# PROCEDURE

**Title:** Antibody Detection Three Cell Screen-Gel Method

**Procedure #:** 2015BLOODBANK06

Institution: Highlands Regional Medical Center

Address: 3600 Highlands Avenue, Sebring Florida 33870

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 - 12/1/2011

Review of procedure every two years

Reviewed by: _____ Date: _____

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Discontinued testing date: _____

# PROCEDURE 6

**Anti-IgG**

## Antibody Detection Method - Three Cell Screen

## Principle

The antibody screen test is used to detect unexpected blood group antibodies. In the gel test, the reagent red blood cells are combined with sample serum/plasma in the upper reaction chamber of the microtube of an MTS™ Anti-IgG Card. Following an incubation period to enhance antigen/antibody interaction, the sensitized red blood cells react with the Anti-IgG incorporated in the gel of the microtube during a centrifugation step. 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.

Fresh serum or plasma collected with or without anticoagulants may be used in indirect antiglobulin procedures for antibody detection. Testing should be performed as soon as possible. Samples that cannot be tested immediately should be stored at 2-8°C or frozen. In the case of potential recipients of blood transfusion, an FDA requirement states that the specimen should not be stored for longer than 3 days before testing. Antibodies dependent for their detection upon the binding of complement may not be detected if aged serum or plasma from an anticoagulated sample is used for antibody detection tests. Serum should be separated from the red blood cells when stored or shipped.

Hemolyzed and grossly icteric blood samples may cause difficulty in interpretation, and test results should be used with caution. Grossly lipemic samples containing particulates that clog the gel, as indicated by diffuse blotches of red blood cells in the microtube, may be clarified by centrifugation or filtration and retested.

## Reagents

- Anti-Human Globulin Anti IgG (Rabbit) MTS™ Anti-IgG Card
- Antibody screen cells comprised of three vials of human red blood cells 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 gel testing
- MTS™ Diluent 2 (a hypotonic buffered saline solution used for in-house red blood cell preparation only)

Do not use reagents beyond 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 and red blood cells 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 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 antibody samples with the appropriate red blood cells. Reactivity must be present with the positive sample only.

### Diluent

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

## Procedure

### Antibody Screen Test Procedure

1. Visually inspect each gel card 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 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 the ID-Micro Typing System™ Interpretation Guide for additional information related to the visual inspection of gel cards before use.

2. Label the MTS™ Anti-IgG Card with the appropriate identification.
3. Remove the foil seal from the gel card or from the individual microtubes used for testing.

**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 50 µL of the 0.8% antibody screen red blood cell suspension to the microtube.

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

5. Add 25µL of serum or plasma to the microtubes.

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

6. Incubate the MTS™ Anti-IgG Card for 15 minutes at 37± 2°C.
7. After incubation, centrifuge the gel card(s) in the MTS™ Centrifuge at the preset conditions.
8. For manual readings, observe the front and the back of each microtube macroscopically and record reactions as described in the interpretation section of the corresponding 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.

### Preparation of 0.8% Suspension of Antibody Screen Cells, if necessary

### Method 1 (For 60 tests, from 3% red blood cell suspensions)

1. Label three test tubes I, II and III; include lot number, date, and time of preparation.
2. Dispense 1.0 mL of each antibody screen cell sample into its appropriately labeled tube and centrifuge to pack the red blood cells.
3. Decant the supernatant and then add 3.0 mL of MTS™ Diluent 2 to each tube.
4. Mix gently to resuspend. The final red blood cell suspensions should be approximately 0.8%. For best results, the red blood cell suspensions should be used on the day of dilution.

### Method 2 (For 20 tests, from packed red blood cells)

1. Label three test tubes I, II, and III; include lot number, date, and time of preparation.
2. Dispense 1.0 mL of MTS™ Diluent 2 into each tube. Add 10 µL of the appropriate packed red blood cell sample.
3. Mix gently to resuspend. The final red blood cell suspensions should be approximately 0.8%. For best results, the red blood cell suspensions should be used on the day of dilution.

## Interpretation of Results (Refer to Instructions for Use and the ID MTS™ 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.

# PROCEDURE 6 Anti-IgG

**Antibody Detection Method - Three Cell Screen**

## Comments

- Interpretation of mixed-field reactions must be done with caution. The presence of fibrin, clots, or particulates may cause some red blood cells to become trapped at the top of the gel. Mixed-field reactions should only be observed in tests containing a dual population of red blood cells, such as a transfused patient, bone marrow recipient or when a pooled cell sample is used for testing. However, not all mixed red blood cell situations have a sufficient minor population to be detected.

## 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.**

- Proper centrifuge calibration is particularly important to the performance of the MTS™ Anti-IgG card. The MTS™ Centrifuge has been exclusively designed to provide the correct time, speed, and angle.
- 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™.
- Variations in the 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 the 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.
- False positive or false negative test results can occur from bacterial or chemical contamination of test materials, inadequate incubation time or temperature, improper centrifugation, improper storage of materials, or omission of test samples.
- 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. Laboratories are advised to consult their approved procedures.
- 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 by the use of EDTA plasma.
- Red blood cells that test as DAT positive should not be used in an indirect antiglobulin procedure.
- The MTS™ Anti-IgG Card is not manufactured to detect Anti-C3 red blood cell sensitizations. It may be used in the compatibility test; however, some literature reports indicate that the Anti-IgG may occasionally fail to detect antibodies that are demonstrable by the use of antiglobulin reagents containing Anti-C3.
- Optimal reaction conditions vary across antibody specificities. No single test method will detect all antibodies. In some low ionic strength test systems, certain antibodies, such as Anti-E and Anti-K, have been reported to be nonreactive.
- 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.
- False positive results may occur if a card that shows signs of drying is used in testing.
- The Anti-H of Para-Bombay individuals may not be detectable in gel.

## References

1. Roback J. (ed) Technical Manual. 16th ed. Bethesda, MD: American Association of Blood Banks, 2008.
2. Package insert: Reagent Red Blood Cells 0.8% Surgiscreen®, current revision. Raritan, NJ: Ortho-Clinical Diagnostics Inc.
3. Instructions for Use: Anti-Human Globulin Anti-IgG (Rabbit) MTS™ Anti-IgG Card (J32848), current revision. Pompano Beach, FL: Micro Typing Systems, Inc.
4. Instructions for Use: MTS™ Diluent 2 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.
6. ID-Micro Typing System™ Interpretation Guide (6902201), Ortho Clinical Diagnostics

## Authorization

Supervisor: _____ Date Instituted: _____

Pathologist: _____ 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, Anti-Human Globulin Anti-IgG (Rabbit) MTS™ Anti-IgG Card (J32848)<br>• Updated product references and trademarks.<br>• Added references to ID-Micro Typing System™ Interpretation Guide<br>• Expanded limitations of the procedure to include guidance for resolving rouleaux test interference in agreement with Instructions for Use.<br>• Added caution against pre-centrifugation of cards that have failed visual inspection.<br>• Replaced "package insert" with "Instructions for Use".<br>• Updated References section. |
| 2005-05-02 | 4.0 | • Changed the format of document.<br>• Changed the date format to YYYY-MM-DD.<br>• Used the term **red blood cells** consistently.<br>• Added step 1 to visually inspect gel cards and the Caution statement.<br>• Added **or within one hour of testing** to the Note about the foil on the gel cards.<br>• Added the sentence **Erroneous results due to carryover may occur.** to the Note stating the pipette tip must not touch the gel card.<br>• Included the Note about the pipette tip not touching the gel card after each Add step.<br>• Changed the wording of the title for preparing 0.8% suspension of antibody screen cell.<br>• 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**... .<br>• Changed the technical manual reference.<br>• Changed the revision dates for the package inserts to **Current Revision.**<br>• Added Revision History. |

Highlands Regional MEDICAL CENTER

**Policy Name:** Type and Screen **Department:** Blood Bank-Lab

**Departmental Review:** **Policy #:** B2.5

**INITIATE DATE** **DATE REVIEWED/REVISED** 07/2013 **PAGE 2 of 2**

| Reviewed by | Reviewed Date | Reviewed by | Reviewed Date |
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| | 8-19-14 | | |
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Initial Implementation Date: _____

Taken out of Service: _____

Reason: _____

Reviewed by: _____ Date: 7/10/13
Department Supervisor

Reviewed by: Angela Lawster Date: 7/8/13
Department Adm. Director

Reviewed by: _____ Date: _____
Department Chief Technologist

Reviewed and Approved by: _____ Date: 7/8/13
Department Medical Director

Highlands Regional MEDICAL CENTER

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| **Policy Name: Type and Screen** | **Department: Blood Bank-Lab** |
| **Departmental Review:** | **Policy #: B2.5** |
| **INITIATE DATE** | **DATE REVIEWED/REVISED 07/2013** |



**PURPOSE:**
Specimens are ABO/Rh typed and screened for unexpected antibodies. Crossmatched units are not set aside for these patients unless certain criteria are met.

**POLICY:**
A Type and screen may be utilized for patients undergoing hospital procedures which do not routinely require transfusion. A crossmatch is performed if the physician requests that specific units be crossmatched for a patient, the patient is undergoing certain surgical procedures, a transfusion is subsequently ordered or if an unexpected antibody is discovered. Refer to Blood Bank Manual for detailed procedures.

**SPECIMEN:**
Samples may be used up to 14 days in pre-admission patients who have not been transfused or pregnant within the past 3 months and may not exceed 3 days for inpatients, patients who have been transfused or pregnant within the past 3 months or have unknown transfusion history. Store patient samples at 1-10°C.

Samples must not be hemolyzed and must be properly labeled with the patient's name, medical record number, date time and collector's identification. The specimen is collected in a blood bank tube and should be labeled with an appropriate blood bank band.

**PROCEDURE:**
1. Inspect the specimen labeling and confirm that all identifying data on the specimen tube matches the information found in the LIS or the manual requisition.

2. Perform an ABO/Rh and antibody screen on the specimen.

3. Negative antibody screens are recorded and reported. Pre-admission samples requiring a crossmatch are stored and compatibility testing completed the day prior to admission.

4. Positive antibody screens are identified, recorded and reported. At least 2 extended crossmatched and antigen negative units corresponding to the antibody(ies) identified should be set up.

5. Subsequent transfusion orders on a negative screen are crossmatched by immediate spin with the Type and Screen specimen.

**PROCEDURE NOTES:**
Once a Type and Screen is completed an immediate spin crossmatch for compatible units can be completed in less than 10 minutes.

**REFERENCE:**
AABB, Technical Manual