

**DEPMEDS LABORATORY PROCEDURES
DEPARTMENT OF CLINICAL SUPPORT SERVICES
U.S. ARMY MEDICAL DEPARTMENT CENTER AND SCHOOL
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MCCS-HCL

STANDING OPERATING PROCEDURE

01 September 02

**A/B/D MONOCLONAL TYPING AND REVERSE GROUPING TEST METHOD
(SIMULTANEOUS FORWARD AND REVERSE TESTING USING A SINGLE GEL CARD)**

1. Principle:

Forward grouping is designed to demonstrate the presence of ABO and Rh blood group antigens (A, B and/or D) by testing the red cells with known antisera (Anti-A, Anti-B and Anti-D). ABO reverse grouping is designed to demonstrate the presence of the expected ABO blood group antibodies (anti-A and/or anti-B) by testing the serum or plasma with known cells (A₁ and B cells). Using a gel card, the presence/absence of A, B and D antigens and the presence/absence of anti-A and anti-B can be detected in the gel microtube. 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.

2. Specimen:

- a. No special preparation of the patient is required prior to specimen collection.
- b. Blood should be collected by approved techniques. A completely clotted or EDTA or ACD anticoagulated sample drawn within five (5) days of testing may be used.
- c. Donor cells collected in CPDA-1 or CPD may be tested up to the expiration date of the unit. Some blood samples, e.g., cord blood, can have particulates such as fibrin clots or Wharton's Jelly. If this occurs, samples may be washed prior to dilution in MTS Diluent 2 PLUS™.

3. Equipment and Reagents

a. Equipment

- 1) Test tubes (12 x 75 mm preferred for preparing cell suspensions)
- 2) Pipettes capable of delivering volumes of red cells 0.5mL and/or 1.0mL
- 3) Micropipettes capable of dispensing 10-12.5µL and/or 25µL and/or 50µL

- 4) ID-MTS Incubator □ (37± 2°C)
- 5) ID-MTS Centrifuge □ (895±25 RPMs)
- 6) ID-MTS Dispenser □ 0.5mL and/or 1.0mL
- 7) ID- MTS Work Rack □ or test tube and gel card racks
- 8) Thermometer capable of measuring 37±2° C
- 9) Serofuge capable of centrifuging 12 x 75 mm or other appropriate test tubes
- 10) Centrifuge capable of separating cells and serum/plasma
- 11) Screening cells, pooled cells, panels cells and/or reverse typing cells
- 12) ID-MTS Gel cards (IgG, Buffered Gel, A/B/D Reverse Grouping, etc.)
- 13) ID-MTS Diluent™ (1,2 or 2 Plus)

b. Reagents

- 1) A₁ and B cells (3±1%)
- 2) MTS Diluent 2 PLUS™, a hypotonic buffered saline solution containing EDTA
- 3) 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)

NOTE: 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.

4. Calibration:

Not applicable. When the analyzer is turned on, it automatically performs a series of self-tests to verify hardware integrity. If an instrument Self-Test fails, an error message is displayed. Consult the Section 7 of the Operator's manual.

5. Quality Control:

To recognize reagent deterioration, the reagents must be tested daily with appropriate controls. To confirm the specificity and reactivity of the A/B/D Monoclonal and Reverse Grouping Card, it is recommended that each lot be tested on each day of use with antigen positive and antigen negative controls red cells. The MTS control microtube should be negative; if positive results occur, quality control testing should be repeated. MTS Diluent 2 PLUS™

should be visually checked to ensure that the liquid is not discolored, turbid or showing any signs of bacterial contamination.

PROCEDURE:

Preparation of a 4% ($\pm 1\%$) suspension of patient or donor red cells in MTS Diluent 2 PLUS™ for forward grouping:

1. In a test tube labeled for the test sample cell suspension, dispense 0.5 mL of MTS Diluent 2 PLUS™.
2. Add 50 μL of whole blood obtained from a well mixed anticoagulated sample or 25 μL of packed red cells obtained from a centrifuged anticoagulated sample.
3. Mix gently. The final cell suspension should be approximately 4%.

Preparation of a 0.8% suspension of reverse typing (A_1 cells and B cells) red cells in MTS Diluent 2 Plus™ for reverse grouping:

Method 1 (For 60 test volume, using 3% cell suspensions)

1. Label two test tubes with A_1 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%.

Method 2 (For 20 test volume, using packed cells)

1. Label two test tubes A_1 and B; include lot number, date and time of preparation. Prepare a volume of cells sufficient to provide 10 μL of packed red blood cells of each reagent red cell sample.
2. In separate labeled tubes, dispense 1.0 mL of MTS Diluent 2 Plus™. Add 10 μL of each of the packed reagent red blood cells.
3. 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%.

ABO and Rh Typing Test Procedure

1. Label the A/B/D Monoclonal and Reverse Grouping Card™ with the appropriate patient or donor identification.
2. Remove the foil seal from the microtubes.
3. 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 pipette to gel card.**
4. 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.
5. Centrifuge the gel card at the preset conditions of 895±25 RPMs for 10 minutes.
6. Read the front and the back of each microtube macroscopically and record reactions as described in the interpretation section of the corresponding MTS Gel Card package insert.
7. If using a gel card reader, refer to the operator's manual.

RESULTS:

Agglutination of the test red cells in a specific microtube containing reagent antisera indicates the presence of the corresponding antigen.

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.

No agglutination in a microtube of the gel card is a negative test result and indicates the absence of an antigen/antibody reaction.

The test cannot be interpreted if agglutination occurs in the control gel microtube.

Examples of Results and Interpretations:

Forward Grouping				Reverse Grouping		Blood Group
Anti-A Microtube	Anti-B Microtube	Anti-D Microtube	Control Microtube	Buffered Gel A ₁ Cell Microtube	Buffered Gel B Cell Microtube	
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
+	+	+	+	+	+	cannot interpret
0	0	0	0	0	0	

COMMENTS:

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. Patient clinical information should be reviewed before concluding a test is mixed-field. However, not all mixed cell situations have a sufficient minor population to be detected.

Since A₁ and B cells have many other blood group antigens, a discrepancy between cell and serum grouping test may occur because the serum under test contains an antibody in addition to, or other than, anti-A and/or anti-B.

SERUM GROUPING TESTS PERFORMED IN CONJUNCTION WITH 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.

A very weak reaction is not an expected result and may represent a false positive or a weak antigenic expression. Further investigation should be conducted before ABO status is determined.

LIMITATIONS:

Some immunocompromised, elderly or newborn patients may have weakened or missing ABO antibodies.

False-positive results may occur in gel cards showing signs of drying.

False-positive results may occur if antibodies, medications, disease states, infections, Wharton's jelly and/or cross-contamination contribute to reactions in the microtubes.

Significant variations in red blood cell suspensions (<0.6 or >1.0%) may result in false-positive or false-negative reactions.

Weak expressions of the A or B antigen may not be detected. Improved reactivity with these weak antigen expressions may be obtained by including the MTS Monoclonal Anti-A,B Cards™ in your testing.

Very weak expressions of D may not be detected. The D^{VI} epitope expression of the D antigen is not detected with this reagent.

In instances where confirmation of D negative antigen status is required, negative D reactions obtained with MTS monoclonal Anti-D should be re-tested with an Anti-D reagent licensed for anti-globulin phase testing.

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. Anomalous results with fresh serum (i.e., a line of red cells on top of the gel) may be observed with serum samples and can be minimized by the use of EDTA plasma.

Adherence to the manufacturer's package insert is critical to test performance.

ABO reverse grouping performed on the serum of an infant may give misleading results until the infant is approximately six months of age. Antibodies found in an infant's circulation prior to this time may be of maternal origin. Cord blood specimens may have weakened or missing ABO antibodies, as the ABH antigens are poorly developed at birth. This may lead to false negative results, particularly with Anti-A reagents.

Sera from patients with agammaglobulinemia may not have normal levels of anti-A and/or anti-B and may not give the expected results.

REFERENCES:

Technical manual. 12th ed. Bethesda, MD: American Association of Blood Banks, 1996: 229-45, 225-6, 633-4.

Current package insert: Reagent Red Blood Cells Affirmagen A₁ and B[®]. Raritan, NJ: Ortho-Clinical Diagnostics.

Current package insert: A/B/D Monoclonal and Reverse Grouping Card[™]. Pompano Beach, FL: Micro Typing Systems, Inc.

Current package insert: MTS Diluent 2 Plus[™] Red Blood Cell Diluent. Pompano Beach, FL: Micro Typing Systems, Inc.

Malyska H, Weiland D. The gel test. *Laboratory Medicine*, 1994;25:81-5.

Standards for blood bank and transfusion services. 18th ed. Bethesda, MD: American Association of Blood Banks, 1997.

AUTHORIZATION:

SUPERVISOR _____ DATE INSTITUTED

PATHOLOGIST _____ DATE REVIEWED

Note:

It may be necessary and is acceptable to modify any or all of these procedures to meet individual facility requirements. A facility may choose to use only those procedures it deems appropriate; however, consideration must be given to the particular product in use and its package insert, reference manual and user's guide prior to altering any portion of this disk. It is the responsibility of the end user to ensure that the procedures, as they are currently written or modified by the end user to meet needs, comply with regulations of local, state and federal agencies and that appropriate documentation is available upon request to demonstrate changes to original documents and effective dates when changes were implemented.