PROCEDURE

Title: ABO & D- Gel Method

Procedure #: 2015BLOODBANK01

Institution: Highlands Regional Medical Center

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Prepared by: Anita Smith Date: 5/29/2015

Title: Laboratory Administrative Director

Accepted by: _____ Date: 6-1-15

Title: Laboratory Medical Director

Date Patient Testing Implemented: 6/1/2015 - 6/1/2008

Review of procedure every two years

Reviewed by: _____ Date: _____

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Discontinued testing date: _____

PROCEDURE 1

Anti-A/Anti-B/Anti-D

ABO Forward Grouping / D Antigen Typing

MTS™ A/B/D Monoclonal Grouping Card

(Two ABO Forward Grouping / D Antigen Typing Tests Using a Single Gel Card)

Principle

The presence or absence of the A, B, and D antigens on human red blood cells can be determined by testing the red blood cells with the respective antisera, specifically Anti-A, Anti-B, and Anti-D. The procedure is based on the principle of agglutination. In the gel test, ABO forward grouping and D antigen typing are performed in the Anti-A, Anti-B, and Anti-D microtubes, which contain the specific antibody incorporated into the gel. Agglutination indicates the presence of an antigen-antibody reaction while lack of agglutination indicates the absence of an antigen-antibody reaction. Agglutinated red blood cells become trapped in the gel at various levels within the microtube, depending on the size of the agglutinates. Free nonagglutinated red blood cells pass through the gel and form a button of red blood cells on the bottom of the microtube.

Specimen

No special preparation of the patient is required prior to specimen collection. Blood should be collected by approved techniques.

Red blood cells freshly collected are preferred for testing and may be collected as clotted samples or in anticoagulants. Clotted samples or those samples collected in ACD may be used up to five (5) days after collection. EDTA and sodium citrate should be tested within fourteen (14) days. Samples collected in heparin or oxalate may be used within two (2) days. Donor red blood cells collected in CPDA-1, CP2D, or CPD may be tested up to the expiration date of the unit. Blood specimens should be stored at 2-8°C if not used immediately. Some blood samples, e.g., cord blood, may have fibrin clots, which may interfere with testing. If this situation occurs, samples should be washed prior to the dilution in MTS™ Diluent 2 PLUS.

Reagents

- MTS™ A/B/D Monoclonal Grouping Card (sequential Anti-A Murine Monoclonal [BIRMA-1 Clone], Anti-B Murine Monoclonal [LB-2 Clone], Anti-D Monoclonal [MS-201 Clone], Anti-A Murine Monoclonal [BIRMA-1 Clone], Anti-B Murine Monoclonal [LB-2 Clone], Anti-D Monoclonal [MS-201 Clone])
- MTS™ Diluent 2 PLUS (a hypotonic buffered saline solution containing EDTA)

Do not use reagents beyond the expiration date.
Do not use gel cards that have not been shipped in an upright position.
Store gel cards upright at 2°C to 25°C.
Store diluent at 2°C to 8°C.
Bring reagents to room temperature (18°C to 25°C) prior to use.
A clear liquid layer should appear on top of the opaque gel in each microtube. Do not use gel cards if the gel matrix is absent or the liquid level in the microtube is at or below the top of the gel matrix. Do not use gel cards that show signs of drying, discoloration, bubbles, crystals, or other artifacts. Do not use cards if foil seals appear damaged or opened.

Note: Refer to the ID-Micro Typing System™ Interpretation Guide for additional information related to the visual inspection of gel cards before use.

Quality Control

Gel Card

To recognize reagent deterioration and to confirm the specificity and reactivity of the MTS™ A/B/D Monoclonal Grouping Card it is recommended that each lot be tested on each day of use with known antigen positive and antigen negative red blood cells. If available, antigen positive red blood cells that exhibit weakened expression of the antigen should be used (for example, A_2B or weak D red blood cells). Otherwise, red blood cells possessing a single dose of the antigen are acceptable. Reagents can be considered to be satisfactory if only antigen-positive red blood cells are agglutinated.

Diluent

MTS™ Diluent 2 PLUS must be visually checked to ensure that the liquid is not discolored, turbid, or showing any signs of bacterial contamination.

Procedure

Preparation of a 4% ±1% suspension of red blood cells in MTS™ Diluent 2 PLUS:

Note: Alternative proportional volumes may be used.

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

Procedure: Red Blood Cell Typing for A, B, and D Antigens

1. Visually inspect each gel cards before use. Each microtube should have a clear liquid layer on top of opaque gel.

CAUTION: **Do not use gel cards if the gel matrix is absent or if the liquid level in the microtube is at or below the top of the gel matrix. Do not use gel cards that show signs of drying, discoloration, bubbles, crystals, or other artifacts. Do not centrifuge cards that have failed the visual inspection. The use of these cards may lead to erroneous test results. Do not use cards if foil seals appear damaged or opened.**

Note: Refer to ID-Micro Typing System™ Interpretation Guide for additional information related to the visual inspection of gel cards before use.

2. Label the gel card with the appropriate sample identification.
3. Remove the foil seal from the gel card.

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.

4. Add 10-12.5 µL of the 4% ± 1% red blood cells diluted in MTS™™ Diluent 2 PLUS to the appropriate microtubes.

CAUTION: **The pipette tip should not touch the gel card. Erroneous results due to carryover may occur.**

5. Centrifuge the gel card in the MTS™ Centrifuge at the preset conditions.
6. For manual readings, observe the front and the back of each microtube macroscopically and record reactions as described in the interpretation section of the MTS™ Gel Card Instructions for Use
   When using automated instruments, follow the procedures that are contained in the operator's manual provided by the device manufacturer.

Interpretation of Results (Refer to the Instructions for Use and the ID-Micro Typing System™ Interpretation Guide for detailed interpretation information.)

- Negative Result – No agglutination and no hemolysis of the red blood cells is a negative test result.
- Positive Result – Agglutination and/or hemolysis of the red blood cells is a positive test result. Red blood cells may remain suspended on the top of the gel or are dispersed throughout the gel in varying degrees. A few red blood cells may form a button in the bottom of the microtube in some positive reactions.
- To rule out possible false positive agglutination in a sample, the MTS™ Monoclonal Control Card should be tested whenever all forward blood grouping results for a given sample demonstrate agglutination. If a positive result occurs with the MTS™ Monoclonal Control Card, testing should be repeated with further investigation; red blood cells should be washed several times in warm saline and retested.

Phenotyping Results			Blood Group
Anti-A Microtube	Anti-B Microtube	Anti-D Microtube	
0	0	+	**Group O, Rh-positive**
0	0	0	**Group O, Rh-negative**
+	0	+	**Group A, Rh-positive**
+	0	0	**Group A, Rh-negative**
0	+	+	**Group B, Rh-positive**
0	+	0	**Group B, Rh-negative**
+	+	+	**Group AB, Rh-positive** Note: **A control should be performed to verify the absence of a false positive result.**
+	+	0	**Group AB, Rh-negative**

+ = Presence of agglutination
0 = Absence of agglutination

Comments

- A very weak reaction on one or both sides of the microtube is not an expected result. Further investigation should be performed before interpretation.
- In instances where confirmation of D-negative antigen status is required, negative reactions obtained with MTS™™ Anti-D (Monoclonal) (IgM) Cardshould be retested with an Anti-D reagent licensed for antiglobulin phase testing.
- Interpretation of mixed-field reactions must be done with caution. The presence of fibrin, clots, or particulates may cause some red blood cells to be trapped at the top of the gel. Mixed-field reactions are generally only observed in tests containing a dual population of red blood cells, such as a transfused patient, bone marrow recipient or when a pooled red blood cell sample is used for testing. However, not all mixed red blood cell situations have a sufficient minor population to be detected.

WARNING: ***ABO serum grouping tests performed in conjunction with ABO red blood cell grouping should always agree. Discrepancies between ABO forward and reverse grouping should be resolved according to routine ABO discrepancy policies and procedures before interpretation of the blood group.***

Limitations of the Procedure (Refer to the Instructions for Use and the ID-Micro Typing System™ Interpretation Guide for detailed product limitations.)

CAUTION: **Adherence to the manufacturer's Instructions for Use is critical to test performance.**

- False positive or false negative test results may occur from bacterial or chemical contamination of test materials, aged blood specimens, inadequate incubation time or temperature, improper centrifugation, improper storage of materials, or omission of test samples.
- False positive results may occur if a card that shows signs of drying is used in testing.
- Proper centrifuge calibration is particularly important to the performance of the MTS™ Gel Cards. The MTS™™ Centrifuge has been exclusively designed to provide the correct time, speed, and angle.
- Red blood cells must be diluted to 4% ± 1% in MTS™™ Diluent 2 PLUS before addition to the microtubes. Variations in red blood cell concentration can markedly affect the sensitivity of test results. If red blood cell suspensions are too concentrated, they can give weaker results due to the increase in the antigen/antibody ratio. In addition, red blood cells may fail to completely migrate to the bottom of the microtube and could cause a false positive interpretation. When red blood cells are too low in concentration, they become difficult to visualize, and, in extreme cases, a weak positive can fail to be detected.
- Aged or hemolyzed blood may yield weaker reactions than those obtained with fresh red blood cells.
- Rouleaux caused by serum or plasma with abnormally high concentrations of protein (such as in patients with multiple myeloma or Waldenstrom's macroglobulinemia or from patients who have received plasma expanders of high molecular weight) may infrequently cause difficulties in Gel Test interpretation. False positive results or hazy reactions may occur with these samples but are rare. If false positive reactions (e.g. rouleaux, cells coated with immunoglobulins, etc.) occur in the control gel, the blood group cannot be established. Additional testing will be necessary to resolve this false positive reaction. If the control test is positive, the test cells should be washed several times in warm saline and retested. If the control test again gives a positive reaction, a valid interpretation of the results obtained cannot be made.
- The MTS™ Anti-A and Anti-B reagents may not detect some weak subgroups of the A and B antigen. The use of the MTS™ Anti-A,B (Murine Monoclonal Blend) Card may better detect these weak antigens.
- Antibodies to preservatives, medications, disease states, Wharton's jelly, and/or cross-contamination of reaction microtubes may cause false positive reactions.
- Occasionally, specimens showing incomplete clotting or excess particulates may require washing prior to testing.
- Suppressed or diminished expression of certain blood group antigens may give rise to false negative reactions. For this reason, caution should always be exercised when assigning the ABO phenotype. The results of forward grouping (red blood cell) testing should be confirmed by reverse grouping (serum) testing.
- Anomalous results may be caused by fresh serum, fibrin, or particulate matter in serum or plasma, or red blood cells that stick to the sides of the microtube. Anomalous results (i.e. a line of red blood cells on the top of the gel) may be observed with serum samples and can be minimized with the use of EDTA plasma.
- The interpretation of reactions obtained when testing infant blood may be complicated by the fact that the infant's serum does not necessarily contain antibody for any antigen absent from the red blood cells, and passive Anti-A and/or Anti-B from the mother's circulation may yield conflicting reactions when tests are performed on cord blood specimens. Cord blood specimens may also give weaker than normal reactions in the red blood cell grouping test. Imperfect development of the ABH antigens at birth may lead to false negative results, particularly with Anti-A reagents.
- Very weak expressions of the D antigen may not be detected. The D^{VI} epitope expression of the D antigen has not been detected with this reagent. Other rare red blood cells with very low copy numbers of the D antigen may need to be tested with antiglobulin and will be negative with this reagent.

References

1. ID-Micro Typing System™ Interpretation Guide (6902201), Ortho Clinical Diagnostics
2. Roback J.. (ed) Technical Manual. 16th ed. Bethesda, MD: American Association of Blood Banks, 2008.
3. Instructions for Use: Blood Grouping Reagent MTS™ A/B/D Monoclonal Grouping Card (J32850), Current version. Pompano Beach, FL: Micro Typing Systems, Inc.
4. Instructions for Use: MTS™ Diluent 2 PLUS Red Blood Cell Diluent, Current revision. Pompano Beach, FL: Micro Typing Systems, Inc.
5. Malyska H, Weiland D. The Gel Test. Laboratory Medicine 1994; 25:81-5.

Authorization

Supervisor: [signature] Date Instituted: 6/1/15

Pathologist: See policy cover sheet 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 Instructions for Use, reference manual and user's guide prior to altering any portion of this information. 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.

Revision History

Date of Revision	Version	Description of Technical Changes
2010-05-31	5.0	• Aligned content with Instructions for Use: MTS™ A/B/D Monoclonal Grouping Card (J32850 • Updated product references and trademarks. • Added warning to not use gel cards that were not shipped upright. • Added references to ID-Micro Typing System™ Interpretation Guide • Deleted statement "Blood grouping should always be performed in conjunction with the MTS™ Monoclonal Control Card™" to align with "Interpretation of Results" section. • Expanded limitations of the procedure to include guidance for resolving rouleaux test interference in agreement with Instructions for Use. • Added caution against pre-centrifugation of cards that have failed visual inspection. • Replaced "package insert" with "Instructions for Use". • Updated References section.
2005-05-02	4.0	• Changed format of document. • Changed the date format to YYYY-MM-DD. • Used the term **red blood cells** consistently. • Added step 1 to visually inspect gel cards and the Caution statement. • Added **or within one hour of testing** to the Note about the foil on the gel cards. • Added the sentence **Erroneous results due to carryover may occur.** to the Caution stating the pipette tip must not touch the gel card. • Included the Caution about the pipette tip not touching the gel card after step 5 of the Procedure: Red Blood Cell Typing for A, B, and D Antigens. • In Interpretation of Results, changed the first sentence in Negative Result to **No agglutination and no hemolysis**... and changed the first sentence in Positive Reaction to **Agglutination and/or hemolysis**... . • Changed the technical manual reference. • Changed the revision dates for the package inserts to **Current Revision.** • Added Revision History.