Anti-Human Globulin Anti-IgG (Rabbit)/Anti-C3b,-C3d (Murine Monoclonal)/DAT Control MTS™ DAT Card

INSTRUCTIONS FOR USE

REF

MTS6014
MTS601402
MTS601410

Rx ONLY

Intended Use

For *in vitro* diagnostic and laboratory professional use

Qualitative procedure for the detection of IgG or C3 components of complement bound to red blood cells.

For use with the ID-Micro Typing System™.

For the testing of 1 to 2 samples per gel card.

Anti-Human Globulin Anti-IgG (Rabbit)/Anti-C3b,-C3d (Murine Monoclonal)/DAT Control, MTS™ DAT Card is intended for the detection of antibodies and or complement on human red blood cells using the direct antiglobulin test. This direct antiglobulin test allows for the differentiation of human red blood cells sensitized *in vivo* by IgG type immunoglobulins or complement C3b and or C3d fractions. Blood samples from patients and or donors intended for direct antiglobulin testing should be drawn into EDTA to prevent *in vitro* complement binding.

MTS™ DAT Card is intended for manual or automated column agglutination technology using ORTHO® Workstation, and the ORTHO VISION® and VISION® Max Analyzers.

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 fetus and 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 a monospecific Anti-IgG and Anti-C3 reagent.

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™ DAT Card is designed to 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™ DAT Card restricts the unbound IgG and/or complement components from moving through the gel during centrifugation. The unbound IgG and complement components do not neutralize the Anti-IgG and/or Anti-C3b,-C3d incorporated in the gel.

Red blood cells sensitized with IgG and/or complement (C3b 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-C3b,-C3d and will form a button at the bottom of the microtube.

Reagents

Anti-Human Globulin, Anti-IgG (Rabbit) and Anti-C3b,-C3d (Murine Monoclonal) are suspended in a diluent and buffered gel solution contained in the following microtubes of the MTS™ DAT Card.

Table 1

Product Code MTS6014, MTS601402 and MTS601410	Component Description
Microtubes 1 and 4	Anti-Human Globulin Anti-IgG (Rabbit)
Microtubes 2 and 5	Anti-Human Globulin Anti-C3b,-C3d (Murine Monoclonal)
Microtubes 3 and 6	Formulation of the diluent used in antibody reagent (used as Control)

The reagents meet present potency and specificity requirements of the FDA.
Sodium Azide (0.1% final concentration) is added as a preservative to all microtubes.

Storage Requirements

Store cards upright at 2–25 °C.

Warnings and 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 [4] 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.
Cell washing is an optional step in specimen preparation. Performance of the device is not compromised by washing cells with saline three times.
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.

INSTRUCTIONS FOR USE

Reagent Preparation

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 the Anti-IgG reagent. Samples coated with complement will then test DAT positive with the Anti-C3b,-C3d reagent.

Hemolyzed 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. [4] 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. [5] Laboratories are advised to consult their approved procedures.

Reagent Preparation

The MTS™ DAT Card is provided ready to use. Each microtube contains antibody or control without antibody 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 Warnings and Precautions).

Procedure

The procedures identified below are for manual testing only. 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 [4] and ID-Micro Typing System™ Implementation Guide and Procedures [6] for additional information. When using Ortho automated instruments, follow the approved procedures that are contained in the operator's manual provided by the device manufacturer.

Materials Provided

Anti-Human Globulin Anti-IgG (Rabbit)/Anti-C3b,-C3d (Murine Monoclonal)/DAT Control (MTS™ DAT 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.
- 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

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 or ORTHO VISION® Max Analyzer

Test Procedure

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 [4] 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).

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 the reaction chamber of each microtube. Ideally, the blood sample should not 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 using 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 [4] 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 (microtubes 1 and 4) or C3b,-C3d (microtubes 2 and 5) on the red blood cells. A negative result must be obtained for the control (microtubes 3 and 6).

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 (microtubes 1 and 4) and/or C3b,-C3d (microtubes 2 and 5) on the red blood cells. If the control (microtubes 3 or 6) is positive, a valid interpretation of the results obtained cannot be made (refer to Limitations of the Procedure).

Reaction Grading Guide (Use in conjunction with Diagram 1)

Reaction Grading	Reaction Grading 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 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).**

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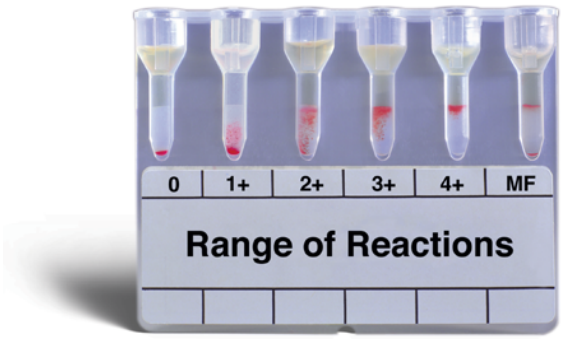
Stability of Reaction

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™ DAT 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.

Limitations of the Procedure

Refer to ID-Micro Typing System™ Interpretation Guide [4] 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. [7]
5. Red blood cells must be suspended in MTS™ Diluent 2 or must be a commercial 0.8% reagent 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 fetus and newborn (HDFN), 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. [4] False positive results or hazy reactions may occur with these samples but are rare. If positive reactions (e.g., rouleaux, cells coated with immunoglobulins, etc.) occur in the control microtube, the specific interpretation (IgG and/or C3b,-C3d) cannot be made. 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. [5] 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 blood samples may cause difficulty in interpretation, and test results should be used with caution.
14. When using Ortho automated instruments, refer to the limitations contained in the operator's manual.

15. DAT positive test results can occur with red blood cells from patients who are undergoing treatment with monoclonal antibody therapies (CD38-directed cytolytic antibodies). Laboratories are advised to consult their approved procedures when interpreting test results if there is a history of monoclonal antibody treatment.

Specific Performance Characteristics

Each lot of MTS™ DAT Card meets FDA requirements for potency. The potency of Anti-IgG is verified by tests with red blood cells tested against decreasing amounts of Anti-D and Anti-Fy^a positive plasmas. Additionally, each lot of Anti-IgG is tested with a known antibody to ensure Anti-IgG sensitivity of 0.1 IU/mL or greater. The potency of Anti-C3b,-C3d are verified by tests with red blood cells sensitized with decreasing amounts of C3b and C3d.

The absence of antibodies to C4 components have been confirmed by methods approved by FDA. [8]

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

Testing was performed on the ORTHO VISION® / VISION® Max Analyzers and ORTHO® Workstation at four sites (two external and two internal sites: Sites 1-4).

Further investigation into the low positive percent agreement (PPA) values obtained on all platforms identified three major contributing factors specific to use of conventional tube test technology as a comparator for column agglutination technology in a direct anti-human globulin test. The major contributing factors identified were method variability of the tube test, operator variability within the tube test, and subjectivity of reaction grading in the tube test (specifically at site 4, with a greater number of different tube method operators). Interactions between these three factors contributed to a low PPA at patient sites due to increased incidence of positive samples within the patient population compared to donors.

When a discrepant result was obtained further investigation was conducted with a referee method; column agglutination technology method for IgG and tube method for C3. A summation of these discrepant results is tabulated below the individual test site results for the automated systems and manual gel test across the four test sites.

Additional testing of patient samples was performed at a third external site (Test Site 5) and has been excluded from the overall performance analysis for all platforms. This site included samples from patients who were undergoing monoclonal antibody therapies. Identification of specific samples from patients undergoing these therapies was not made available. An investigation was performed on the impact of monoclonal antibody therapies on the gel DAT test. This investigation demonstrated that exposure of DAT negative samples to CD38-directed cytolytic antibodies can lead to unexpected positive reactions in the DAT test as the CD38 antigen is present on red blood cells (Refer to Limitation of Procedures, item 15). It was not able to be concluded whether monoclonal antibody therapies cause equivalent interference in tube technology versus column agglutination technology. It was also not able to be concluded if the high number of positive samples were from patients undergoing this therapy or if the results were due to a combination of the factors related to tube test variability observed at the other test sites in addition to the CD38-directed cytolytic antibody therapy.

Performance Characteristics on ORTHO VISION® / VISION® Max Analyzer

Clinical equivalency testing was performed at four sites (two external and two internal sites) that routinely perform immunohematology testing. Random clinical specimens including donors and patients (N=2778) were tested on the ORTHO VISION® / VISION® Max Analyzer.

Random sample results were assessed on a microtube-to-tube test basis using a paired sample comparison between the comparator tube method and the gel card under evaluation. For reaction grades to be concordant between methods either both results had to be negative, or both had to be positive (any reaction grade 1+ through 4+). For the tube test method, a weak reaction was considered positive when comparing to the gel card microtube. The combined results from all sites are summarized in the following table. Percent agreement indicates concordance between the two assays and does not indicate which method gave the correct result. The results below do not reflect testing to resolve initial discrepant results between methods.

Anti-IgG and Anti-C3b,-C3d Microtube Concordance - Random Clinical Specimens - All Sites Combined*

Test	Total Sample Size	Total Percent Agreement	Lower Bound of 95% CI	Positive Sample Size**	Positive Percent Agreement	Lower Bound of 95% CI	Negative Sample Size	Negative Percent Agreement	Lower Bound of 95% CI
Anti-IgG	2778	98%	98%	28	64%	47%	2750	99%	98%
Anti-C3b, -C3d	2778	99%	99%	9	56%	25%	2769	99%	99%

* All samples demonstrated negative results in the Control microtube.

** The expected frequency of positive DAT results has been reported as 1 in 1,000 to 1 in 14,000 for blood donors and 1 to 15% of hospital patients with the wide variation most likely due to different DAT techniques. [5] In the clinical studies performed, the frequency of random positive DAT results among the clinical sample set was 1.3%. The observed variability of the comparator tube method, as discussed at the beginning of the Specific Performance Characteristics section, also had an impact in the calculation of the 95% CI summarized in the discordant investigation discussion below.

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Specific Performance Characteristics

The results of positive percent agreement and negative percent agreement for each individual test site is summarized in the following table. The results below do not reflect testing to resolve initial discrepant results between methods.

Anti-IgG and Anti-C3b,-C3d Microtube Concordance - Individual Test Site Results*

Test Site	Sample Type	Test	Total Sample Size	Overall Agree	Overall Percent Agreement	Positive Sample Size	Positive Agreement	Positive Percent Agreement	Negative Sample Size	Negative Agreement	Negative Percent Agreement
Site 1 - External	Donor	Anti-IgG	659	653	99%	1	1	100%	658	652	99%
Site 1 - External	Donor	Anti-C3b, -C3d	659	653	99%	0	N/A	N/A	659	653	99%
Site 2 - External	Donor	Anti-IgG	765	762	>99%	4	2	50%	761	760	>99%
Site 2 - External	Donor	Anti-C3b, -C3d	765	763	>99%	0	N/A	N/A	765	763	>99%
Site 3 - Internal	Patient	Anti-IgG	548	538	98%	15	12	80%	533	526	99%
Site 3 - Internal	Patient	Anti-C3b, -C3d	548	545	99%	5	5	100%	543	540	99%
Site 4 - Internal	Patient	Anti-IgG	806	779	97%	8	3	38%	798	776	97%
Site 4 - Internal	Patient	Anti-C3b, -C3d	806	795	99%	4	0	0%	802	795	99%

* All samples demonstrated negative results in the Control microtube.

N/A = Not applicable

There were 68 discrepant results between the MTS™ DAT Card and an FDA licensed Anti-IgG tube reagent and Anti-C3b, -C3d tube reagent method across the four sites. Discordant investigation for each microtube formulation consisted of sample testing with a second, different FDA licensed column agglutination technology method or tube method followed by repeat testing of the sample using the initial discrepant test method.
Of the 68 discrepant results, 46 were discrepant with Anti-IgG. Thirty-six of these were positive in the test gel card and negative with the comparator; ten discrepant results were negative with the test gel card and positive with the comparator. As shown in the table below, when the referee method was used, the majority of IgG results agreed with the test gel card. C3 results with the referee method were equally divided between the test and comparator when comparator was positive. Overall, the table shows that the referee test (IgG card method) results, when the comparator is positive, which are associated with low PPA, favor the MTS™ DAT Card testing.

Anti-IgG and Anti-C3b, -C3d Microtube Discrepant Sample Referee Test Results

Microtube	Total Discrepant Samples	Test Positive, Comparator Negative	Referee Positive	Referee Negative	Test Negative, Comparator Positive	Referee Positive	Referee Negative
Anti-IgG	46	36	32	4	10	0	10
Anti-C3b, C3d	22	18	5	13	4	2	2

In the repeat testing of the sample using the initial discrepant test method, six initial IgG and one initial C3 positive tube reagent results and nine initial IgG and four initial C3 negative tube reagent results changed to become concordant when repeated as part of discordant investigation. No MTS™ DAT Card results in the IgG microtube changed when repeated as part of discordant investigation. One MTS™ DAT Card result in the C3 microtube changed to become concordant when repeated as part of discordant investigation.

The following table summarizes the additional patient testing results from the third external testing site that have been excluded from the combined Anti-IgG and Anti-C3b, -C3d Microtube Concordance.

Anti-IgG and Anti-C3b, -C3d Microtube Concordance - Additional Patient Test Site*

Test Site	Sample Type	Test	Total Sample Size	Overall Agree	Overall Percent Agreement	Positive Sample Size	Positive Agreement	Positive Percent Agreement	Negative Sample Size	Negative Agreement	Negative Percent Agreement
Site 5 - External	Patient	Anti-IgG	668	628	94%	64	42	66%	604	586	97%
Site 5 - External	Patient	Anti-C3b, -C3d	668	652	98%	19	18	95%	649	634	98%

* All samples demonstrated negative results in the Control microtube.

Anti-IgG and Anti-C3b, -C3d Microtube Agreement - Contrived Positive Samples

Due to the small positive sample size, IgG and C3 contrived positive samples (N=203 unique samples) were used and met a 100% point estimate agreement for each ID-MTS test system. The characterization of the contrived samples covered a range of reactivity from 1+ to 3+.

Anti-IgG and Anti-C3b, -C3d Microtube Agreement - Contrived Positive Samples*

Test	N	Percent Agreement
Anti-IgG	103	100%
Anti-C3b,-C3d	100	100%

* All samples demonstrated negative results in the Control microtube.

Performance Characteristics on ORTHO® Workstation

Clinical equivalency testing was performed at four sites (two external and two internal sites) that routinely perform immunohematology testing. Random clinical specimens including donors and patients (N=2767) were tested on the ORTHO® Workstation.

Random sample results were assessed on a microtube-to-tube test basis using a paired sample comparison between the comparator tube method and the gel card under evaluation. For reaction grades to be concordant between methods either both results had to be negative, or both had to be positive (any reaction grade 1+ through 4+). For the tube test method, a weak reaction was considered positive when comparing to the gel card microtube. The combined results from all sites are summarized in the following table. Percent agreement indicates concordance between the two assays and does not indicate which method gave the correct result. The results below do not reflect testing to resolve initial discrepant results between methods.

Anti-IgG and Anti-C3b,-C3d Microtube Concordance - Random Clinical Specimens - All Sites Combined*

Test	Total Sample Size	Total Percent Agreement	Lower Bound of 95% CI	Positive Sample Size**	Positive Percent Agreement	Lower Bound of 95% CI	Negative Sample Size	Negative Percent Agreement	Lower Bound of 95% CI
Anti-IgG	2767	98%	98%	29	55%	38%	2738	99%	98%
Anti-C3b, -C3d	2767	99%	99%	10	60%	30%	2757	99%	99%

* All samples demonstrated negative results in the Control microtube.

** The expected frequency of positive DAT results has been reported as 1 in 1,000 to 1 in 14,000 for blood donors and 1 to 15% of hospital patients with the wide variation most likely due to different DAT techniques. [5] In the clinical studies performed, the frequency of random positive DAT results among the clinical sample set was 1.4%. The observed variability of the comparator tube method, as discussed at the beginning of the Specific Performance Characteristics section, also had an impact in the calculation of the 95% CI summarized in the discordant investigation discussion below.

The results of positive percent agreement and negative percent agreement for each individual test site is summarized in the following table. The results below do not reflect testing to resolve initial discrepant results between methods.

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Specific Performance Characteristics

Anti-IgG and Anti-C3b,-C3d Microtube Concordance - Individual Test Site Results*

Test Site	Sample Type	Test	Total Sample Size	Overall Agree	Overall Percent Agreement	Positive Sample Size	Positive Agreement	Positive Percent Agreement	Negative Sample Size	Negative Agreement	Negative Percent Agreement
Site 1 - External	Donor	Anti-IgG	727	724	>99%	1	1	100%	726	723	>99%
Site 1 - External	Donor	Anti-C3b, -C3d	727	725	>99%	0	N/A	N/A	727	725	>99%
Site 2 - External	Donor	Anti-IgG	669	666	>99%	3	0	0%	666	666	100%
Site 2 - External	Donor	Anti-C3b, -C3d	669	668	>99%	0	N/A	N/A	669	668	>99%
Site 3 - Internal	Patient	Anti-IgG	548	535	98%	17	12	71%	531	523	98%
Site 3 - Internal	Patient	Anti-C3b, -C3d	548	545	99%	6	6	100%	542	539	99%
Site 4 - Internal	Patient	Anti-IgG	823	798	97%	8	3	38%	815	795	98%
Site 4 - Internal	Patient	Anti-C3b, -C3d	823	810	98%	4	0	0%	819	810	99%

* All samples demonstrated negative results in the Control microtube.

N/A = Not applicable

There were 63 discrepant results between the MTS™ DAT Card and an FDA licensed Anti-IgG tube reagent and Anti-C3b, -C3d tube reagent methods across the four sites. Discordant investigation for each microtube formulation consisted of sample testing with a second, different FDA licensed column agglutination technology method or tube method followed by repeat testing of the sample using the initial discrepant test method.

Of the 63 discrepant results, 44 were discrepant with Anti-IgG. Thirty-one of these were positive in the test gel card and negative with the comparator; thirteen discrepant results were negative with the test gel card and positive with the comparator. As shown in the table below, when the referee method was used, the majority of IgG results agreed with the test gel card. C3 results with the referee method were equally divided between the test and comparator when comparator was positive. Overall, the table shows that the referee test (IgG card method) results, when the comparator is positive, which are associated with low PPA, favor the MTS™ DAT Card testing.

Anti-IgG and Anti-C3b, -C3d Microtube Discrepant Sample Referee Test Results

Microtube	Total Discrepant Samples	Test Positive, Comparator Negative	Referee Positive	Referee Negative	Test Negative, Comparator Positive	Referee Positive	Referee Negative
Anti-IgG	44	31	29	2	13	3	10
Anti-C3b, C3d	19	15	5	10	4	2	2

In the repeat testing of the sample using the initial discrepant test method, six initial IgG and one initial C3 positive tube reagent results and seven initial IgG and four initial C3 negative tube reagent results changed to become concordant when repeated as part of discordant investigation. Two MTS™ DAT Card results in the IgG microtube changed when repeated as part of discordant investigation. No MTS™ DAT Card results in the C3 microtube changed to become concordant when repeated as part of discordant investigation.

The following table summarizes the additional patient testing results from the third external testing site that have been excluded from the combined Anti-IgG and Anti-C3b, -C3d Microtube Concordance.

Anti-IgG and Anti-C3b, -C3d Microtube Concordance - Additional Patient Test Site*

Test Site	Sample Type	Test	Total Sample Size	Overall Agree	Overall Percent Agreement	Positive Sample Size	Positive Agreement	Positive Percent Agreement	Negative Sample Size	Negative Agreement	Negative Percent Agreement
Site 5 - External	Patient	Anti-IgG	708	663	94%	73	45	62%	635	618	97%
Site 5 - External	Patient	Anti-C3b, -C3d	708	695	98%	20	19	95%	688	676	98%

* All samples demonstrated negative results in the Control microtube.

Anti-IgG and Anti-C3b, -C3d Microtube Agreement - Contrived Positive Samples

Due to the small positive sample size, IgG and C3 contrived positive samples (N=203 unique samples) were used and met a 100% point estimate agreement for each ID-MTS test system. The characterization of the contrived samples covered a range of reactivity from 1+ to 3+.

Anti-IgG and Anti-C3b, -C3d Microtube Agreement - Contrived Positive Samples*

Test	N	Percent Agreement
Anti-IgG	103	100%
Anti-C3b,-C3d	100	100%

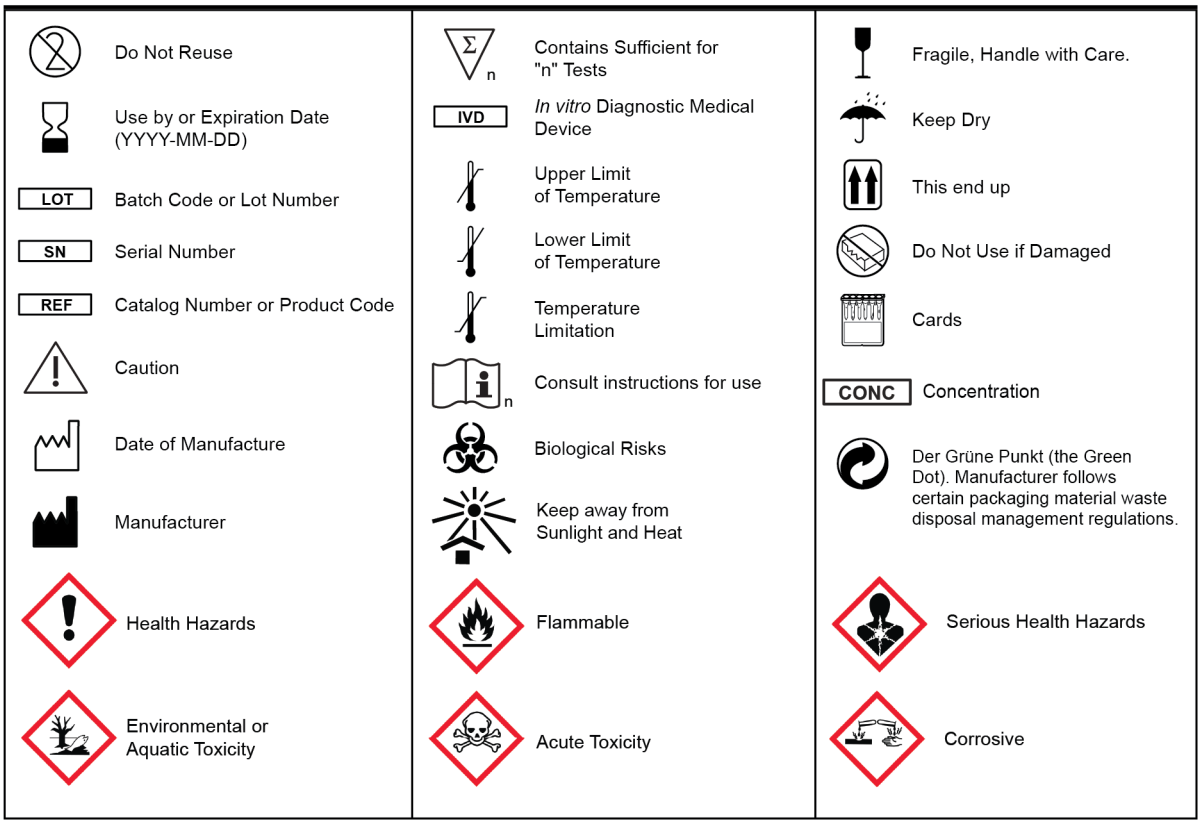
* All samples demonstrated negative results in the Control microtube.

References

1. Coombs RRA, Mourant AE, 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. ID-Micro Typing System™ Interpretation Guide (6902201), Ortho Clinical Diagnostics.
5. Cohn CS, Delaney M, Johnson S, et al, eds. Technical Manual. 21st Edition. Bethesda, MD: AABB, 2023.
6. ID-Micro Typing System™ Implementation Guide and Procedures (6902200), Ortho Clinical Diagnostics.
7. Nasongkla M, Hummert J, Chaplin Jr. H. Weak "false positive" direct antiglobulin test C3d. *Transfusion.* 1982;22:273-275.
8. Office of Biologics Research and Review, FDA. Recommended methods for Anti-Human Globulin Evaluation. Docket No. 84S-0182.

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*
2025-09-03	1.0	Initial release of Instructions for Use

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

Patents: www.quidelortho.com/us/en/patents

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