1. **Principle**

 The direct antiglobulin test is used to demonstrate the presence or absence of immunoglobulin on the surface of red blood cells. Cells that have globulin adsorbed to their surfaces are said to be sensitized.

The sensitizing globulin may be gamma globulin (antibody) and/or beta globulin (components of complement). The direct antiglobulin test demonstrates whether or not red blood cells have become sensitized in vivo with IgG and/or complement.

In this gel test, the patient’s red blood cells, in a hypotonic buffered saline solution, are centrifuged through a gel microtube containing anti-IgG. The detection of IgG sensitization occurs when the sensitized red blood cells react with the Anti-IgG in the gel of the microtube during centrifugation. This method only detects sensitization with IgG.

1. **Scope and Related Policies**
	1. The Direct Antiglobulin Test (DAT) may be performed for investigation of:
* hemolytic disease of the newborn
* autoimmune hemolytic anemia
* transfusion reactions
* sensitization caused by drugs
	1. A DAT is required if an auto control is not done in the antibody screen and:
* antibody identification is required (e.g., limited volume of plasma)
* antigen typing of the patient cells is required
	1. The antiglobulin used for this direct antiglobulin test only contain antibodies to IgG and will not identify the C3d component of complement.
1. **Specimens**

 EDTA anticoagulated whole blood, the blood sample should be tested within 24 hours after collection. Clotted samples are not recommended for testing. See procedure Notes 8.5 regarding hemolysis and sample. Some blood samples, such as cord blood, blood stored for an extended time, or blood that has been incompletely anticoagulated, may develop fibrin clots or particulates. Red cells from such samples may be washed before testing to remove the clots and particulates.

Red blood cells that are stored for extended periods of time may become coated in vitro with complement and globulin proteins. Those samples coated with IgG will then test as DAT positive with this reagent.

1. **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 Diluent 2, a hypotonic buffered saline solution

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

 Do not use beyond expiration date. Store cards at 2 to 25°C. Store the 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 recognize reagent deterioration, the reagents must be tested daily with appropriate controls.
	2. 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™.
	3. 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 IgG coated red cell samples. 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. 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.
8. **Procedures**

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| --- | --- |
| * 1. Preparation of 0.8% Test Cells, from packed red blood cells
 | * + 1. Dispense 1.0 mL of MTS Diluent 2 into a labeled test tube.
 |
| * + 1. Add 10µL of packed red blood cells to the labeled tube.
 |
| * + 1. Mix gently. Final cell suspension should be approximately 0.8% and be stable for 24 hours. For best results, suspensions should not be less than 0.6% or exceed 1.0%.
 |
| * 1. Direct Antiglobulin Test Procedure
 | * + 1. Label the MTS Anti-IgG Card with the appropriate identification and test information.
 |
| * + 1. Bring reagents to room temperature (18-25°C) prior to use
 |
| * + 1. Remove the foil seal from the microtube 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 the 0.8% red cell suspension to the labeled microtube. Do not touch pipette to gel card.
 |
| * + 1. Centrifuge the gel card 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:
* 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.

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| --- | --- |
| **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. |
| 6.2.9 Report results |

1. **Reporting**
	1. Agglutination of any of the red blood cells in the microtube is a positive reaction.
	2. Absence of agglutination indicates lack of detectable IgG components on the red blood cells.
	3. If the DAT is positive with anti-IgG and the control is negative, obtain a patient medication and recent (past three months) transfusion history. It may be necessary to ask the patient or patient’s family, nurse and/or physician to obtain an accurate history. See NRT.005 – Investigation of a Positive Direct Antiglobulin Test (DAT).
	4. In a neonatal specimen, if the DAT is negative, the neonate is jaundiced, the maternal antibody screen is negative and neonatal cells are ABO incompatible with the maternal plasma, report:

Although the DAT is negative, HDFN due to maternal anti-A or anti-B cannot be excluded.

1. **Procedural Notes**
2. If a direct antiglobulin test performed on a clotted sample identifies complement, the result shall be verified using an EDTA sample.9.1
3. 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’s history.
4. Too few or too many cells in the microtube may cause falsepositive or false negative reactions. This may be due to one or both of the following errors:
* Improperly prepared cell suspension
* 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. Reports in the literature indicate that Anti-IgG may fail to detect antibodies that are demonstrable by the use of a polyspecific anti-human globulin reagent. Antibodies not detected by Anti-IgG may be clinically significant.
7. Adherence to the manufacturer’s package insert is critical to test performance.

**9.0 References**

1. CSTM Stds , Version 3 February 2011.5.3.6.2
2. Roback JD, ed. American Association of Blood Banks Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011:497-499
3. Implementation Guide and Procedures -Procedure 13 Direct Antiglobulin Test Version 5 2010-05-31, ID-Micro Typing System
4. ID-Micro Typing SystemTM Interpretation Guide-2010-06-04
5. *INSTRUCTIONS* FOR USE Anti-Human Globulin Anti-IgG (Rabbit) MTS™ Anti-IgG Card, Version 2.0
6. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| April 30, 2014 | * Revised name of manual
* Changed document number from GM.007 to GM.008
* Addition of IgG and/or compliment added to section
* Section 3 updated procedure note reference, and additional wording
* Section 5.2 additional wording added for clarity
* Section 6.2.2 additional step added
* Section 6.2.8 removed reference to “weak” grading from chart
* Removed steps 6.3-6.5
* Section 8 revised and renumbered and 8.5 – 8.10 added
* Updated list of references with most recent editions
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