PROCEDURE

Title: Direct Antiglobulin Test with C3d-Gel Method

Procedure #: 2015BLOODBANK14

Institution: Highlands Regional Medical Center

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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

Review of procedure every two years

Reviewed by: _____ Date: _____

Reviewed by: _____ Date: _____

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Reviewed by: _____ Date: _____

Discontinued testing date: _____

PROCEDURE 14

Direct Antiglobulin Test

Anti-IgG,-C3d

Principle

The direct antiglobulin test (DAT) is used to demonstrate the presence or absence of IgG and C3 on the surface of red blood cells. Red blood cells that possess IgG and/or C3 adsorbed to their surfaces are referred to as sensitized red blood cells. The direct antiglobulin test demonstrates the sensitization of red blood cells in vivo. The MTS™ Anti-IgG,-C3d Card can be used to detect the presence of IgG and/or C3 on red blood cells. In the gel test, the sensitized red blood cells react with the Anti-IgG,-C3d 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.

Samples intended for direct antiglobulin testing should be drawn into EDTA to prevent in vitro complement binding. If EDTA is unavailable, specimens drawn into ACD, CPD, or CPDA-1 are preferable to non-anticoagulated clotted specimens. Red blood cells should be tested within 24 hours after collection.

Clotted samples should not be refrigerated.

Some samples such as cord blood, blood stored for extended periods of time, or blood that has been incompletely anticoagulated may develop fibrin clots or particulates. The fibrin clots or particulates may interfere with the gel test and cause red blood cell entrapment at the top of the microtube. Testing should be repeated using red blood cells that have been washed to remove the clots or particulates.

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

Reagents

- Anti-Human Globulin Anti-IgG,-C3d Polyspecific (Rabbit) MTS™ Anti-IgG,-C3d Card
- MTS™ Diluent 2™ (a hypotonic buffered saline solution)

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 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 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,-C3d Card, it is recommended that each lot be tested on each day of use with known positive and negative samples. Reactivity must be present with the positive sample only. The anti-complement reactivity of this reagent can be assessed with complement-coated red blood cells.

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

Preparation of 0.8% Suspension of Test Cells from packed red blood cells

1. Dispense 1.0 mL of MTS™ Diluent 2 into a labeled test tube.
2. Add 10 µL of packed red blood cells to the labeled tube.
3. Mix gently to resuspend. The final red blood cell suspension should be approximately 0.8%.

Direct Antiglobulin 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 the 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 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,-C3d 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% 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. Centrifuge the gel card(s) in the MTS™ Centrifuge at the preset conditions.
6. 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.

Interpretation of Results (Refer to the Instructions for Use and the ID-Micro Typing System™ 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. A positive test result indicates the presence of IgG and/or C3d on the red blood cells.

Comments

- Direct antiglobulin tests are useful in the investigation of hemolytic disease of the newborn, autoimmune hemolytic anemia, and transfusion reactions.
- Interpretation of the results of a direct antiglobulin test depends on the specificity of the anti-human globulin reagent in use. To further investigate positive results, monospecific antiglobulin reagents and elution techniques may be used.
- The use of various drugs and certain disease states are known to be associated with positive direct antiglobulin tests.
- Interpretation of mixed-field reactions must be done with caution. The presence of fibrin, clots or particulates may cause some red blood cells to be 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 red blood cell sample is used for testing. However, not all mixed 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 package insert is critical to test performance.**

- This card is intended for the direct antiglobulin test only.
- Not all positive reactions imply the presence of clinically significant antibodies. It is important to distinguish between nuisance reactions, in which red blood cell bound serum globulins are present but have no clinical significance, from positive reactions due to clinically significant antibodies. To further investigate positive results, monospecific antiglobulin reagents and elution techniques may be used.
- 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.
- Proper centrifuge calibration is particularly important to the performance of the MTS™ Anti-IgG,-C3d 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 before addition to the MTS™ Anti-IgG,-C3d Card.
- 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.
- 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.

References

1. Roback, J. (ed) Technical Manual. 16th ed. Bethesda, MD: American Association of Blood Banks, 2008.
2. Instructions for Use: Anti-Human Globulin Anti-IgG, -C3d Polyspecific (Rabbit) MTS™ Anti-IgG,-C3d Card J32849)™ Current revision. Pompano Beach, FL: Micro Typing Systems, Inc.
3. Instructions for Use: MTS™ Diluent 2™ Red Blood Cell Diluent Current revision. Pompano Beach, FL: Micro Typing Systems, Inc.
4. Malyska H, Weiland D. The Gel Test. Laboratory Medicine, 1994; 25:81-5.

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, -C3d Polyspecific (Rabbit) MTS™ Anti-IgG,-C3d Card J32849)™ • Updated product references and trademarks. • Added reference to ID-Micro Typing System™ Interpretation Guide • Expanded limitations of the procedure to include guidance for resolving rouleaux test interference in agreement with Instructions for Use. • Added caution against the pre-centrifugation of cards that have failed visual inspection. • Replaced "package insert" with "Instructions for Use". • Updated References section.
2005-05-02	4.0	• Changed the format of document. • Changed the date format to YYYY-MM-DD. • Used the term **red blood cells** consistently. • Added step 1 to visually inspect gel cards and the Caution statement. • Added **or within one hour of testing** to the Note about the foil on the gel cards. • Added the sentence **Erroneous results due to carryover may occur** to the Caution stating the pipette tip must not touch the gel card. • Included the Caution about the pipette tip not touching the gel card after step 4 of the Direct Antiglobulin Test Procedure. • 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**... . • Changed the technical manual reference. • Changed the revision dates for the package inserts to **Current Revision**. • Added Revision History.