1. **Principle**

This antibody identification test is used to identify unexpected blood group antibodies with the use of enzyme treated cells to help in the recognition and differentiation of some weak or multiple antibody specificities. Since some antigens are destroyed, weakened, or otherwise altered by enzyme modification, reactivity of antibodies to these antigens will be eliminated or reduced. Enzyme treatment of red blood cells can increase the reactivity of some blood group antibodies The MTS™ Buffered Gel Card is designed for use with enzyme-treated red blood cells. In this test, the reagent red blood cells in a hypotonic buffered saline solution are combined with patient serum/plasma to allow antigen/antibody interaction in the upper chamber of the microtube. Following incubation the panel and serum/plasma sample are centrifuged. Agglutination indicates the presence of an antigen/antibody reaction while lack of agglutination indicates no antigen/antibody reaction. The inclusion of an autocontrol facilitates recognition of the presence of autoantibodies in the plasma sample being tested. Both IgM and IgG antibodies may cause direct agglutination of enzyme-treated red blood cells.

1. **Scope and Related Policies**
   1. A panel of cells is tested when the initial antibody screen is positive.
   2. When a patient has a clinically significant antibody or a previous history of clinically significant antibodies, red cells lacking the corresponding antigen(s) should be tested using an antiglobulin (or comparable) technique.9.1
   3. “Selected cells” from a panel of cells are tested to exclude the presence of other clinically significant antibody(ies) when:
      1. An antibody, previously identified, reacts with the screening cells.
      2. Additional cells are required to exclude an antibody (i.e. after exclusion procedure has been performed on an initial antibody panel).
   4. Enzyme treatment of red cells enhance reactivity of an antibody with the corresponding antigen by increasing antibody uptake onto red cells due to either exposure of latent antigen sites or decreasing stearic hindrance through the removal of carbohydrates and polypeptides from the red cell surface.
   5. Reduction of reactivity of an antigen, with the appropriate antibody, can be due to total cleavage of the antigen site or to the removal of a constituent close to the reaction site which affects the stearic or charge configuration of the antigen so that it is no longer recognised by the antibody.
   6. A panel of cells is pretreated with enzyme and tested when the initial antibody screen is weakly positive or inconclusive.
2. **Specimens**

EDTA anticoagulated whole blood drawn within 14 days of testing is the preferable sample although serum can be used.

If the patient has been recently transfused, or is pregnant, the sample should be within three days of collection.

Hemolyzed and grossly icteric specimens may cause difficulty in interpretation. See 8.4 in Procedure 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. **Materials**

**Equipment:** Centrifuge Dispenser

Incubator Set-up workstation, optional

Pipettor Serologic centrifuge

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

Test tubes – 10 x 75 mm Package insert

**Reagents:** MTS Buffered Gel Card - buffered gel suspension

Antibody identification panel comprised of human red blood cells as 0.8%, enzyme-treated Reagent Red Blood Cells, ready for use **or** 3%, enzyme-treated, Reagent Red Blood Cells, **or** 3%, to be enzyme-treated Reagent Red Blood Cells

MTS Diluent 2 Plus, a hypotonic buffered saline solution containing EDTA (for in-house preparation only)

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.

**Note:** This procedure does not include directions for   
enzyme treatment of red blood cells. See SP.008 Papain Treatment of Red Cells and SP.007 Ficin Treatment of Red Cell

1. **Quality Control**
   1. Appropriate controls must be tested with the enzyme treated cells to validate adequate treatments.
   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 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.
   7. MTS Diluent 2™ must be visually checked to ensure that the liquid is not discolored, turbid or showing 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™.
   8. To confirm the specificity and reactivity of the IgG gel card the manufacturer recommends that each lot be tested each day of use with known positive and negative antibody samples with the appropriate red cell. Reactivity must be present in the positive specimen only.
   9. 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.
2. **Procedures**

|  |  |
| --- | --- |
| * 1. Antibody Panel Cell Preparation, if necessary. | |
| * 1. Method 1 (For 2-4 tests, from 3% enzyme pretreated cell suspension) | * + 1. Label test tubes for panel cells to be tested; include lot number, date and time of preparation. |
| * + 1. With an appropriate pipette, dispense one (1) volume (suggested minimum 100μL) of each antibody panel cell sample to the appropriately labeled tube. Add a small volume of MTS Diluent 2 Plus™ to each test tube for volume. |
| * + 1. Centrifuge one (1) minute to pack the red blood cells. |
| * + 1. Decant the supernatant (a dry cell button is recommended) and then add two (2) volumes of MTS Diluent 2 Plus™ (i.e., 200μL if the initial volume of 3% cells was 100μL) to each tube. |
| * + 1. Mix gently. Final cell suspensions should be approximately 0.8% and stable for 24 hours. For best results, suspensions should not be less than 0.6% or exceed 1.0%   **Note:** The preparation of a small volume of a 0.8% red cell suspension has been modified to best target 0.8%, within a range of 0.6-1.0%. |
| * 1. Method 2 (For 20 tests, from enzyme-pretreated packed cells) | * + 1. Label test tubes for panel cells to be tested; include lot number and date and time of preparation. Prepare a volume of cells sufficient to provide 10μL of packed red blood cells of each panel cell sample. |
| * + 1. In separate labeled tubes, dispense 1.0 mL of MTS Diluent 2 Plus™. Add 10μL of each of the packed red blood cells from the panel to its labeled tube. |
| * + 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. Antibody Identification Test Procedure (Enzyme Treated Cells) | * + 1. Label the MTS Buffered Gel Cards™ with the appropriate identification and test information. |
| * + 1. Remove the foil seal from the microtubes to be used.   **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 50μL of each 0.8% antibody panel cell suspension to the correct microtube. Do not touch pipette to gel card. |
| * + 1. Using an appropriate pipette, add 25μL of serum or plasma to the correct microtubes. |
| * + 1. Incubate at 37±2ºC for 15 minutes. Refer to package insert for comment on extending incubation times. |
| * + 1. Centrifuge the gel cards at the pre-set conditions of 895 ±25 rpm for 10 minutes. |
| * + 1. After centrifugation, remove the card(s) from the centrifuge and observe each card for the following signs.  1. Unagglutinated red cells observed in the gel are usually caused by an interrupted centrifuge cycle. These red cells will appear dark pink and hazy. 2. A line of red cells streaming down one side and forming a “J” Appearance is caused by improperly seated card in the card holder.   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 from the chart below.  |  |  | | --- | --- | | **Grade** | **Description of Reaction\*** | | Neg | Unagglutinated red blood cells form a well-defined button at 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. | | 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**
  2. No agglutination or hemolysis of the test cells in the gel card is a negative result and indicates the absence of an antigen/antibody reaction. Unexpected antibodies were not present or were undetected. Interpret and report the antibody screen as negative.
  3. Agglutination in any or all cells indicates the presence of an antibody directed against the corresponding antigen that is present on the reagent cell sample.
  4. Hemolysis in the absence of a hemolyzed sample or agglutination of any of the cells in the gel card indicates the presence of an antibody directed against the corresponding antigen which is present on that reagent cell sample.
  5. Reactivity of the serum/plasma with the autologous control red cells may indicate the presence of autoantibody. Clinical history regarding recent red cell transfusion may be helpful.
  6. Identification of the antibody present in the plasma or serum may be made by matching the reactions obtained with the antigen profiles of red blood cells on the panel sheet. Additional cells may be needed to identify multiple antibodies. See NRT.008 - Exclusion of Antibodies.
  7. Report Antibody(ies)

1. **Procedural Notes**
   1. 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.
   2. 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:
      1. Improperly prepared cell suspension
      2. Adding the incorrect quantities of cells to the upper chamber.

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 can occur from bacterial contamination of test materials, inadequate incubation time or temperature, improper centrifugation, improper storage of materials or omission of test samples.
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. Addition of cells and plasma or serum
7. Red cell suspension should be added before the plasma or serum because the volume of red cell suspension is greater than the volume of plasma. Insufficient mixing may occur if the smaller volume of plasma is added before the red cell suspension.
8. Plasma or serum should be added within 10-15 minutes of adding the red cell suspension to the reaction chambers. Any red cells that come in contact with the gel column prior to centrifugation may not have the opportunity to come in contact with the plasma and may begin to migrate through the gel potentially giving a weaker reaction after centrifugation.
9. Incubation times in low ionic strength solutions between 5 – 40 minutes have been recommended in the literature. No single incubation time will be optimal for all antibodies. If the incubation time is changed from the manufacturer’s recommendation, validation studies are required.
10. There is the potential for IgM antibodies to react in this test. Some patient antibodies that are IgM in nature may react with corresponding antigens in the upper portion of the microtube and be trapped in the top portion of the gel at the time of centrifugation resulting in a positive reaction.
11. Adherence to the manufacturer’s package insert is critical to test performance.
12. Antibodies specific for low-incidence antigens not represented on the test cells will not be detected
    * 1. Antibodies below the threshold level may not be detected by this test.
      2. Anti-IgG may occasionally fail to detect antibodies that are demonstrable by the use of antiglobulin reagents that contain anti-C3.
      3. False-positive results may occur if antibodies to components of the preservative solution are present in the serum tested.
      4. Adherence to the manufacturer’s instruction for use is critical to test performance.
13. **References**
    1. CSTM Standards, Version 3 February 2011 5.3.5.
    2. Implementation Guide and Procedures -Procedure 8 Antibody Identification Method Using MTS Buffered Card-Enzyme treated Panel Cells Version 5 2010-05-31, ID-Micro Typing System
    3. Roback JD, ed. American Association of Blood Banks Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011;473-476
    4. ID-Micro Typing SystemTM Interpretation Guide-2010-06-04
14. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| April 30th, 2014 | * Revised name of manual * Changed document number GM.005 to GM.006 * Revised wording in sections 1.0 and 2.0 * Section 3.0 revised to state use of serum * Section 5.0 reformatted and renumbered * Section 6.0 reformatted and renumbered, reaction grading table updated to remove “w” as a reaction grade * Section 7.0 reformatted and renumbered * Section 8.0 revised wording and renumbered * Revised list of references |