Methodist Health Services Corporation
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Department of Pathology
Peoria, IL 61636

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INDIRECT ANTIGLOBULIN TEST
GEL CARD TEST METHOD

PRINCIPLE

The Indirect Antiglobulin Test is used to detect unexpected blood group antibodies. 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. This results in promoting antibody uptake. The detection of an antibody occurs when the sensitized red blood cells react with the Anti-IgG gel in the microtube during centrifugation.

SPECIMEN

Patient Preparation: No special preparation of the patient is required prior to specimen collection. Blood should be collected by approved techniques.
Note for Antibody Screens and/or Compatibility Testing: If patient has been transfused with blood or blood component(s) containing red cells, or has been pregnant within the preceding three months, or if transfusion history is unknown or uncertain, sample must be drawn within three days of transfusion. Otherwise, sample can be drawn within 7 days of transfusion.
Requirements: K_2 EDTA pink or lavender top tube is preferred.
Minimum Volume:

- Adult: 3.0 mL whole blood
- Pediatric: 2 - K_2 EDTA microtainers (each with 300-500 uL) or cord blood specimen

Specimen Stability:

If stored at room temperature 15-30°C, stable for testing 24 hours.
If stored 2-8°C, stable for testing for 72 hours.

Storage: 2-8°C for a minimum of 7 days after transfusion, or 10 days post crossmatch.

BEFORE ACCEPTING A TYPE AND SCREEN SPECIMEN, the technologist should visually verify that enough plasma would be left in the tube after performing the T&S to crossmatch approximately 12 units of blood. This will avoid another sample having to be drawn and repeating testing during an emergency.

Rejection Criteria: Hemolysis. **In rare occasions where sample cannot be redrawn, hemolyzed specimens may be used for testing as long as the testing personnel can accurately interpret the reactions.

REAGENTS

Antibody screen cells comprised of two vials of human red blood cells as:

1. 0.8%, ready for use in MTS Anti-IgG Gel testing, **OR**
2. 3%, to be prepared in-house for use in MTS Anti-IgG testing

Reagent	Storage	Stability
Selectogen I and II Reagent Red Blood Cells, OrthoClinical, 0.8% ready to use	2-8°C	Exp date
Biotestcell 1 and 2. BioRad, 3% to be converted.	2-8°C	Exp date
MTS Anti-IgG Gel Card	2-25°C	Exp. date
MTS Diluent 2	2-8°C	Exp. date

- Do not use beyond expiration date. Bring reagents to room temperature (18 to 25°C) prior to use.

INSTRUMENTS/EQUIPMENT

1. Pipettes for 25uL and 50uL dispensing
2. Pipette tips
3. MTS Centrifuge
4. MTS Incubator

QUALITY CONTROL

0.8% Cell Suspension Antibody Screen:

- To confirm the specificity and reactivity of the screen cells, run 1 positive control and 1 negative control for each lot of cells to be used, once per day of testing.
- Converted 4% suspension cells must have quality control performed when prepared.
- Refer to Blood Bank Daily QC Procedure for more information.

MTS Anti-IgG Card:

- To confirm the specificity and reactivity of the gel card, it is recommended that each lot be tested on each day of use with known positive and negative antibody samples with the appropriate red cells. Reactivity must be present with the positive sample only.
- Visually check gel cards: do not use if the liquid level in the microtube is at or below the top of the gel matrix, or if drying, bubbles, crystals, and/or artifacts are seen.

PROCEDURE

1. Label the MTS Anti-IgG Card appropriately,
   ex: with the patient's last name, first initial and test information.
2. Remove the foil seal from the microtubes to be used.
3. Add 50uL of each 0.8% antibody screen cell suspensions to its labeled microtube. Do not touch pipette to gel card.
4. Add 25uL of serum or plasma to the labeled microtubes. There should not be more than a 15 minute delay before adding plasma/serum to the testing cells in the microtube.
5. Incubate at 37±2°C for 15 minutes (not longer than 40 minutes).
6. Centrifuge the gel card at 895±25 RPMs for 10 minutes.
7. Read the front and the back of each microtube macroscopically and record reactions as described below.

*If 3-4% reagent cells are to be used for screen, **convert to 0.8% suspension** as follows:

a. Pipette 167uL of 3-4% reagent cells into labeled test tube
b. Centrifuge for 60 seconds
c. Using a pipette, remove the supernatant without disturbing the red cells
d. Dispense 0.5mL of MTS Diluent 2 to tube of packed cells
e. Mix to resuspend
f. Visually compare suspension to reagent 0.8% cells to confirm proper preparation
g. Proceed with Step 1 above.

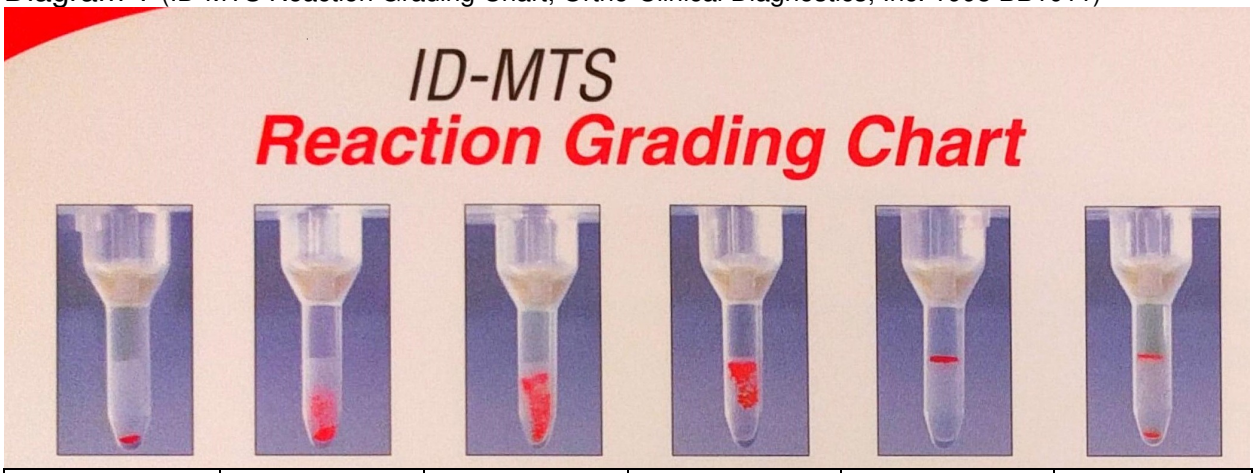
INTERPRETATION OF RESULTS

Negative Result	No agglutination and no hemolysis of the red blood cells is a negative test result. A complete sedimentation of all red blood cells is present in the bottom of the microtube.
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.

Reaction Grading Guide (Use in conjunction with Diagram 1 below)

REACTION	DESCRIPTION
0 Negative	Unagglutinated red blood cells form a well-defined button at the bottom of the microtube.
1+ Reaction	Red blood cell agglutinates are observed predominantly in the lower half of the gel microtube. Unagglutinated red blood cells form a button in the bottom of the microtube.
2+ Reaction	Red blood cell agglutinates are dispersed throughout the length of the gel microtube. Few unagglutinated red blood cells may be observed in the bottom of the microtube.
3+ Reaction	The majority of red blood cell agglutinates are trapped in the upper half of the gel microtube.
4+ Reaction	Solid band of red blood cell agglutinates on top of the gel. A few agglutinates may filter into the gel but remain near the predominant band.
Mixed Field	Red blood cell agglutinates at the top of the gel or dispersed throughout the gel microtube accompanied by a button of negative red blood cells in the bottom of the microtube. ****See Comments*

Diagram 1 (ID-MTS Reaction Grading Chart, Ortho-Clinical Diagnostics, Inc. 1998 BB1011)



Negative	1+ Reaction	2+ Reaction	3+ Reaction	4+ Reaction	Mixed Field

RESULTS

Absence of hemolysis or agglutination indicates a negative reaction and should be resulted as such.

Agglutination or hemolysis (from a non-hemolyzed specimen) indicates a positive reaction and should be resulted as such. Positive reactions warrant further investigation, including antibody identification if indicated.

Mixed field reactions must be interpreted with caution. Possible causes are coagulopathy, cold or warm autoantibodies, and medications. Antibodies to the preservative solution present in Ortho reagent cells can also cause a mixed field reaction.

If a type and screen is ordered and the antibody screen is positive, perform an antibody identification (if indicated), and prepare two units pRBCs (antigen negative if indicated) to be Coombs crossmatched and allocated to the patient during the admission.

RESULT ENTRY

1. Enter results in LIS appropriately

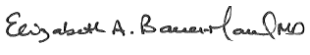
LIMITATIONS

1. Antibodies specific for low-incidence antigens not represented on the test cells will not be detected.
2. Antibodies below the threshold level may not be detected with this test.
3. False-positive or mixed field results may occur if antibodies to components of the preservative solution are present in the plasma/serum tested.
    a. These are typically characterized by panreactive mixed field patterns on the gel panel with a negative patient auto control and negative gel crossmatches.
    b. Converted 3-4% to 0.8% cell suspensions run in gel will show negative results when tested with patient plasma showing preservative reactivity.
4. Significant variations in red blood cell suspensions (<0.6 or >1.0%) may result in false-positive or false-negative reactions.
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. Anomalous results with fresh serum (i.e. a line of red cells on top of the gel) may be minimized by the use of EDTA plasma.
6. Adherence to the manufacturer's package insert is critical to test performance.
7. It may help to spin the sample for 10 minutes and repeat testing if a mixed field reaction is observed.

REFERENCES

Technical manual. 16th ed. Bethesda, MD: American Association of Blood Banks, 2008:437-460.
Current package insert: Reagent Red Blood Cells SELECTOGEN®. Raritan, NJ: Ortho-Clinical Diagnostics Inc.
Current package insert: Anti-Human Globulin Anti-IgG (Rabbit) MTS Anti-IgG Card. Pompano Beach, FL: Micro Typing Systems, Inc.
Current package insert: MTS Diluent 2 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. 26th ed. Bethesda, MD: American Association of Blood Banks, 2009.

POLICY CREATION :		
Author:	***Mary McCallister***	***July 9, 2003***
Medical Director:	***Devendra Trivedi, M.D.***	***July 9, 2003***

MEDICAL DIRECTOR		
DATE	NAME	SIGNATURE
	Elizabeth A. Bauer-Marsh, M.D.	
SECTION MEDICAL DIRECTOR		

REVISION HISTORY (began tracking 2011)			
Rev	**Description of Change**	**Author**	**Effective Date**
1	Minor formatting, added document ID to header, updated pretransfusion sample to 30 day acceptability. Added the Resulting for HBB	S.Schaffer	12/6/11
2	Clarification under results for setting up units if antibody screen is positive.	Kathy Turpin	11/19/12
3	Added BioRad reagents. Changed specimen acceptability from 30 days to 7. Added number 7 to limitations section.	Kathy Turpin	12/22/14
4	Clarified MTS diluent QC. Removed LIS specific references	Vincent Strow	2/10/16
5	Removed redundancies, inserted better diagram 1 for reaction results, added information on mixed field reactions and resolution. Clarified antibody ID reflex when screen positive.	Vincent Strow	5/11/17

Reviewed by

Lead	Date	Coordinator/Manager	Date	Medical Director	Date
K. Maher	12/30/11		12/27/11		12/30/11
			11/19/12		11/20/12
D. Allen	11/4/14		12/22/14		1/30/15
V. Strow	2/10/16		7/8/16		7/21/16
V. Strow	5/11/17		5/18/17		5/22/17