1. **Principle**

Simultaneous forward and reverse testing using a single gel card.

The Gel procedure is based on the principle of hemagglutination in which red cell antigens react with corresponding antibodies incorporated into the gel medium. Each tube is prefilled with gel and its corresponding antibody. As the red cells pass through the gel, the antigen/antibody reaction takes place and creates agglutination. This agglutination of red cells is trapped in the buffered gel during centrifugation.

Agglutination indicates the presence of antigen/antibody reaction while lack of agglutination indicates the absence of antigen/antibody reaction. The control microtube must be negative for the results to be valid.

1. **Scope and Related Policies**

Note: ABO grouping, Rh typing and antibody screen together make up the
Type and screen procedure.

* 1. The ABO group shall be determined by testing the patient’s red cells with anti-A and anti-B reagents.9.1 The Rh type shall be determined by testing the patient’s red cells with anti-D reagent. 9.1
	2. The patient’s plasma shall be tested with A1 and B reagent red cells. This test should be omitted in neonates.9.1
	3. The result of the red cell and plasma tests should agree. Any discrepancy should be investigated and resolved with appropriate documentation before issuing red cells.9.1 See NRT.003 – ABO Group Problem Solving.
	4. If an Rh typing problem is detected and transfusion is necessary before resolution, for pre-menopausal females and children, Rh negative blood products must be issued until the problem is resolved. Other patients, in the absence of a known anti-D, may receive Rh positive blood in emergency situations or when there is a shortage of Rh negative blood.
	5. When testing potential blood product recipients, the test for weak D is unnecessary except as stated in 2.11.9.1
	6. Obstetrical patients that type as Rh positive or weak D positive should be designated as Rh positive. Patients whose red cells type as Rh negative should be designated as Rh negative.9.1
	7. Previous transfusion records shall be reviewed. Previous results must be compared with current results.9.2
	8. All reagents shall be used and controlled according to the supplier’s recommendations and procedures.9.1

2.8.1 Although some manufacturers do not recommend an Rh
 control, all manufacturers list as a test limitation the possibility
 that false positive reactions may occur if the test red cells are
 strongly agglutinated prior to the addition of the reagent. This
 is also true with bacterial contamination of the specimen.
 Therefore an Rh control should be tested concurrently with
 anti-D antisera.

2.8.2 A control system, appropriate to the anti-D reagent in use,
 shall be included. If this control is positive, the Rh typing must
 be repeated with an appropriate anti-D reagent and control.9.1

* 1. If a discrepancy is detected between the forward and reverse ABO grouping and transfusion is necessary before resolution, only group O red cells shall be issued.9.1
	2. For neonatal patients:

2.10.1 Testing for weak D should be performed on infants who type
 as Rh negative if their mother is Rh negative and has no
 evidence of Rh alloimmunization.9.1

2.10.2 A venous or capillary blood specimen should be used for all
 pre-transfusion testing. Cord blood must not be used for
 pre-transfusion testing.9.3

2.10.2.1 For other purposes (e.g., investigation of Hemolytic
 Disease of the Fetus and Newborn or to assess the
 need for injection of Rh Immune Globulin to the
 mother), a cord blood or a peripheral specimen may
 be used to determine Rh typing.

2.10.3 The initial pre-transfusion blood specimen shall be tested for
 ABO and Rh antigens and for clinically significant
 antibodies.9.1 If a neonatal sample is not available, a
 maternal sample may be used.

2.10.4 For ABO, only anti-A and anti-B reagents are required.9.2
 (I.e. forward grouping only)

2.10.5 Repeat ABO and Rh typing may be omitted for the
 remainder of the neonatal period during any one hospital
 admission, provided that transfused red cells are group O.9.3

2.10.6 When indicated (e.g. positive DAT) IgG Anti-A and Anti-B
 screening should be done.

* 1. For apparent Rh negative obstetrical patients, testing for weak D should be performed.
1. **Specimen**

EDTA anticoagulated whole blood drawn within five (5) days of testing collection is the preferable sample although serum can be used.

Some blood samples (e.g. cord blood) can develop fibrin clots when diluted. These samples may be washed prior to dilution in MTS Diluent 2 Plus TM

Hemolyzed and grossly icteric specimens may cause difficulty in interpretation. See 8.10 in Procedural Notes.

Grossly lipemic specimens containing particles that clog the gel, as indicated by diffuse blotches of red cells, may be clarified by centrifugation or filtration and re-tested.

1. **Material**

**Equipment:** ID – Micro Typing System™:

 Centrifuge

 Incubator

 Pipettor

 Dispenser

 Set-up workstation, optional

**Supplies:** ID-Tips (pipette tips)

 Test tubes – 10 x 75mm

 Serologic pipettes

 Package insert

**Reagents:** A1 and B cells (4±1%)

 MTS Diluent 2 PLUS, a hypotonic buffered saline solution containing EDTA

 A/B/D Monoclonal and Reverse Grouping Card, (sequential Anti-A murine monoclonal, Anti-B murine

 monoclonal, Anti-D monoclonal, Control Gel, Buffered Gel and Buffered Gel)

Do not use beyond expiration date. Store cards at 2 to 25°C. Store diluent and red cells at 2 to 8°C. Bring reagents to room temperature (18 to 25°C) prior to use.

1. **Quality Control**
	1. To confirm the specificity and reactivity of the MTS Anti-A, MTS Anti-B, MTS Anti-D and MTS Control Gel Card, it is recommended that each lot be tested on each day of use with known positive and negative controls. Expected reactivity should be seen with the controls. Reactivity must be present in the positive specimen only.
	2. Do not freeze or expose cards to excessive heat. Store upright at 2 to 25°C. If the cards have not been stored in an upright position, centrifuge the cards before use.
	3. Do not use cards that show signs of drying. A clear liquid layer should appear on top of the gel in each microtube.
	4. Do not use cards in which the microtubes show discoloration, bubbles or crystals.
	5. Do not use the microtube cards where the seal to the microtube appears to be damaged or opened.
	6. Do not remove the foil seal to the microtubes until ready to use.

* 1. MTS Diluent 2 Plus™ should be visually checked each day of use to ensure it does not become discolored, turbid or show any signs of bacterial contamination Red blood cells must be suspended in MTS™ Diluent 2 or be a commercial 0.8% red blood cell in low ionic strength diluent specifically approved for use with the ID-Micro Typing System™.
	2. The manufacturer recommends that, following centrifugation, results should be read immediately. Results may be affected by drying of the gel, hemolysis of the red cells and slanting of the reaction patterns due to storage in a non-upright position.
1. **Procedure**

|  |  |
| --- | --- |
| * 1. Preparation of a 4%±1% suspension of patient or donor red cells in MTS Diluent 2 PLUS for forward grouping: (Note: Alternative proportional volumes may be used.)
 | * + 1. In a test tube labeled for the test sample cell suspension, dispense 0.5 mL of MTS Diluent 2 PLUS.
 |
| * + 1. Add 50 µL of whole blood obtained from a well-mixed anticoagulated sample or 25 μL of packed red cells.
 |
| * + 1. Mix gently. The final cell suspension should be approximately 4%.
 |

Preparation of a 0.8% suspension of A1 and B cells in MTS Diluent 2 PLUS for ABO reverse grouping

|  |  |
| --- | --- |
| * 1. Method 1 (For 60 test volume, using 3% cell suspensions)
 | * + 1. Label two test tubes with A1 and B; include lot number, date and time of preparation.
		2. With an appropriate pipette, dispense 1.0 mL of each reagent red cell to appropriately labeled tubes and centrifuge to pack.
		3. Decant the supernatant and then add 3.0 mL of MTS Diluent 2 PLUS to each tube.
		4. Mix gently. Final cell suspensions should be approximately 0.8% and are stable for 24 hours. For best results, suspensions should not be less than 0.6% or exceed 1.0%.
 |
| * 1. Method 2 (For 20 test volume, using packed red cells)
 | * + 1. In separate, labeled test tubes, prepare a volume of A1 and B cells sufficient to provide 10 µL of packed red blood cells.
 |
| * + 1. Label two test tubes A1 and B; include lot number, date and time of preparation. Dispense 1.0 mL of MTS Diluent 2 PLUS into each. Add 10 µL of the appropriate reagent red cell sample.
 |
| * + 1. Mix gently. Final cell suspensions should be approximately 0.8% and are stable for 24 hours. For best results, suspensions should not be less than 0.6% or exceed 1.0%.
 |
| * 1. ABO and Rh Typing Test Procedure
 | * + 1. Bring sample s and reagents to room temperature (18-25ºC).
 |
| * + 1. Visually inspect each gel tube before use. Ensure that microtube has clear liquid on top and opaque gel.
 |
| * + 1. Label the A/B/D Monoclonal and Reverse Grouping Card with the appropriate patient or donor identification.
 |
| * + 1. Remove the foil seal from the microtubes.

**Note**: Foil should be removed immediately before testing or within one hour of testing. Once opened, the gel may begin to dry out which could affect test results. Ensure that residual foil does not block the opening of any microtube after removal of the foil. |
| * + 1. Using an appropriate pipette, add 50 µL of each of the 0.8% reverse grouping cells to the labeled Buffered Gel microtubes. Add 50 µL of serum or plasma to the Buffered Gel microtubes.
 |
| * + 1. Using an appropriate pipette, add 10-12.5 µL of 4%±1% red cells diluted in MTS Diluent 2 PLUS to the Anti-A/-B/-D and Control microtubes. Do not touch gel card by pipette.
 |
| * + 1. Centrifuge the gel card at the preset conditions of 895±25 rpm for 10 minutes.
 |
| * + 1. After centrifugation, remove the card(s) from the centrifuge and observe macroscopically each card for the following signs:
* Unagglutinated red cells observed in the gel are usually caused by an interrupted centrifugation cycle. These red cells will appear dark pink and hazy.
* A line of red cells streaming down one side and forming a “J” appearance is caused by improperly seated card in the card holders.
* If the card(s) show a sign of improper centrifugation, repeat the test. Do not re-centrifuge the card(s).
 |
| * + 1. Read the front and the back of each microtube.
 |
| * + 1. Record reactions as described from the chart below

|  |  |
| --- | --- |
| **Grade** | **Description of Reaction\*** |
| Neg | Unagglutinated red blood cells form a well-defined buttonat the bottom of the microtube. See Procedural Notes 8.1 if a few unagglutinated cells are trapped at the top or sides of the gel. |
|  |  No W in updated guide |
| 1  | Agglutinates predominantly observed in the lower half of the microtube. Unagglutinated red cells form a button in the bottom of the microtube. |
| 2  | Agglutinates dispersed throughout the length of the gel column. A few unagglutinated cells may be observed in the bottom of the microtube.  |
| 3  | Majority of agglutinates trapped in the upper half of the microtube.  |
| 4 | A solid band of red cell agglutinates on top of the gel. A few agglutinates may filter into the gel, but remain near the predominant band.  |
| H | Hemolysis with few or no red cells in the gel. Report if hemolysis is present in the microtube but not in the specimen.  |
| mf | A band of red cell agglutinates at the top of the gel or dispersed throughout the gel, accompanied by unagglutinated cells in the bottom of the microtube.  |
| NT or ND | Not tested or not done |

\*Do not use half grade, superscript or “plus signs.” See the discussion section of each grading in the MTS Interpretation Guide for more information on grading. |

1. **Reporting**
	1. Agglutination of the test red cells in a specific microtube containing reagent antisera indicates the presence of the corresponding antigen.
	2. Agglutination of the red cells in a microtube of the gel card containing the A1 and/or B reagent cells indicates the presence of an antibody directed toward an antigen present on the reagent red cell sample.
	3. No agglutination in a microtube of the gel card is a negative test result and indicates the absence of an antigen/antibody reaction.
	4. The test cannot be interpreted if agglutination occurs in the control gel microtube.
	5. Interpretation:

|  |  |  |
| --- | --- | --- |
| Red Cell Typings | Reverse Grouping | Blood Group |
| Anti-A Microtube | Anti-B Microtube | Anti-D Microtube | Control Microtube | Buffered Gel A1 Cell Microtube | Buffered GelB CellMicrotube |  |
| 0 | 0 | + | 0 | + | + | O positive |
| 0 | 0 | 0 | 0 | + | + | O negative |
| + | 0 | + | 0 | 0 | + | A positive |
| + | 0 | 0 | 0 | 0 | + | A negative |
| 0 | + | + | 0 | + | 0 | B positive |
| 0 | + | 0 | 0 | + | 0 | B negative |
| + | + | + | 0 | 0 | 0 | AB positive |
| + | + | 0 | 0 | 0 | 0 | AB negative |

* 1. ABO serum grouping tests performed in conjunction with ABO red cell grouping should always agree. Discrepancies between reverse and forward grouping should be resolved according to routine ABO discrepancy policies and procedures before interpretation of the blood group. The control microtube must be negative for valid interpretation of the ABO and Rh tests.
	2. Weak reaction is not an expected result and may represent a false positive or a weak antigenic expression. Further investigation should be performed before interpretation
1. **Procedural Notes**
	1. Some immunocompromised, elderly or newborn patients may have weakened or missing ABO antibodies.
	2. False-positive results may occur if antibodies, medications, disease states, infections, Wharton’s jelly and/or cross-contamination contribute to reactions in the microtubes.
	3. Weak expressions of the A or B antigen may not be detected. Improved reactivity with these weak antigen expressions may be obtained by testing the MTS Monoclonal Anti-A, B Card.
	4. Very weak expressions of D may not be detected. The DVI epitope expression of the D antigen is not detected with this reagent. In instances where confirmation of D negative antigen status is required, negative reactions obtained with MTS monoclonal Anti-D
	5. ABO reverse grouping antibodies found in cord blood samples may be of maternal origin. Cord blood specimens may give weaker-than-normal reactions in ABO red cell grouping tests since the ABH antigens are poorly developed at birth.
	6. For the resolution of ABO group or Rh type problems refer to: NRT.003 ABO Group Problem Solving or NRT.004 Rh Typing Problem Solving.
	7. Interpretation of mixed-field reactions must be done with caution. The presence of fibrin, clots or particulates may result in some cells layering at the top of the gel. Mixed-field reactions are generally only observed in tests containing a dual population of red cells, such as a transfused patient, bone marrow recipient or when a pooled cell sample is used for testing. However, not all mixed cell situations have a sufficient minor population to be detected. Check patient history.
	8. Too few or too many cells in the microtube may cause false positive or false negative reactions. This may be due to one or both of the following errors:

 8.8.1 Improperly prepared cell suspension

 8.8.2 Adding the incorrect quantities of cells to the upper chamber.

 8.8.3 In this case repeat the test(s) ensuring correct quantities
 using new cell suspensions.

* 1. Rouleaux is a property of test plasma resulting in a characteristic pattern of red cell aggregation. It can occur if sufficient quantities of abnormal proteins are present in the test sample and may infrequently cause difficulties in gel test interpretation. Rouleaux must be confirmed using tube hemagglutination methods and saline replacement performed when necessary.
	2. Red cells present in the gel and hemolysis in the liquid portion is usually due to a hemolyzed specimen. In this case, hemolysis should not be reported as a positive test result. If hemolysis occurs during centrifugation, the liquid portion above the gel will appear pink or red but there will be few or no cells in the gel.
	3. False positive or false negative test results from bacteria or chemical contamination of test materials, inadequate incubation times or temperature, improper storage of materials.
	4. False-positive results may occur in gel cards showing signs of drying.
	5. Anomalous results may be caused by fresh serum, fibrin or particulate matter in serum or plasma, or red cells that stick to the sides of the microtube. Use of EDTA plasma will minimize the problem.
	6. Adherence to the manufacturer’s instructions for use is critical to test performance
1. **References**
	1. Standards for Hospital Transfusion Services Version 3– September 2011. Canadian Society for Transfusion Medicine, 5.3.2., 5.3.3.
	2. Roback JD, ed. American Association of Blood Banks Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011:545-546
	3. CSA Z902-10 Blood and Blood Components, Mississauga, ON: Canadian Standards Association; February 2010; 10.9.1
	4. Implementation Guide and Procedures Procedure 2 ABO Forward and Reverse Grouping/D Typing Version 5 (2010-05-31), ID-Micro Typing System TM Ortho Clinical Diagnostics
	5. ID-Micro Typing SystemTM Interpretation Guide-2010-06-04
2. **Revision History**

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| --- | --- |
| **Revision Date** | **Summary of Revision** |
| April 30, 2014 | * Revised name of manual
* Changed document number from GM.001 to GM.002
* Revised wording of section 1.0
* Updated reference in section 2.7 from 9.1 to 9.2
* Updated reference in section 2.9 from 9.2 to 9.1
* Updated reference in section 2.10 from 9.1 to 9.3
* Added “See 8.10 Procedural Notes” to section 3.0
* Section 4.0 *Reagents-* changed from 3±1% to 4±1%
* Renumbered section 5.0
* Revised wording in section 5.3 to specify a “clear liquid layer.”
* Revised and renumbered section 6.0
* Revised and renumbered section 8.0
* Revised wording of section 8.14 to include reference to manufacturer’s instructions for use
* Revised reference list
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