodies that do not produce direct agglutination (sensitizing antibody). The antiglobulin test uses a secondary antibody, made in another species and directed against human globulins, that attaches and agglutinates the sensitized red cells.

4.1.4 An IAT is used in antibody detection, antibody identification, crossmatching, and blood group phenotyping.

4.2 **Specimen:**

4.2.1 EDTA tube (4 ml). Clotted sample (serum) is accepted especially in emergency cross match or when the patient is outside the hospital (OPD cases).

4.3 **Procedure:**

4.3.1 Using Gel Microtyping System (Refer to the chapter "Column Technology & the Gel Microtyping System")

4.3.2 Tube Method:

4.3.2.1 Materials:

4.3.2.1.1 Glass tubes
4.3.2.1.2 Saline (0.9%)
4.3.2.1.3 22% bovine albumin
4.3.2.1.4 Antihuman globulin (AHG) reagent; polyspecific or anti-IgG may be used.
4.3.2.1.5 Group O antibody detection cells.
4.3.2.1.6 Coombs Control Cells "CCC" (IgG sensitized RBC's).
4.3.2.1.7 Automatic cell washer.
4.3.2.1.8 37 °C water bath or dry bath.
4.3.2.1.9 Microscope, glass slides, covers.
4.3.2.1.10 Disposable pipettes.

4.3.2.2 Procedure:

4.3.2.2.1 Place two drops of plasma to be tested into four properly labelled test tubes (one tube for each screening cell I, II, III) and one tube as autocontrol (AC).
4.3.2.2.2 Add to the first three tubes one drop of the corresponding test cells and add one drop of 2-5% patient's/ donor's RBC's suspension to the autocontrol tube.
4.3.2.2.3 Mix gently and incubate for 5 minutes at room temperature (20-24 °C).
4.3.2.2.4 Centrifuge for 20 seconds at 1000 g, resuspend gently, read macroscopically, grade and record the results on the antigen table.
4.3.2.2.5 Incubate at 37 °C for 30 to 60 minutes. (If albumin is used, add 3 drops of 22% bovine albumin and mix gently, then incubate at 37°C for 15-30 minutes).
4.3.2.2.6 Centrifuge for 20 seconds at 1000 g, resuspend gently, read macroscopically, grade and record the results on the antigen table.
4.3.2.2.7 Wash the red cells three or four times with saline, and completely decant the final wash.
4.3.2.2.8 Add two drops of AHG. Mix gently, centrifuge for 20 seconds at 1000 g, resuspend gently, read, grade and record the results on the antigen table.
4.3.2.2.9 Confirm the validity of negative tests by adding IgG-coated red cells (CCC) to all negative tests, centrifuge for 20 seconds at 1000 g., resuspend gently and examine for agglutination.

4.3.2.3 Interpretation of results:

4.3.2.3.1 The presence of agglutination at any phase constitutes a positive test. Enter the reactions obtained on the antigen table. Verify that the lot number of the test cell reagents corresponds to the lot number indicated on the antigen table.

4.3.2.3.2 A positive reaction with the autocontrol may be due to the presence of an autoantibody.

4.3.2.3.3 Positive test should be followed by antibody identification testing.

4.3.2.3.4 Test are negative when no agglutination is observed followed by agglutination with the addition of IgG-coated red cells and centrifugation. If the IgG-coated red cells are not agglutinated, the negative result is invalid and the test must be repeated.

4.4 **Notes:**

4.4.1 Antibody Detection Red Cells:

4.4.1.1 Group O red cells suitable for pre-transfusion antibody screening are commercially available and are offered as sets of either two or three bottles of single-donor red cells.

4.4.1.2 Pooled antibody detection red cells may be used only when testing donor serum/ plasma.

4.4.1.3 Reagent red cells must express the following antigens: D, C, E, c, e, M, N, S, s, P1, Le^a, Le^b, K, k, Fy^a, Fy^b, Jk^a, and Jk^b.

4.4.1.4 If the commercially antibody screening red cells are not available, phenotyped donor O cells may be selected for antibody screen. After suspension preparation, they can be used for only 24 hours.

4.4.2 Antibodies in the Rh, MNS, Duffy, and Kidd systems most commonly demonstrate dosage. The three-cell set usually offers red cells from presumed homozygous donors with double-dose expression for the following common antigens: D, C, E, c, e, M, N, S, s, Fy^a, Fy^b, Jk^a, and Jk^b.

4.4.3 For saline or albumin techniques, 30 min incubation at 37 °C is adequate to detect the clinically significant coating antibodies. For some weak reactive antibodies, extended incubation period may be needed.

4.4.4 Increasing the ratio of serum to red cells increases the degree of antibody coating on red cells. A commonly used ratio is 2 drops serum to 1 drop of 2-5% cell suspension.

4.4.5 Controls: The cells used for the detection of unexpected antibodies in pre-transfusion testing should be checked daily with weak examples of antibody. Control sera can be prepared from reagent grade typing sera (as per availability) diluted with 6% bovine albumin to give 2+ reactions by an IAT.

4.4.6 Steps 3 and 4 may be omitted to avoid the detection of antibodies reactive at room temperature.

4.4.7 In general, an antibody is considered potentially clinically significant if antibodies of its specificity have been associated with hemolytic disease of the fetus and newborn (HDFN), with a hemolytic transfusion reaction, or with notably decreased survival of transfused red cells. Antibodies reactive at either 37 °C or in the AHG test phase are more likely to be clinically significant than are cold reactive antibodies.

4.4.8 Albumin may reduce repulsive forces between cells and thus may promote agglutination

4.4.9 Clinically significant red cell alloantibodies may become undetectable in a recipient's serum over time. Between 30% and 35% of antibodies become undetectable within 1 year, and nearly 50% become undetectable after 10 or more years.

4.4.10 If you use serum (clotted sample) instead of plasma, look for agglutination or hemolysis in I.S., or 37°C phases and agglutination in AHG phase.

4.4.11 Record the result in Careware system of the laboratory blood transfusion request and cross match register, donor blood group register or test request.

4.5 **Sources of False-Negative Results in Antiglobulin Testing:**

4.5.1 Neutralization of Antihuman Globulin (AHG) Reagent:

4.5.1.1 Failure to wash cells adequately to remove all serum or plasma. Fill tube at least ¾ full of saline for each wash. Check dispense volume of automated washers.

4.5.1.2 If increased serum volumes are used, a routine wash may be inadequate. Wash additional times or remove serum before washing.

4.5.1.3 Contamination of AHG by extraneous protein. Do not use finger or hand to cover tube. Contaminated droppers or wrong reagent dropper can neutralize the entire bottle of AHG.

4.5.1.4 High concentration of IgG paraproteins in test serum; protein may remain even after multiple washes.

4.5.2 Interruption in Testing:
- 4.5.2.1 Bound IgG may dissociate from red cells and either leave too little IgG to be detected or may neutralize AHG reagent.

4.5.3 Improper Reagent Storage:
- 4.5.3.1 AHG reagent may lose reactivity if frozen.
- 4.5.3.2 Reagent may become bacterially contaminated.
- 4.5.3.3 Excessive heat or repeated freezing or thawing may cause loss of reactivity of test serum.
- 4.5.3.4 Reagent red cells may lose antigen strength on storage.

4.5.4 Improper Procedures:
- 4.5.4.1 Over centrifugation may pack the red cells so tightly that agitation required to resuspend these red cells may break up agglutinates. Under centrifugation may not be optimal for agglutination.
- 4.5.4.2 Failure to add test serum, enhancement medium, or AHG may cause negative test.
- 4.5.4.3 Too heavy red cell concentration may mask weak agglutination. Too light suspension may be difficult to be read.
- 4.5.4.4 Improper or insufficient serum: cell ratios.
- 4.5.4.5 Incubation at lower temp (<37 ºC) decreases the rate of antigen –antibody reaction while incubation at higher temp. (> 37 ºC) may damage the red cells or the antibody molecules.

4.5.5 Saline:
- 4.5.5.1 Optimal saline wash solution for most antibodies is pH 7.0 to 7.2.
- 4.5.5.2 Low pH of saline solution can decrease sensitivity.
- 4.5.5.3 Some antibodies may require a specific temperature of the saline to be retained on the cell. Use 37 ºC or 4 ºC saline.

4.6 **Sources of False-Positive Results in Antiglobulin Testing:**

4.6.1 Cells Agglutinated Before Washing:
- 4.6.1.1 If potent agglutinins are present, agglutinates may not disperse during washing. Observe red cells before the addition of antihuman globulin (AHG) or use control tube substituting saline for AHG; reactivity before the addition of AHG or in the saline control invalidates AHG reading.

4.6.2 Particles of Contaminants:
- 4.6.2.1 Dust or dirt in glassware may cause clumping (not agglutination) of red cells. Fibrin or precipitates in test serum may produce red cell clumps that mimic agglutination.

4.6.3 Improper Procedures:
- 4.6.3.1 Over centrifugation may pack cells so tightly that they do not easily disperse and appear positive.

4.6.4 Cells That Have Positive Direct Antiglobulin Test (DAT):
- 4.6.4.1 Cells that are positive by DAT will be positive in any indirect antiglobulin test.

4.6.5 Complement:
- 4.6.5.1 Complement components, primarily C4, may bind to cells from clots or from CPDA-1 donor segments during storage at 4 ºC and occasionally at higher temperatures.
- 4.6.5.2 Complement may attach to red cells in specimens collected from infusion lines used to administer dextrose containing solutions.

4.7 **Antibody identification:**

4.7.1 MCH blood bank doesn't perform Ab identification using the tube method. Upon availability of reagents, Ab identification is done using Gel Microtyping System (see chapter of "Column Technology & the Gel Microtyping System").

4.7.2 Principle:
- 4.7.2.1 A panel of RBC's of known antigenic configuration used to identify any antibody detected in patient's plasma/elute by:
  - 4.7.2.1.1 Positive antibody screen.
  - 4.7.2.1.2 Elution from red cells which give positive DAT.
- 4.7.2.2 Antibody identification helps in:
  - 4.7.2.2.1 Pre-transfusion testing to select antigen negative blood for transfusion.

4.7.2.2.2 Prenatal testing to predict the type of HDN.

4.7.2.3 The serum or eluate is tested at all test phases.

4.7.3 Specimen:

4.7.3.1 Plasma / Serum:

4.7.3.1.1 For adult patients: Two Properly labelled EDTA blood tubes (4 ml) from patient. Clotted sample is accepted for Ab identification in emergency cross match or if the patient is outside the hospital (OPD cases).

4.7.3.1.2 For infant and pediatric cases: Two Properly labeled EDTA blood (3 ml)

4.7.3.2 Eluate.

4.7.4 Materials

4.7.4.1 Glass tubes.

4.7.4.2 Saline (0.9%).

4.7.4.3 22% bovine albumin.

4.7.4.4 Antihuman globulin (AHG) reagent. Polyspecific or anti-IgG may be used.

4.7.4.5 Group O antibody identification detection cells (Cell panel for Ab. Identification).

4.7.4.6 Coombs Control Cells "CCC" (IgG sensitized RBCs) .

4.7.4.7 Automatic cell washer.

4.7.4.8 37°C water bath or dry bath.

4.7.4.9 Microscope, glass slides, covers.

4.7.4.10 Disposable pipettes.

4.7.5 Procedure:

4.7.5.1 Select an in-date panel and its sheet with the same lot number.

4.7.5.2 Fill in all information on the panel sheet:

4.7.5.2.1 Date of testing

4.7.5.2.2 Technician/ specialist name

4.7.5.2.3 Patient name and medical record (ID) number

4.7.5.2.4 Phases of testing, additive and conditions:

4.7.5.2.4.1 IS = immediate spin phase

4.7.5.2.4.2 37 = 37°C incubation phase

4.7.5.2.4.3 AHG = Antihuman globulin phase

4.7.5.2.4.4 CCC = Coombs control cells or (check cells).

4.7.5.3 Select set of cell panel of minimum 11 cell panel.

4.7.5.4 1st step- saline phase (Immediate spin "IS"):

4.7.5.4.1 Label all tubes with the patient number, the first 11 tubes (1-11), and the last tube "AC" (Auto control).

4.7.5.4.2 Mix the cell panel gently, add one drop of the corresponding reagent red cells (e.g. 1-11) to the labelled tubes, and mix well gently with the specimens.

4.7.5.4.3 Add one drop of patient 2-5% RBCs cell suspension to 'AC" tube, and mix well gently with serum.

4.7.5.4.4 Incubate for 5 minutes at room temperature.

4.7.5.4.5 Centrifuge for 20 seconds at 1000 g., resuspend gently and examine macroscopically for agglutination and record the result in I.S. column.

4.7.5.5 2nd step- albumin phase (37°C incubation phase):

4.7.5.5.1 Add three drops of bovine albumin 22 % to each tube and mix gently.

4.7.5.5.2 Incubate the tubes for 15- 30 minutes at 37°C.

4.7.5.5.3 Centrifuge for 20 seconds at 1000 g., resuspend gently and examine macroscopically for agglutination and record the result in 37°C column on the panel sheet.

4.7.5.6 3rd step- anti human globulin (AHG) phase:

4.7.5.6.1 Wash all tubes 4 times with saline. After the last wash, decant completely.

4.7.5.6.2 Add 2 drops of AHG to all tubes, and mix gently, then centrifuge for 20 seconds at 1000 g., resuspend gently, examine for agglutination and record the results in AHG column in panel sheet.

4.7.5.6.3 To all negative tests add one drop of C.C.C, then centrifuge for 20 seconds at 1000 g., examine for agglutination. If no agglutination, repeat the test.

4.7.5.7 Grade reactions and record on panel sheet.

4.7.6 Interpretation:

4.7.6.1 Positive result is the presence of agglutination which indicates the presence of antibodies.

4.7.6.1.1 Presence of hemolysis in IS, or 37°C phases indicate positive results.

4.7.6.2 Negative result is absence of agglutination which indicates the absence of antibodies.

4.7.6.2.1 Absence of hemolysis in IS, or 37°C phases indicate negative results.

4.7.6.3 If a pattern of positive and negative reactions is observed, antibodies can be eliminated to the antigens present on the nonreactive cells .

4.7.6.4 Patient should be negative for the antigen corresponds to the identified antibody unless the patient has been transfused in the past 3 months .

4.7.6.5 Phase of reactivity:

4.7.6.5.1 Each antibody has optimum phase of reactivity, e.g. anti-Fya reacts well in AHG phase.

4.7.6.5.2 Do not consider the antibody as the first choice if it reacts in another phase different from its optimum one.

4.7.6.5.3 IS phase indicates the presence of cold Ab as Anti M; N; P or Lewis.

4.7.6.5.3.1 An immediate centrifugation reading, may enhance the detection of certain antibodies (anti-M, -N, -P1, -I, -Le^a, or -Le^b).

4.7.6.5.4 37°C and AHG phases indicate the presence of warm Ab as Anti S, s; Rh; Kell: Duffy or Kidd.

4.7.6.5.4.1 Test observation after 37°C incubation may detect some antibodies (e.g. potent anti- D, -E, or -K) that can cause direct agglutination of red cells.

4.7.6.5.5 Other antibodies (e.g. anti- Le^a, -Jk^a) may be detected by their lysis of antigen-positive red cells during the 37 °C incubation if serum is tested.

4.7.6.6 Auto Control:

4.7.6.6.1 If the plasma/serum reacts with both the panel cells and autologous cells, this suggests the presence of autoantibody but does not exclude an alloantibody.

4.7.6.6.2 If auto control is positive in the AHG phase, perform DAT and if positive perform eluate (upon availability).

4.7.6.6.3 If the DAT is positive, the result must be interpreted with careful attention to the transfusion history. Recently transfused patients with RBC may have positive DAT, as the circulating donor red cells could be coated with alloantibody.

4.7.6.7 Dosage:

4.7.6.7.1 Anti M, Anti c and Anti JK^a may react only with cells homozygous for same antigens (show dosage).

4.7.6.8 Antibody to high frequency antigen:

4.7.6.8.1 Reactivity with all cells except auto control cells may indicate reactivity to high frequency antigen

4.7.6.9 Panagglutination:

4.7.6.9.1 Reactivity with all cells, including autocontrol cells, indicates non-specific panagglutination. This may be due to reactivity to preservative solution.

4.7.6.10 Selected cells: Selected red cells with different antigen combinations can be used to confirm or rule out antibodies. Upon availability, other panels may be used for confirmation.

4.7.7 Notes:

4.7.7.1 Many institutions omit I.S. step because antibodies that react only at lower temperatures have little or no clinical significance.

4.7.7.2 Clinically significant antibody is one that associated with hemolytic transfusion reaction or HDN.

4.7.7.2.1 This includes Rh antibodies, Anti-S, -s, -U, Lub, K, K-, Kpa, Kpb, Jsa, Jsb, Fya ,Fyb, Jka, JKb, Dia, Dib, Doa, Dob, Coa and occasionally anti-M or Yta .

4.7.7.3 Weak reactions can be enhanced by:

4.7.7.3.1 Increase incubation time

4.7.7.3.2 Increase serum to cell ratio.

4.7.7.3.3 Perform dry button technique by washing each panel cell and decant to dry button, then add 2 drops of the serum to the dry button of cells.

4.8 **Antibody titration:**

4.8.1 Antibody titration is not routinely done in MCH blood bank.

4.8.2 Principle:

4.8.2.1 It is a semi quantitative method to determine antibody concentration in a serum sample.

4.8.2.2 Applications:

4.8.2.2.1 Estimation of antibody activity in alloimmunized pregnant women for early detection of haemolytic disease of newborn (HDN).

4.8.2.2.2 Elucidating autoantibody specificity.

4.8.2.2.3 Titre and avidity of antibodies to certain antigens.

4.8.2.2.4 Determine antibody class (IgG or IgM) by the effect of sulfhydryl reagents (upon availability).

4.8.3 Specimen:

4.8.3.1 Serum or plasma antibody to be titrated.

4.8.4 Reagents:

4.8.4.1 Red cells (2-5% suspension) that express the antigens corresponding to the antibody specificity.

4.8.4.2 Saline or albumin for dilution.

4.8.5 Procedure:

4.8.5.1 Label 10 test tubes according to the serum dilution I, 1/2, 1/4 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512.

4.8.5.2 Deliver one volume of saline to all test tubes except the 1st tube (1/1).

4.8.5.3 Add an equal volume of serum to 1st and 2nd tubes.

4.8.5.4 Using a clean pipette, mix the contents of 2nd tube (1/2) and transfer one volume into the 3rd tube (1/4).

4.8.5.5 Continue the same procedure from 3rd tube (1/4) to tube number 10, then remove one volume from 10th tube (1/512) and save it for further dilution.

4.8.5.6 Label 10 tubes for the appropriate dilutions.

4.8.5.7 Transfer 2 drops from each dilution to the appropriate tube and add 1 drop for RBC's suspension to all tubes.

4.8.5.8 Mix well and screen for antibodies by immediate spin, 37°C water bath and AHG steps.

4.8.5.9 Examine for agglutination macroscopically from higher to lower dilution to exclude prozone phenomena in lower dilution (higher concentration).

4.8.6 Interpretation:

4.8.6.1 Observe the highest dilution that produces 1 + macroscopic agglutination.

4.8.6.2 Report the titre 1, 2, or 4 and so on.

4.8.6.3 If agglutination observed in all 10 tubes, prepare more dilutions and test it.

4.8.6.4 Observe strength and agglutination and give score (1+ =5, 2+=8, 3+=10, 4+=12).

4.8.6.5 From titre and strength, determine antibody titre and avidity, (e.g. high titre, low avidity antibody has high titre and weak reaction).

4.8.7 Notes:

4.8.7.1 Careful pipetting is essential.

4.8.7.2 Consistent time, temperature and centrifugation.

4.8.7.3 Age of patient, phenotype and concentration of cell suspension will influence results.

4.8.7.4 Same serum dilutions must be used for different RBC's samples
4.8.7.5 Adequate volumes of serums needed to attain accurate results.

4.9 **Acid elution test and acid elution stain:**

4.9.1 Those tests are not performed in MCH blood bank.

5. MATERIALS AND EQUIPMENT:

5.1 **Forms and Records:**

5.1.1 Careware system of the laboratory
5.1.2 Coomb's test register.
5.1.3 Donor blood group register.
5.1.4 Cross match register.
5.1.5 Blood & Blood Products Request & Release Form
5.16 Clinically significant Antibody file.

5.2 **Materials:**

5.2.1 As previously mentioned with each specific procedure.

6. RESPONSIBILITIES:

6.1 It is the responsibility of the blood bank technicians/ specialists in the pre-transfusion area to follow the detailed procedures and to ask help of supervisor and blood bank doctor in time of need.

6.2 If any unexpected blood group antibody is detected, inform the supervisor or blood bank physician for further investigations.

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.

9. APPROVALS:

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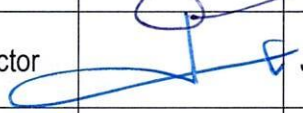
Reviewed by:	Dr. Tamer Mohamed Naguib	Medical Director		January 13, 2025
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