1. **Principle**

This antibody identification test is used to identify warm reacting clinically significant antibodies while preventing the reactivity of cold clinically insignificant antibodies.

 In this gel test, the reagent red blood cells in a hypotonic saline solution are combined with patient plasma to allow antigen/antibody interaction in the upper chamber of the microtube. The cards are then incubated to enhance antigen/antibody interaction, and if the cells become sensitized, the sensitized cells will react with the Anti-IgG incorporated in the gel of the microtube during centrifugation. Agglutination indicates the presence of an antigen/antibody reaction while lack of agglutination indicates no antigen/antibody reaction. A modification of this technique, referred to as prewarming, can be employed to diminish or eliminate the reactivity of unwanted cold reactive antibodies, while maintaining sensitivity for clinically significant, warm-reactive antibodies. The inclusion of an autocontrol facilitates recognition of the presence of autoantibodies in the plasma sample being tested.

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. A prewarm technique is usually done when a cold reactive, clinically insignificant antibody is present or suspected in a patient’s specimen.
	5. The prewarm technique must be identical, other than the warming, to the method that gave positive results (i.e., same type of card, incubation time, etc.).
2. **Specimens**

EDTA anticoagulated whole blood within 14 days of collection 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 8.10 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.

**4.0 Materials**

**Equipment:** Centrifuge

 Incubator

 Pipettor

 Dispenser

 Set-up workstation, optional

 Serologic centrifuge

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

 Test tubes – 10 x 75 mm

 Serologic pipettes

 Package insert

**Reagents:** MTS Anti-IgG Card, Anti-IgG (Rabbit) suspended in gel

 Antibody identification panel as:

 0.8%, ready for use in MTS Anti-IgG Gel testing, or 3%, to be prepared in-house for use in MTS Anti-IgG testing

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

Saline

 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-IgG Card™, it is recommended that each lot be tested on each day of use with known positive and negative samples with the appropriate red cell. Reactivity must be present with the positive sample 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.
	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. 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.

**6.0 Procedures**

|  |  |
| --- | --- |
| * 1. Antibody Panel Cell Preparation, if necessary.
 | * + 1. Method 1 (for 2-4 tests, from 3% cell suspensions)
* Label test tubes for panel cells to be tested; include lot number, date and time of preparation.
* With an appropriate pipette, dispense one (1) volume (suggested minimum 100μL) of each antibody panel cell sample to its appropriately labeled tube. Add a small volume of MTS Diluent 2™ to each test tube for volume.
* Centrifuge one (1) minute to pack the red blood cells.
* Decant the supernatant (a dry cell button is recommended) and then add two (2) volumes of MTS Diluent 2™ (200μL if the initial volume were 100μL) to each tube.
* 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%.
 |
| * 1. Method 2 (for 20 tests, from 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™. 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. Autocontrol 0.8% cell suspension preparation
 | * + 1. Place 1.0 mL of MTS Diluent 2 in a test tube labeled with the test sample identification.
 |
| * + 1. Add 10μL of packed red cells from the sample to be tested.
 |
| * + 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
 | * + 1. Label the MTS Anti-IgG 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. Place small aliquot of the screening/panel cells in a test tube in the MTS incubator for 5-10 minutes.
 |
| * + 1. Place the card and an aliquot of the test plasma in the MTS incubator for 5 to 10 minutes.
 |
| * + 1. Using an appropriate pipette, add 50μL of the 0.8% antibody screen/panel cell suspension(s) to the labeled microtube(s). Do not touch pipette to gel card.
 |
| * + 1. While keeping the card in the incubator, add 25цL of prewarmed plasma or serum to the labeled microtubes.
 |
| * + 1. Incubate at 37±2ºC for minimum of 15 minutes. Refer to the instructions for use for comment on extending incubation times and Procedural Notes 8.9.
		2. Centrifuge the gel cards at the preset 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.
* Unagglutinated red cells observed in the gel are usually caused by an interrupted centrifuge 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 holder.
* If the card(s) show a sign of improper centrifugation, repeat the test. Do not recentrifuge 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. |
|  |  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. No agglutination or hemolysis of red cells, by prewarm technique, usually indicates that unexpected clinically significant antibodies were not present or were undetected.
	2. Agglutination or hemolysis of red cells by prewarm technique usually indicates the presence of unexpected clinically significant antibodies.
	3. 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 that is present on that reagent cell sample.
	4. Reactivity of the plasma with the autologous control red cells may indicate the presence of autoantibody. Clinical history regarding recent red cell transfusion may be helpful.
	5. Identification of the antibody present in the plasma 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.
	6. Report antibody(ies).
2. **Procedural Notes**
	1. Although centrifugation is performed at room temperature, prewarming of the reactants prior to mixing may eliminate reactivity due to cold antibodies.
	2. As with the implementation of any new procedure, the performance of this prewarm technique should be verified prior to implementation as part of the institution’s standard operating procedure.
	3. Some antibodies of clinical importance may be eliminated by prewarm technique.
	4. Antibodies below the threshold level may not be detected with this test.
	5. Antibodies specific for low-incidence antigens not represented on the test cells will not be detected.
	6. False-positive results may occur if antibodies to components of the preservative solution are present in the plasma or serum tested.
	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.
	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:
		1. Improperly prepared cell suspension.
		2. Adding the incorrect quantities of cells to the upper chamber.
		3. In this case repeat the test(s) ensuring correct quantities using new cell suspensions.
	9. 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.
	10. 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.
	11. 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.
	12. False-positive results may occur in gel cards showing signs of drying
	13. 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.
	14. Addition of cells and plasma or serum
		1. 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.
		2. 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.
	15. 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.
	16. 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.
	17. Adherence to the manufacturer’s instructions for use is critical to test performance.
	18. Anti-IgG may occasionally fail to detect antibodies that are demonstrable by the use of antiglobulin reagents that contain anti-C3.
	19. Adherence to the manufacturer’s package insert is critical to test performance.
3. **References**
	1. CSTM STDS Version 3 5.3.5.3
	2. Implementation Guide and Procedures -Procedure 8 Antibody Identifcation Method with( Auto Control) 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; 463-470,489
	4. ID-Micro Typing SystemTM Interpretation Guide-2010-0604
	5. INSTRUCTIONS FOR USE Anti-Human Globulin Anti-IgG (Rabbit) MTS™ Anti-IgG Card, Version 2.0
4. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| April 30, 2014 | * Revised name of manual
* Changed document number from GM.004 to GM.005
* Revised the wording of section 1.0
* Revised and renumbered section 2.0
* Specified that the preferred sample is EDTA anticoagulated whole blood within 14 days of collection in section 3.0
* Revised wording to include “See 8.10 in Procedure Notes” in section 3.0
* Revised wording in section 5.3 to specify a “clear liquid layer.”
* Revised and renumbered sections 5.0, 6.0, 7.0 and 8.0
* Revised list of references
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