Anti-Human Globulin Anti-IgG, -C3d Polyspecific (Rabbit)

MTS™ Anti-IgG, -C3d Card

INSTRUCTIONS FOR USE

REF MTS084014

Rx ONLY

Intended Use

For *in vitro* diagnostic use only
For Direct Antiglobulin Test
For use with the ID-Micro Typing System™
Contains: 6 tests per card

Observable Indications

Drying, discoloration, bubbles, crystals, other artifacts, opened or damaged seals may indicate product alteration.

Summary and Explanation of the Test

Anti-Human Globulin was described in 1945 by Coombs, Mourant, and Race. [1] Blood group antibodies of the IgG class, that were previously undetectable, reacted in the direct or indirect antiglobulin test (also known as the Coombs test). Anti-IgG reagents remain important tools for determining the presence or absence of IgG on human red blood cells. The reagent is used in the investigation of hemolytic disease of the newborn (refer to Limitations of Procedure, item 11), transfusion reactions, and autoimmune hemolytic anemia in a direct antiglobulin test (DAT). The DAT detects IgG and/or C3 using either a polyspecific reagent solely or monospecific Anti-IgG and Anti-C3.

Principles of the Procedure

The combination of the antiglobulin reagent incorporated into gel, known as the ID-MTS™ Gel Test [2] was first described by Dr. Yves Lapierre. [3] The MTS™ Anti-IgG,-C3d Card can be used in the direct antiglobulin test. [2]

Red blood cells that are coated with IgG and/or complement due to *in vivo* sensitization are detected with the direct antiglobulin test.

The MTS™ Anti-IgG,-C3d Card restricts the unbound IgG from moving through the gel during centrifugation. The unbound IgG and complement components do not neutralize the Anti-IgG,-C3d incorporated in the gel.

Red blood cells sensitized with IgG and/or C3d react with the corresponding antiglobulin component in the microtube during centrifugation. Strongly positive agglutination reactions produce a line of red blood cells layered at the top of the gel. Positive reactions will have varying degrees of visible red blood cell agglutinates suspended in the gel. Uncoated (unsensitized) red blood cells are not agglutinated by the Anti-IgG and/or Anti-C3d and will form a button at the bottom of the microtube.

Reagents

Anti-Human Globulin Anti-IgG,-C3d; Polyspecific (Rabbit) for the MTS™ Anti-IgG,-C3d Card is prepared from blended pools of sera obtained from rabbits that have been immunized with either human IgG or human C3. The sera is adsorbed to remove unwanted heterospecific antibodies and is suspended in a buffered gel solution. The reagent meets present potency and specificity requirements of the FDA. [4]

Sodium Azide (0.1% final concentration) is added as a preservative.

Anti-Human Globulin, Anti-IgG,-C3d; Polyspecific (Rabbit) suspended in a diluent and buffered gel solution is contained in the 6 microtubes of the MTS™ Anti-IgG,-C3d Card.

Storage Requirements

Store cards upright at 2–25 °C.

Precautions

- Do not use beyond expiration date.
- Do not freeze or expose cards to excessive heat.
- Use reagents as furnished.

Caution:	**All blood products should be treated as potentially infectious. Source material from which this product was derived was found negative when tested in accordance with current FDA required tests. No known test methods can offer assurance that products derived from human blood will not transmit infectious agents.**

Caution:	**Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive compounds. If discarded into sink, flush with a large volume of water to prevent azide buildup.**

WARNING:	***Once a gel card is used in testing, it may contain infectious material and should therefore be handled and disposed of as biohazard waste.***

- 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 [5] for additional information related to the visual inspection of gel cards before use.

- Do not remove foil seal until ready to use. Foil should be removed immediately before testing or within 1 hour of testing. Once opened, the gel may begin to dry out which could affect test results (refer to Limitations of the Procedure, item 10).
- After removing the foil, visually inspect all gel cards to ensure that residual film does not block the opening of any microtube.

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

- Customers who choose to use commercial antisera in an off-label manner must ensure that the test method is appropriate by validating its intended use.
- Do not use gel cards that have not been shipped in an upright position.

Specimen Collection and Preparation

No special preparation of the patient is required prior to specimen collection. Collect all blood samples using acceptable phlebotomy techniques.

Samples for Direct Antiglobulin Test (DAT)

Samples intended for direct antiglobulin testing should be drawn into EDTA to prevent *in vitro* complement binding. Red blood cells should be tested within 24 hours after collection. 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 ID-MTS™ 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 become coated *in vitro* with complement and/or globulin proteins. Those samples coated with IgG will then test as DAT positive with this reagent.

Hemolyzed and grossly icteric blood samples may cause difficulty in interpretation, and test results should be used with caution.

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 the ID-MTS™ Gel Test interpretation. [5] False positive results or hazy reactions may occur with these samples but are rare. Samples exhibiting rouleaux should be washed several times in saline and retested. [6] Laboratories are advised to consult their approved procedures.

Reagent Preparation

The MTS™ Anti-IgG,-C3d Card is provided ready to use. Each microtube contains Anti-IgG,-C3d suitable for one test. The gel card is heat-sealed with aluminum foil to preserve the integrity of the reagents. Variations in the liquid and/or gel levels between microtubes may normally be observed. However, do not use cards if the liquid level in the microtube is at or below the top of the gel matrix (refer to Precautions).

Procedure

The procedures identified below are for manual testing only. When using automated instruments, follow the procedures that are contained in the operator's manual provided by the device manufacturer. Laboratories must follow their approved validation procedures and are advised to consult the appropriate regulatory agencies to determine validation requirements. Refer to ID-Micro Typing System™ Interpretation Guide [5] and ID-Micro Typing System™ Implementation Guide and Procedures [7] for additional information.

Materials Provided

Anti-Human Globulin, Anti-IgG,-C3d; Polyspecific (Rabbit) suspended in a final diluent and buffered gel solution is contained in the 6 microtubes of the MTS™ Anti-IgG,-C3d Card.

Materials Required but Not Provided

For manual gel card processing:

- Quality Control Material known to give the appropriate positive and negative test results for each reagent requiring quality control. Examples include, but are not limited to, Hemo bioscience HBS – C3 Control Cells
- MTS™ Diluent 2
- Pipets: 10 µL and 50 µL
- Pipet Tips
- Test Tubes
- Dispenser pipet capable of delivering 1.0 mL
- Marking Pen
- ORTHO® Workstation
- ORTHO Optix™ Reader

For automated gel card processing with the ORTHO VISION® Analyzer or ORTHO VISION® Max Analyzer:

- Quality Control Material known to give the appropriate positive and negative test results for each reagent requiring quality control
- MTS™ Diluent 2
- ORTHO VISION® Analyzer
- ORTHO VISION® Max Analyzer

Test Procedure

Direct Antiglobulin Test

1. Bring samples and reagents to room temperature (18–25 °C).
2. Visually inspect gel cards 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 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 [5] for additional information related to the visual inspection of gel cards before use.

3. Prepare a red blood cell suspension of approximately 0.8% in MTS™ Diluent 2 (e.g., deliver 1.0 mL of MTS™ Diluent 2 into a test tube and pipet 10 µL packed red blood cells into the diluent), mix gently.
4. Label the gel card appropriately.
5. Remove the foil seal from the MTS™ Gel Card or from the individual microtubes to be used for testing. After removing the foil, visually inspect all gel cards to ensure that residual film does not block the opening of any microtube.

Note:	Foil should be removed immediately before testing or within 1 hour of testing. Once opened, the gel may begin to dry out which could affect test results (refer to Limitations of the Procedure, item 10).

6. Add 50 µL of red blood cells (cells must be diluted in MTS™ Diluent 2 to approximately 0.8% or be a commercial 0.8% red blood cell in a low ionic strength diluent specifically approved for use in the ID-Micro Typing System™) to each microtube. It is not necessary that the blood come into contact with the gel.

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

7. Centrifuge the prepared cards in the ORTHO® Workstation at the preset conditions installed by the manufacturer.

8. After centrifugation, remove the gel card(s) from the centrifuge. Observe, read macroscopically the front and back of each microtube for agglutination and/or hemolysis and record reactions. If either side of the microtube is positive, the reaction is to be considered positive. See Diagram 1.

Interpretation of Results

Refer to ID-Micro Typing System™ Interpretation Guide [5] for additional information.

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. A negative test result indicates the absence of detectable IgG or C3 on the red blood cells.

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 C3 on the red blood cells.

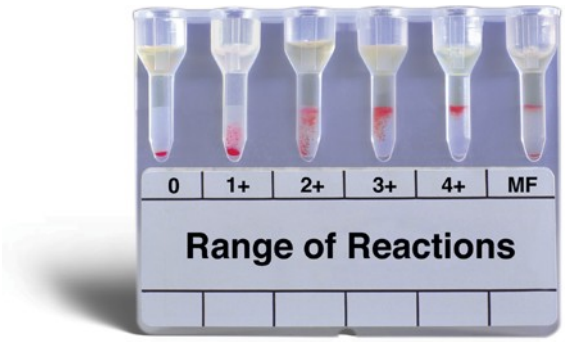
Reaction Grading Guide (Use in conjunction with Diagram 1)

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 Note below.

Note:	Caution must be taken in interpreting a reaction as mixed field. Additional patient history and testing will be necessary for resolution. However, not all mixed cell situations have a sufficient minor population to be detected.

Caution:	**Clots, particulates or other artifacts may cause some red blood cells to be entrapped at the top of the gel that may cause an anomalous result in a negative test (refer to Limitations of the Procedure, item 8).**

Diagram 1: Examples of Reaction Grades



Stability of Reaction

For best results, it is recommended that reactions should be read immediately following centrifugation. Interpretations may be affected by the drying out of the gel, hemolysis of the red blood cells, and slanting of the reaction patterns due to storage in a non-upright position.

Quality Control

To confirm the specificity and reactivity of the MTS™ Anti-IgG,-C3d Card, it is recommended that each lot be tested 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 by using complement coated cells. [1]

Limitations of the Procedure

Refer to ID-Micro Typing System™ Interpretation Guide [5] for additional information.

1. Strict adherence to the procedures and recommended equipment is essential.
2. Proper centrifuge calibration is particularly important to the performance of the MTS™ Gel Test. The ORTHO® Workstation, ORTHO VISION® Analyzer and ORTHO VISION® Max Analyzer have been exclusively designed to provide the correct time, speed and angle.
3. This card is intended for direct antiglobulin testing only.
4. Not all positive reactions imply the presence of clinically significant antibodies. It is important to distinguish between "nuisance" reactions in which cell bound serum globulins are present, but which have no clinical significance from positive reactions due to clinically significant antibodies. [11]
5. Red blood cells must be suspended in MTS™ Diluent 2 or be a commercial 0.8% red blood cell in a low ionic strength diluent specifically approved for use in the ID-Micro Typing System™.
6. Variations in red blood cell concentration can markedly affect the sensitivity of test results. [1] 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 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.
7. 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.
8. Anomalous results may be caused by fibrin or other particulate matter in blood samples that could stick to the sides of the microtube.
9. Red blood cells that test as DAT positive should not be used in an indirect antiglobulin test procedure.
10. False-positive results may occur if a card that shows signs of drying is used in testing.
11. Negative direct antiglobulin test results do not necessarily rule out hemolytic disease of the newborn (HDN), especially if ABO incompatibility is suspected.
12. 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 the ID-MTS™ Gel Test interpretation. [5] 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. [6] 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.
13. Hemolyzed and grossly icteric blood samples may cause difficulty in interpretation, and test results should be used with caution.
14. When using automated instruments, refer to the limitations contained in the operator's manual provided by the device manufacturer.

Specific Performance Characteristics

Each lot of MTS Anti-IgG,-C3d Card™ meets FDA requirements. The potency of Anti-IgG and Anti-C3d are verified by tests with red blood cells sensitized with decreasing amounts of Anti-D, Anti-Fy^a and Anti-C3d according to methods approved by FDA. Additionally, each lot is tested with a known antibody to ensure Anti-IgG sensitivity of 0.1 IU/mL or greater.

The presence of Anti-C3b and the absence of antibodies to C4 components have been confirmed by methods approved by FDA. [1]

The absence of contaminating heterophile agglutinins has been verified in tests employing group A_1, B, and O red blood cells.

Performance Characteristics on ORTHO VISION® Analyzer

Method comparison testing was performed at one site that routinely performs immunohematology testing. Patient specimens (N=65) were tested on the ORTHO VISION® Analyzer and the ORTHO ProVue® Analyzer. Additional DAT IgG, -C3D results (N=298) were derived from whole blood control samples tested as part of a reproducibility study conducted at three sites. Individual microtube results were evaluated for agreement between the analyzers. For microtube reaction grades to be in agreement with expected results, microtube reaction grades were either negative in the absence of IgG

and/or -C3d coated cells or positive (1+ through 4+) in the presence of IgG and/or -C3d coated cells. The combined results from all sites are summarized in the following table

	Total			Positive			Negative		
Test	N	% Agreement	Lower Bound One Sided 95% CI	N	% Agreement	Lower Bound One Sided 95% CI	N	(%) Agreement	Lower Bound One Sided 95% CI
DAT IgG, -C3d	363	99.4%	98.3%	115	99.1%	95.9%	248	99.6%	98.1%

Agreement between two methods does not indicate which method gave the correct results.

Performance Characteristics on ORTHO VISION® Max Analyzer

Method comparison testing was performed at five sites that routinely perform immunohematology testing. Patient specimens were tested on the ORTHO VISION® Max Analyzer and the ORTHO VISION® Analyzer. Individual microtube results were evaluated for agreement between the analyzers. For microtube reaction grades to be in agreement with expected results, microtube reaction grades were either negative in the absence of IgG and/or -C3d coated cells or positive (1+ through 4+) in the presence of IgG and/or -C3d coated cells. The combined results from all sites are summarized in the following table.

	Total			Positive			Negative		
Test	N	% Agreement	Lower Bound One Sided 95% CI	N	% Agreement	Lower Bound One Sided 95% CI	N	(%) Agreement	Lower Bound One Sided 95% CI
DAT IgG, -C3d	438	99.8%	98.9%	172	100.0%	98.3%	266	99.6%	98.2%

Agreement between two methods does not indicate which method gave the correct results.

Performance Characteristics on ORTHO Optix™ Reader

Method comparison testing was performed at three sites (two external and one internal site), that routinely perform immunohematology testing. Individual microtube results were evaluated for agreement between ORTHO Optix™ Reader and the ORTHO VISION® Analyzer. For microtube reaction grades to be in agreement between the systems, microtube reaction grades were either both negative or both positive (1+ through 4+). Microtube results for a given test were combined across applicable ID-MTS™Gel Cards. The combined results from all sites are summarized in the following table.

	Total			Positive			Negative		
Test	N	% Agreement	Lower Bound One Sided 95% CI	N	% Agreement	Lower Bound One Sided 95% CI	N	(%) Agreement	Lower Bound One Sided 95% CI
DAT IgG -C3d	1007	100.0%	99.7%	364	100.0%	99.2%	643	100.0%	99.5%

Agreement between two methods does not indicate which method gave the correct results.

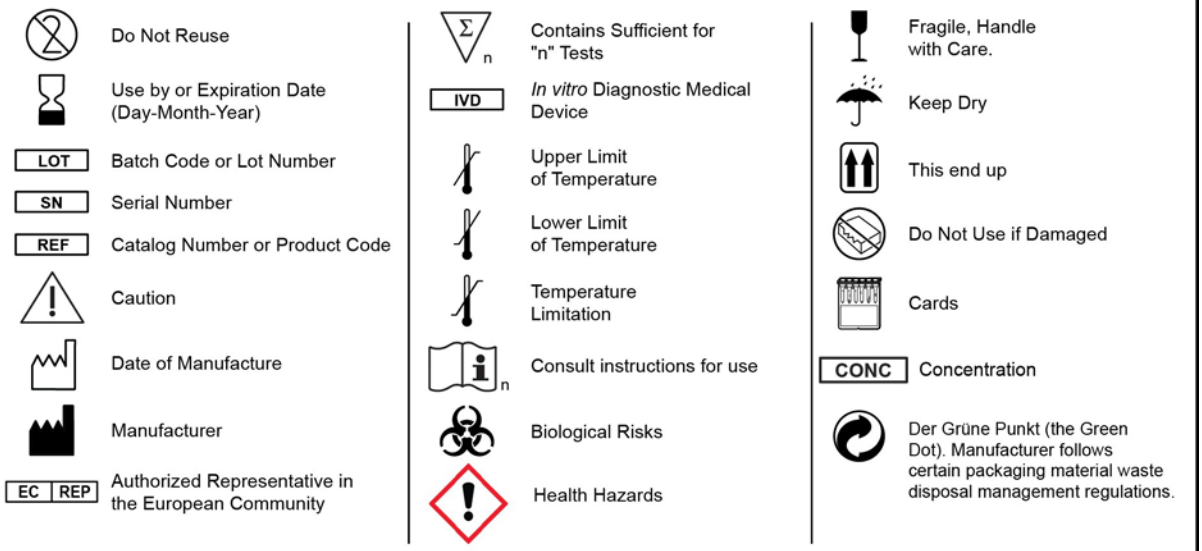
References

1. 1. Coombs RRA, Mourant EE, Race RR. Detection of weak and "incomplete" Rh agglutinins. A new test. *Lancet.* 1945;ii:15.
2. Malyska H, Weiland D. The gel test. *Laboratory Medicine.* 1994;25:81-85.
3. Lapierre Y, Rigal D, Adam J, et al. The gel test: a new way to detect red cell antigen-antibody reactions. *Transfusion.* 1990;30:109-113.
4. Code of Federal Regulations; FDA, April 1, 2007; 21 CFR 606.151 (b).
5. ID-Micro Typing System™ Interpretation Guide (6902201), Ortho Clinical Diagnostics.
6. Brecher M. (ed) Technical Manual, 16th Ed. Bethesda, MD: American Association of Blood Banks, 2008.
7. ID-Micro Typing System™ Implementation Guide and Procedures (6902200), Ortho Clinical Diagnostics.
8. Howard JE, Winn LC, Gottlieb CE, et al. Clinical significance of the anti-complement components of antiglobulin antisera. *Transfusion.* 1982;22:269-272.
9. Moore HC, Mollison PL. Use of a low-ionic strength medium in manual tests for antibody detection. *Transfusion.* 1976;16:291-296.

10. Office of Biologics Research and Review, FDA. Recommended methods for Anti-Human Globulin Evaluation. Docket No. 84S-0182.
11. Nasongkla M, Hummert J, Chaplin Jr. H. Weak "false positive" direct antiglobulin test C3d. *Transfusion.* 1982;22:273-275.

Glossary of Symbols

The following symbols may have been used in the labeling of this product.



Revision History

Date of Revision	Version	Description of Technical Changes*
2020-11-12	5.0	• Removed reference to MTS™ Centrifuge throughout document • Corrected trademark for ORTHO Workstation from (™) to (®) throughout document • Materials Required but not Provided: Added ORTHO Optix™ Reader • Specific Performance Characteristics: Added Performance Characteristics for ORTHO Optix™ Reader • New format; technically equivalent to V4.0

* The change bars indicate the position of a technical amendment to the text with respect to the previous version of the document.

Made under one or more of the following U.S. Patents:
5,338,689
5,460,940
5,512,432
5,863,802
6,114,179
Other Patents Pending

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