STANDARD OPERATING PROCEDURE FOR RESOLVING ABO & Rh BLOOD GROUP DISCREPANCIES
TITLE: STANDARD OPERATING PROCEDURE FOR RESOLVING ABO & RH BLOOD GROUP DISCREPANCIES

1.0 Principle

1.1 To resolve ABO and Rh blood group discrepancies.

1.2 Blood group discrepancies exist when the reaction in the forward group (red cells) does not match the reactions in the reverse grouping (plasma/serum), when expected reactions are weak or negative, or if the previous and current results do not match. Discrepancies may be caused by intrinsic problems with the red cells, serum or technical errors when performing the procedure.

2.0 Scope and Related Policies

2.1 The facility shall develop and maintain operating procedures for each activity that affects the safety of recipients.

2.2 ABO & Rh blood group discrepancies should be investigated when:

   2.2.1 The reactions in the forward grouping do not match the reactions in the reverse grouping.
   2.2.2 Expected results are less than 2+ or negative.
   2.2.3 Mixed field reactions are suspected.
   2.2.4 When discrepancies are found between current and previous results.

2.3 The results of the red cell and plasma test should agree. Current and previous results should be compared to identify any blood group discrepancy. The discrepancy shall be resolved and the resolution documented before issuing red cells.

   2.3.1 If transfusion is necessary before resolving the ABO discrepancy the recipient should receive group O red cells and AB plasma.
   2.3.2 If transfusion is necessary before resolving the Rh typing discrepancy the recipient should receive Rh negative blood components.

2.4 ABO & Rh blood group testing must be preformed on properly collected and labelled blood samples from the recipients.

2.5 A patient history check must be performed to compare current and previous results and to determine if any information in the patient’s history could explain the reason for the discrepancy (e.g. bone marrow transplant, previous transfusions).
2.6 Related Standard Operating Procedures:
   2.6.1 NL2010.014 Patient Identification and Specimen Labeling
   2.6.2 NL2010.013 Patient History Check
   2.6.3 NL2010.012 Determining Specimen Suitability
   2.6.4 NL09-005 Direct Antiglobulin Test
   2.6.5 NL2012-033 Preparation of Red Cell Suspensions

3.0 Specimens
   3.1 Blood sample collected in EDTA anticoagulant.
   3.2 Red cells from clotted samples (SST tubes with gel separator should not be used).
   3.3 Venous or capillary blood sample from neonates.
      Note: Cord blood must not be used for pre-transfusion testing

4.0 Materials
   Reagents:
   Anti-A anti-sera
   Anti-B anti-sera
   Anti-D monoclonal anti-sera
   Monoclonal control
   A1 reagent red cells
   B reagent red cells
   Group O screening cells
   Isotonic Saline
   Other reagents as required

   Supplies:
   Test tubes (10x75mm)
   Transfer pipettes
   Test tube rack

   Equipment:
   Serological centrifuge
   Waterbath 37(±1) °C
   Cellwasher
   Interval Timer
5.0 Quality Control

5.1 All reagents shall be used and controlled according to the manufacturer’s written instructions.

5.2 All anti-sera must be visually inspected for contamination such as discoloration, cloudiness, turbidity and/or particulate matter.

5.3 All reagent red cells must be visually inspected for hemolysis and/or discoloration.

5.4 The results of the visual inspection, reagent lot number, expiry date, date of the inspection and the individual performing the inspection must be documented.

5.5 The expiry date should be checked on each reagent used. Do not use reagents beyond expiry date.

5.6 A control consisting of 6-8% bovine serum albumin or a diluent control may be used with the recipient’s red cell suspension.

5.7 To detect a false positive reaction with the anti-D reagent a control that is appropriate for the anti-D reagent being used shall be set-up when performing an Rh typing.

5.8 The reactivity of blood grouping reagents shall be confirmed each day of use by control tests with known antigen positive and negative red cells. Positive control cells should be selected to represent weak expression of the specific antigen.

5.9 Automated instruments shall be maintained as per manufacturer’s instructions. There must be documentation of preventive maintenance, calibration testing, and daily, weekly and monthly quality control results.
6.0 Procedure

Type of discrepancy
- Preliminary checks for technical errors. See 6.1
- Problems with the ABO forward grouping. See 6.2
- Problems with the ABO reverse grouping. See 6.3
- Problems with the Rh grouping. See 6.4
- Previous and current results do not match. See 6.5

For selection of red cells following resolution of blood group discrepancies see 6.6.

6.1 Preliminary checks for technical errors:
See Procedural Note 8.1

6.1.1 Recheck suitability of specimen. (e.g. specimens contaminated with IV fluid may give weak reactions in the reverse grouping).
  - If there is any doubt about the identity or the quality of the specimen collect a new sample and repeat ABO and/or Rh grouping.

6.1.2 Check the label (s) on the vial (s) to ensure the correct reagents were used in the initial testing.

6.1.3 Check the reagent (s) for appearance of possible contamination. Use a new reagent vial if there is evidence of contamination in the reagent being used.

6.1.4 Prepare a new 3-5% patient red cell suspension using cells that have been washed a minimum of 3 times with isotonic saline.

6.1.5 Repeat ABO and/or Rh grouping.

6.1.6 If the problem is resolved:
  - Perform a final clerical check to ensure the information on the specimen, ABO and/or Rh tubes and worksheet coincide.
  - Interpret the ABO and/or Rh grouping and record the results.

6.1.7 If the problem is not resolved:
  - Do not interpret the ABO and/or Rh group. Perform a patient history check to obtain diagnosis, transfusion history, obstetrical history, transplantation history and current medications. It may be necessary to consult with other facilities to obtain a thorough patient history.
  - Document any information obtained.

NOTE: If a transfusion is necessary before resolution of the discrepancy issue Group O Rh negative red cells and Group AB plasma products.
6.2 Problems with forward grouping:
   6.2.1 Unexpected or extra reactions in the forward grouping:
      See Procedural Note 8.2.1.
      6.2.1.1 Perform patient history check to determine if the patient has
            been transfused with non-group specific RBC components in
            the past 3 months or received an ABO-mismatched stem cell
            or bone marrow transplant.
      6.2.1.2 Repeat the ABO grouping using a 3-5% washed cell
            suspension.
      6.2.1.3 If the problem is resolved:
            • Perform a final clerical check to ensure the information
              on the specimen, ABO tubes and worksheet coincide.
            • Interpret the ABO grouping and record the results.
      6.2.1.4 If the problem is not resolved:
            • Suspect a cold agglutinin and perform a cold agglutinin
              investigation.
      6.2.1.5 If the cold agglutinin is not a clinically significant
            alloantibody, perform ABO grouping using cells and reagents
            that have been pre-warmed separately.
            Perform the following steps:
            • Label 3 tubes: A/A and patient identification number,
              A/B and patient identification number and a tube with the
              patient’s identification number.
            • Add reagent antisera to each of the corresponding labeled
              tubes.
            • Add approximately 1 ml of patient’s 3-5 % washed cell
              suspension to a tube labeled with patient identification.
            • Incubate all tubes at 37(±1) °C for 5 -10 minutes.
            • Add 1 drop of the patient’s pre-warmed cells to each of
              the pre-warmed tubes.
            • Mix and centrifuge. Place tubes back in incubator.
            • Read tubes macroscopically one at a time.
      6.2.1.6 If the problem is resolved:
            • Perform a final clerical check to ensure the information
              on the specimen, ABO tubes and worksheet coincide.
            • Interpret the ABO grouping and record the results.
      6.2.1.7 If the discrepancy is still not resolved, do not interpret the
            ABO group. Consult with a senior technologist for further
            direction.
            Select Group O red cells and Group AB plasma for transfusion.
6.2.2  **Forward group is weak (<2), missing reactions or has mixed field reactions:**  See Procedural Note 8.2.2 - 8.2.4.

6.2.2.1 Read the forward grouping results microscopically checking for mixed field reactions.
- If mixed field reactions are present, review the patient’s transfusion history to determine if the patient has been transfused with non-group specific RBC components in the past 3 months or received an ABO-mismatched stem cell or bone marrow transplant.

**Note:** It might be necessary to contact other facilities that the patient may have been admitted to in the past 3 months to obtain the transfusion history.

6.2.2.2 If mixed field reactions are not present, repeat the forward grouping using a 3-5% washed cell suspension to remove any soluble A or B substances that may interfere with the antiserum.

6.2.2.3 If the problem is resolved:
- Perform a final clerical check to ensure the information on the specimen, ABO tubes and worksheet coincide.
- Interpret the ABO grouping and record the results.

6.2.2.4 If the discrepancy is still not resolved, do not interpret the ABO group. Consult with a senior technologist for further direction.

6.3  **Problems with the reverse grouping:**

6.3.1  **Reverse grouping has unexpected or extra reactions.**  See Procedural Note 8.3.1

6.3.1.1 Repeat the reverse grouping using saline replacement method. Saline replacement can be used to distinguish rouleaux from agglutination and to identify ABO antibodies.
- Re-centrifuge the original tubes for A1 and B cells.
- Remove the supernatant leaving the red cell “button” undisturbed.
- Add 2 drops of saline to each tube.
- Examine the tubes for appropriate volume and appearance.
- Mix and centrifuge the tubes.
- Re-suspend the cells and observe for agglutination.
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**Note:** Rouleaux will disperse when suspended in saline. True agglutination is stable when suspended in saline, therefore, further investigation will be required.

6.3.1.2 If the extra actions are eliminated and the patient history reveals a protein abnormality (e.g. multiple myeloma, macroglobulinemia or infusion of dextran) record results.

6.3.1.3 If the extra reactions are not eliminated the discrepancy may be caused by an Anti-A₁ and/or a cold agglutinin.

6.3.1.4 Check for Anti-A₁ if the patient’s group is A or AB.

- Perform A₁ lectin testing to determine if the patient is a subgroup of A. (Patient may be Group A₂ or A₂B with Anti-A₁).
- If the patient results are positive with Anti-A₁ lectin, the patient is Group A₁ or A₁B. Extra reactions in the reverse grouping are likely due to a cold agglutinin. Perform a cold agglutinin procedure.
- If the patient is negative with Anti-A₁ lectin, the patient is a subgroup of A (most likely an A₂ or A₂B with Anti-A₁) or the patient may also have a cold agglutinin.

**Select Group O or Group A₂ red cells for transfusion.**

6.3.1.5 If cold agglutinin investigation reveals that the cold agglutinin is not a clinically significant alloantibody, perform ABO reverse grouping using pre-warm technique.

- Label 3 tubes: A₁ and patient identification number, B and patient identification number and a tube with the patient’s identification number.
- Add 1 drop of A₁ and B cells to the corresponding labeled tubes.
- Add approx. 0.5 ml of patient plasma to a tube labeled with patient identification.
- Incubate all 3 tubes at 37°C for 5-10 minutes.
- Add 2 drops of the pre-warmed plasma to each of the pre-warmed tubes containing A₁ and B cells.
- Mix and centrifuge.
- Resuspend the cells and read macroscopically.

6.3.1.6 If the problem is resolved:

- Perform a final clerical check to ensure the information on the specimen, ABO tubes and worksheet coincide.
- Interpret the ABO grouping and record the results.
6.3.1.7 If pre-warm technique does not eliminate the extra reactions in the reverse grouping, repeat the reverse grouping using cells negative for any identified cold-reacting alloantibodies in the patient (e.g. anti M, anti A₁), if cells are available.

6.3.1.8 If the discrepancy is still not resolved, consult with a senior technologist for further direction.

**Select Group O red cells and Group AB plasma for transfusion.**

6.3.2 **Missing or weak reactions (< 2) in the reverse group**

See Procedural Note 8.3.2

6.3.2.1 Perform patient history check to determine if the patient has been transfused with non-group specific RBC components in the past 3 months or received an ABO-mismatched stem cell or bone marrow transplant.

6.3.2.2 Add 2 additional drops of patient plasma/serum to the A₁ and B cells.

6.3.2.3 Mix and centrifuge the tubes. After centrifuging re-suspend, read macroscopically, grade and record results.

6.3.2.4 If the reactions are enhanced to at least a grade 2

- Perform a final clerical check to ensure the information on the specimen, ABO tubes and worksheet coincide.
- Interpret the ABO grouping and record the results.

6.3.2.5 If the reactions are still not enhanced to at least a grade 2, repeat the reverse grouping as follows:

- Label 6 tubes with the patient’s identification number.
- Label tubes, auto, I, II, III, A₁ and B.
- Add 4 drops of patient plasma/serum to each tube.
- Add 1 drop of patient’s red cell suspension to the tube labeled “auto”.
- Add 1 drop of the appropriate screening cells to the corresponding labeled tubes.
- Add 1 drop of A₁ and B cells to the corresponding labelled tubes.
- Mix all tubes and incubate at room temperature for 30 minutes.
- Centrifuge, re-suspend the cells and read macroscopically.
- If the expected reactions are still not achieved, incubate tubes at 4°C for 15-30 minutes.
- Centrifuge, re-suspend the cells and read macroscopically.
Note: Acceptable results: if the auto-control and Group O screening cells are negative and the reverse group reactions are enhanced to at least grade 2.

6.3.2.6 If the discrepancy is still not resolved, consult with a senior technologist for further direction.

Select Group O red cells and Group AB plasma for transfusion.

6.4 Problems with Rh grouping.

6.4.1 Anti-D reaction less than 2+ with a negative control.

See Procedural Note: 8.4.1

6.4.1.1 Read the tubes microscopically and look for mixed field reactions.

6.4.1.2 If mixed fields reactions are present:

- Perform patient history check for diagnosis and transfusion history.
- If the patient received blood of a different Rh group within the past 3 months, record with an explanation for the discrepancy. It may be necessary to consult with other facilities to obtain a thorough patient history.
- Document any information obtained.
- If the patient is pregnant or was recently pregnant and there is clinical suspicion of massive maternal hemorrhage, Kleihauer testing may be required.

6.4.1.3 If mixed field reactions are not present:

- Add an extra drop of anti-D reagent to the tube.
- Incubate at room temperature for 5 minutes.
- Centrifuge and read macroscopically.
- If reaction is positive report as Rh Positive.

6.4.1.4 If the Rh grouping discrepancy is still not resolved, consult with a senior technologist for further direction.

Select Rh negative red cells for transfusion.

6.4.2 If the Rh control is positive.

See Procedural Note: 8.4.2

6.4.2.1 Prepare a new 3-5% cell suspension using cells that have been washed a minimum of 3 times with isotonic saline. If a cold agglutinin is suspected, repeat with isotonic saline warmed to 37ºC.

6.4.2.2 Repeat the Rh grouping on the washed cell suspension.
6.4.2.3 If the control is negative when using the washed cell suspension:
   • Perform a final clerical check to ensure the information on the specimen, Rh tubes and worksheet coincide.
   • Report the Rh group.

6.4.2.4 If the control remains positive perform a Direct Antiglobulin Test (DAT).
   • If the DAT is positive due to IgG, treat the cells with EDTA Glycine-Acid (Gamma EGA Kit) to remove any bound antibody and then repeat the Rh grouping. Report result.
   • If the DAT is negative or positive only for complement, perform a cold agglutinin investigation. If the investigation reveals that the cold agglutinin is not a clinically significant alloantibody, repeat the Rh grouping on a 3-5% washed cell suspension using the pre-warm technique.

6.4.2.5 If the problem is resolved:
   • Perform a final clerical check to ensure the information on the specimen, Rh grouping tubes and worksheet coincide.
   • Interpret the Rh grouping and record the results.

6.4.2.6 If the Rh grouping discrepancy is still not resolved, consult with a senior technologist for further direction.

**Select Rh negative red cells for transfusion.**

6.5 Previous and current results do not match:

*See Procedural Note 8.5*

6.5.1 Repeat the ABO and/or Rh group on the current specimen using blood from the original sample tube.

6.5.2 If the discrepancy still exists, perform a clerical check.
   • Ensure that the specimen was labeled properly.
   • Ensure that the specimen information matches the information on the request. The phlebotomists should be consulted to determine if there is an identification error.
   • Ensure information on specimen and the corresponding ABO and/or Rh test tubes match.
6.5.3 If there are no errors in the clerical check, review the previous ABO and/or Rh group results in the patient’s history to determine the date of the previous results. If that sample is still available repeat ABO and/or Rh group on that sample.

6.5.4 If the previous sample is not available, retrieve the previous blood group worksheets to confirm the blood group reactions were transcribed correctly.
- If the previous grouping was transcribed incorrectly, report the results obtained from the current specimen.
- If the previous grouping was transcribed correctly, another sample must be collected from the patient. Enter the repeat sample as a new requisition in the LIS (if applicable).
- If the results of the tests on the recollected sample are the same as the current ABO and/or Rh grouping results, report the results. Attempt to determine if an interpretation error was made on the previous test results.
- If the results of the tests on the recollected sample are the same as the previous records then it must be determined if there was a patient identification or sample labelling error on the current sample. There must be two matching blood groups to change the patient’s blood group in the LIS or in the patient’s medical chart.

6.5.5 Document the process and conclusion of the investigation in the computer (if applicable) and on the laboratory worksheets and in the patient’s medical record.

6.5.6 Complete an occurrence form as per facility procedure.

6.6 Selection of Red Cells following resolution of Blood Group Discrepancy

6.6.1 When the blood group discrepancy has been resolved select red cells for crossmatching as follows:

6.6.1.1 If the patient has received greater than 8 units of non-group specific red cell components in the last 24 hours:
- Request a new sample 24 hours post transfusion and perform a Direct Antiglobulin Test (DAT).
- If the DAT is negative, the patient may be switched back to group specific red cells.
- If the DAT is positive; perform an eluate. Test with A₁ cells or B cells, depending on the patients group, and I, II, III screening cells to exclude all significant antibodies.
- If anti-A or anti-B is eluted from the patient’s red cells then the patient should receive only group O red cells.
• If a clinically significant antibody is identified select red cells that are negative for the corresponding antigen.

6.6.2 If the patient received less than 8 units of non-group specific red cells in the last 24 hours, group specific red cells may be given.

7.0 Reporting

7.1 Interpret and report all resolved discrepancies.

7.2 Document all procedures(s) used to resolve the discrepancy (e.g. additional plasma, pre-warm technique, saline replacement technique, washing of red cell suspension etc).

7.3 Document all results obtained.

7.4 If the discrepancy is not resolved, the report should state “the ABO and/or Rh cannot be determined at this time”.

7.5 The patient’s transfusion history should be updated with the blood group discrepancy for future testing.

7.6 If it is necessary to contact another facility for a patient’s history, that facility must provide documentation of the information. The name of the person providing the information and the date must be documented.

7.7 Unresolved ABO and/or Rh grouping discrepancies must be reviewed by the Divisional Chief or designate.

8.0 Procedural Notes

8.1 Technical causes for ABO group discrepancies:
• Procedural errors
• Specimen error
• Cell suspension too heavy or too light
• Anti-sera not added or wrong anti-sera used
• Improper centrifugation
• Red cell “button” resuspended too vigorously, dispersing small agglutinins
• Failure to re-suspend entire cell “button”
• Inappropriate reading (microscopic)
• Missed observation of hemolysis.
8.2 Forward group:

8.2.1 Unexpected or extra reactions resolved by washing the patients red cells, possible causes:

- Strong cold autoagglutinin
- Rouleaux
- Wharton’s jelly (cord blood specimens)
- Fibrin, contamination with other debris.

Other causes of unexpected or extra reactions in forward group:

- Antibody-sensitized red cells may agglutinate with ABO antisera due to the colloidal nature of the reagents.
- Human source of ABO antisera may contain antibodies to a low incidence antigen.
- Acquired B antigen (rare condition reported only in Group A₁ patients who have colon cancer, inflammatory bowel disease, or septicemia).
- Polyagglutinable red cells may agglutinate with human sourced anti-A, anti-B and/or anti-A,B. Use of monoclonal antisera may resolve the discrepancy.
- Antibodies in patient plasma against dyes or drugs in ABO antisera.

8.2.2 Weaker than 2+ or missing reactions, possible causes:

- Mixed field agglutination
- Excess blood group substance in patient causing neutralization of anti-A and/or anti-B (e.g. mucin producing adenocarcinomas).

8.2.2.1 To enhance the detection of weakly expressed antigens:

- Incubate the washed patient cells with anti-A and anti-B for 15 minutes at 18-25º C.
- Use enzyme treated cells (follow manufacturer’s insert).
- Use adsorption and elution methods (follow manufacturer’s insert).
8.2.3 **Forward group: mixed field agglutination, possible causes:**
- Recent transfusion of non-group specific red cells.
- Feto-maternal hemorrhage.
- Patients who have received an allogeneic bone marrow or hematopoietic stem cell transplant may give mixed field results during the transplant period. For some patients, the mixed field will remain indefinitely.
- Weak subgroups, e.g. A$_3$ subgroup of A and other subgroups of A or B may not react as mixed field with anti-A and/or anti-B.
- Altered expression of A and/or B antigens due to disease. When the current ABO group does not agree with the historical group, the patient diagnosis may be the explanation for the change.
- Polyagglutinable red cells such as Tn-activated cells. The reverse group fails to confirm the forward group.
- Twin chimerism (very rare).

8.3 **Reverse Grouping:**
8.3.1 **Reverse group: unexpected or extra reaction(s), possible causes:**
- Rouleaux
- Cold agglutinin
- Anti-A$_1$

8.3.2 **Missing or weak reactions (<2), possible causes:**
- If the patient is elderly or immune suppressed, suspect hypogammaglobulinemia or agammaglobulinemia.
- Neonatal patients sometimes do not demonstrate anti-A and/or anti-B until 4-6 months of age.
- Patient may have received a bone marrow or hematopoietic stem cell transplant.

8.4 **Rh grouping problems:**
8.4.1 **Possible causes of mixed field reactions:**
- Recent transfusion with different Rh donor unit
- Contaminated specimen
- Unusual Rh phenotypes that may or may not be associated with production of Rh alloantibodies.
- Large feto-maternal bleed.
- Genetic anomalies such as dispermy and chimerism.
8.4.2 **Positive control may be due to:**
- Rouleaux
- Strong autoagglutinins
- Positive Direct Antiglobulin Test (DAT).

9.0 **Records Management**

9.1 The recipient transfusion data file in the Transfusion Medicine Laboratory shall be retained indefinitely.

9.2 All transfusion records in the recipient’s medical chart shall be retained in accordance with health care facility policy.

9.3 Quality control of blood components, blood products, reagents and equipment shall be retained for 5 years.

9.4 Date and time of specimen collection and phlebotomist’s identification shall be retained for 1 year.

9.5 Request form for serologic tests shall be retained for one month.

9.6 Documentation of staff training and competency must be kept for a minimum of ten years.
10.0 References


10.4 Chaulk, Barbara: Resolving ABO & Rh blood group discrepancies. Corner Brook, NL: Western Health; 2012.


