BLOOD GROUPING REAGENT

Anti-N (Murine Monoclonal) (IgG)

ORTHO™ Sera

MEETS FDA POTENCY REQUIREMENTS

Intended for Use in the United States

REF **6904555**

INSTRUCTIONS FOR USE

Intended Use

For *in vitro* diagnostic use only
For use with the ID-Micro Typing System™ Buffered Gel Card
For Direct Agglutination Test

The Anti-N reagent (Anti-MNS2) is for the qualitative *in vitro* detection of human N positive red blood cells by the direct agglutination test.

Summary and Explanation

The N status of red blood cells is defined by the amino acid sequence of the major red blood cell sialoglycoprotein, glycophorin A.[1-5] Anti-N (Anti-MNS2) reacts with antigens on glycophorin A, causing agglutination of the red blood cells and classifying these cells into three distinct phenotypes: M+N-, M+N+ and M-N+.[1-5] Additionally, irrespective of the MN status of their major glycoprotein, almost all human red blood cells carry the N antigen on a minor red blood cell sialoglycoprotein, glycophorin B.[1-5]

Principles of Procedure

When used by the recommended technique, the reagent will cause agglutination (clumping) of red blood cells carrying the N antigen. Lack of agglutination of the red blood cells demonstrates the absence of the N antigen.

Reagents

Anti-N (Murine Monoclonal) (IgG) is supplied as one reagent.

- 1 vial containing 3 mL of murine monoclonal antibodies of type IgG (cell line BO3) containing 0.1% (w/v) sodium azide and bovine material (i.e., bovine serum albumin, fetal bovine serum).

Any bovine material used in the manufacture of this product is sourced from USDA approved facilities.

No preparation of the reagent is required. Use directly from the vial. Do not dilute.

Storage Requirements

Store at 2–8 °C.
Do not freeze.
Do not use beyond expiration date. The format of the expiration date is expressed as YYYY-MM-DD (year-month-day).
May be at 20–25 °C while in use.
Replace cap when not in use.

Specimen Collection

- No special preparation of the patient/donor is required prior to specimen collection.
- Specimens should be collected by aseptic technique with an anticoagulant.
- The specimen should be tested as soon as possible after collection. If testing is delayed, the specimen should be stored at 2–8 °C.
- Do not use collection tubes that contain plasma/cell separation media.
- Samples collected in EDTA should be tested within seven days from collection.
- Donor blood collected in ACD, CPD, CP2D, CP2D with AS-3 and CPDA-1 may be tested until the expiration date of the donation.
- Clotted, hemolyzed, grossly icteric or contaminated blood specimens should not be used.
- 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.
- Specimens should not be exposed to extreme heat.

Precautions

Do not use if turbid.
Do not dilute.
Do not freeze.
Do not use beyond the expiration date.
This reagent contains 0.1% (w/v) sodium azide.
Handle and dispose of reagents as potentially infectious, in accordance with local, state, and national laws.
This reagent is for *in vitro* diagnostic use only.

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.**
CAUTION:	**Appropriate care should be taken in the use and disposal of this product. Source materials may include human components and antibody producing cells that are used in the manufacture of polyclonal and monoclonal products.**
CAUTION:	**Contains material of murine origin; therefore, handle appropriately as the absence of murine viruses has not been determined.**

Procedure

Materials Provided

ORTHO™ Sera Anti-N

Materials Required but not Provided

- Isotonic saline
- Reagent red blood cells suitable for the control of Anti-N
- MTS™ Buffered Gel Card

NOTE:	Store cards upright at 2–25 °C.
CAUTION:	**Inspect the condition of the card before use.** **Do not use gel cards that have not been shipped in an upright position.** **Do not use cards beyond expiration date.** **Do not freeze or expose cards to excessive heat.** **Use reagents as furnished.**

- Micropipetters for delivery of 25 µL and 50 µL
- Pipet tips
- Marking pen
- MTS™ Centrifuge
  or ORTHO™ Workstation
  or ORTHO VISION® Analyzer

Test Procedure

NOTE:

The reagent has been standardized for use by the technique described below.

The direct agglutination test procedure listed below is for manual testing only. When using instruments (see Materials Required but not Provided), follow the procedures that are contained in the operator's manual provided by the device manufacturer.

Direct Agglutination Test

1. Prepare an approximate 0.8% red blood cell suspension from patient or donor cells, using isotonic saline.
2. Allow the card and reagent to come to 20–25 °C before use. A clear liquid layer should appear on top of the opaque gel in each microtube.
3. Visually inspect gel cards before use.

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 the ID-Micro Typing System™ Interpretation Guide[6] for additional information related to the visual inspection of gel cards before use.

4. Label the card appropriately with a sample identifier.
5. Remove the foil seal from the MTS™ Buffered Gel Card or from the individual microtubes to be used for testing.

CAUTION:	**Do not remove card 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). After removing the foil, visually inspect all gel cards to ensure that residual film does not block the opening of any microtube.**

6. Add 25 µL of the reagent to the appropriate reaction chamber(s) of the opened card.

CAUTION:	**Do not touch the pipet to the side of the reaction chamber. If this occurs, change the pipet tip before proceeding to the next chamber.**

7. Add 50 µL of 0.8% red blood cell suspension to the appropriate reaction chamber(s) of the card.

CAUTION:	**Do not touch the pipet to the side of the reaction chamber. If this occurs, change the pipet tip before proceeding to the next chamber.**

8. Observe that the contents of the reaction chamber(s) are combined. If necessary tap gently.

NOTE:	Assure that the reagents remain in the reaction chamber. There should be no mixing of reactants with reagents in the column prior to centrifugation.

9. Centrifuge the card at the preset conditions, as installed by the instrument manufacturer.
10. Read the front and back of the individual columns for macroscopic agglutination or hemolysis upon test completion.
11. Record the reaction strength.

Interpretation of Results

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

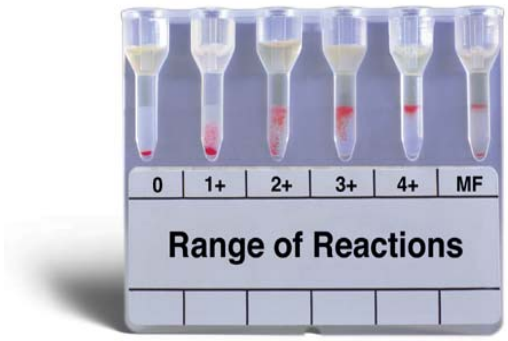
Positive Result = Agglutination of the red blood cells is a positive test result. Red blood cells may remain suspended on the top of the gel or are dispersed throughout the gel in varying degrees. A few red blood cells may form a button in the bottom of the microtube in some positive reactions.

Reaction Grading Guide (Use in conjunction with Diagram 1)	
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 5.)**

Diagram 1: Examples of Reaction Grades



NOTE: Refer to ID-Micro Typing System™ Interpretation Guide[6] for additional information.

Stability of Reaction

For best results, it is recommended that reactions should be read immediately following centrifugation.

Quality Control

Quality Control (QC) of reagents is required. Quality Control should be performed on each lot of reagent on each day of use according to standard operating procedures.

Reagent red blood cells may be used direct from the vial as control cells in ORTHO Sera tests, including 0.8% Resolve® Panel A, 0.8% Resolve® Panel B, 0.8% Resolve® Panel C (Untreated Only), 0.8% Selectogen® and 0.8% Surgiscreen®.

Limitations of the Procedure

1. Strict adherence to the procedures and recommended equipment is essential.
2. Proper centrifuge calibration is particularly important to the performance of the MTS™ Buffered Gel Card. The MTS™ Centrifuge and ORTHO™ Workstation have been exclusively designed to provide the correct time, speed, and angle.
3. The expression of certain red blood cell antigens may diminish in strength during storage, particularly in EDTA samples. Better results will be obtained with fresh samples.
4. Suppressed or weak expression of blood group antigens may give rise to false-negative reactions.
5. Anomalous results may be caused by the following:
   - Fibrin or particulate matter
   - Red blood cells sticking to the sides of the reaction chamber
   - Do not use cards that appear damaged (i.e., break in foil seal or break, crack or bubble in the column), exhibit drying (i.e., liquid level is at or below the top of the gel matrix) or exhibit discoloration (due to bacterial contamination, which can cause false reactions).
   - Loss of fluid in the card column may cause (weak) false positive results.
   - J reactions may occasionally be observed with high red blood cell concentrations. J reactions may also be observed if during centrifugation the card is not seated properly in the holder or not allowed to spin at a 90° angle.

     NOTE: A J reaction consists of cells forming a button at the bottom of the gel matrix or microtube when either end of the cell button goes up the side of the column. The cell button may be disrupted. A J reaction may represent a weakly positive reaction.
   - 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.
6. Tests with these or other anomalous results should be repeated.
7. Erroneous results could occur if final reactions are not read upon completion of centrifugation.
8. Mixed cell populations may be encountered as a result of, for example, transfusion, fetal maternal hemorrhage, or transplantation. Consult patient history when results of this nature are encountered before assigning an antigen type.
9. Donor/Patient red blood cells must be diluted with isotonic saline, before adding the red blood cells to the microtube.

Performance Characteristics

Expected Results

In performance evaluation studies (data on file at Alba Bioscience Limited), blood samples were tested with ORTHO™ Sera Anti-N (Murine Monoclonal) (IgG) by ID-Micro Typing System™ Column Agglutination Technology (CAT) as follows:

Including all samples:

Test	Positive			Negative		
	N	% Agreement*	One-Sided Exact 95% LCL (%)	N	% Agreement*	One-Sided Exact 95% LCL (%)
Anti-N	804	98.4	97.4	342	100	99.1

Excluding DAT positive samples:

Test	Positive			Negative		
	N	% Agreement*	One-Sided Exact 95% LCL (%)	N	% Agreement*	One-Sided Exact 95% LCL (%)
Anti-N	799	99.0	98.2	342	100	99.1

LCL: lower confidence limit

* % Agreement between ORTHO™ Sera Anti-N (Murine Monoclonal) (IgG) and comparator reagent only and does not indicate which reagents gave the correct results.

In performance evaluation studies, 1146 samples were tested with ORTHO™ Sera Anti-N (Murine Monoclonal) (IgG) using the MTS™ Centrifuge.The one-sided exact 95% LCL of positive percent agreement (PPA) was 97.4% for agglutination tests based on a comparison of interpreted results. The PPA did not meet the acceptance criteria due to 13 discrepant results (see sample classification and comments in the summary table below). The discordance between the trial and the comparator reagent could be attributed in five cases to the sample itself having a positive DAT result. Eight discrepant samples which confirmed the initial result on repeat testing have no rational explanation that can be attributed to the discrepant results. In 11 out of 13 discrepancies the resolver reagent obtained the same result as the trial reagent. The one-sided exact 95% LCL of negative percent agreement was ≥99% for agglutination tests based on a comparison of interpreted results.

Classification	Number of Discrepancies	Comment
DAT Positive	5	DAT positive following investigation.
Unresolved	8	ORTHO™ Sera Anti-N reagent and comparator reagent continued to show different result following repeat testing.

Results were evaluated against comparable FDA approved products using the appropriate methods for the comparators.

Migration studies have been performed using the ORTHO™ Workstation and results were as follows:

Reagent	Number of samples tested	Concordance*	Positive Samples	
			N	Frequency (%)
Anti-N	100	100%	64	64

*Concordance indicates agreement between the ORTHO™ Workstation and the MTS™ Centrifuge only and does not indicate which systems gave the correct results.

Further migration studies have been performed using the ORTHO VISION® Analyzer and results were as follows:

Test	Positive			Negative		
	N	% Agreement*	One-Sided Exact 95% LCL (%)	N	% Agreement*	One-Sided Exact 95% LCL (%)
Anti-N	606	100.0	99.5	623	99.8	99.2

LCL: lower confidence limit

*Concordance indicates agreement between the ORTHO™ Workstation and the ORTHO VISION® Analyzer only and does not indicate which system gave the correct results.

In these migration studies, 1229 samples were tested with ORTHO™ Sera Anti-N (Murine Monoclonal) (IgG) using the ORTHO™ Workstation and the ORTHO VISION® Analyzer. The one-sided exact 95% LCL of positive percent agreement was 99.5% for agglutination tests based on a comparison of interpreted results. The one-sided exact 95% LCL of negative percent agreement (NPA) was 99.2% for agglutination tests based on a comparison of interpreted results.

Precision Study Results

As part of the performance evaluation, precision and lot to lot studies were performed using multiple operators, days and runs to confirm repeatability and reproducibility of test results in the same run, day and with the same operator and between runs, days and operators. The study took account of variables such as days of the week, times of day and supplementary reagents used in the testing. There were no discordant results; all expected positive test outcomes generated unequivocal positive reactions and all expected negative test outcomes generated unequivocal negative reactions.

Specific Performance Characteristics

Prior to release, each lot of ORTHO™ Sera Anti-N (Murine Monoclonal) (IgG) is tested in alignment with FDA recommendations against a panel of antigen-positive and antigen-negative red blood cells to ensure suitable reactivity.

ORTHO™ Sera Anti-N (Murine Monoclonal) (IgG) Blood Grouping Reagent has been tested using the ID-Micro Typing System™ and when stored and used according to the recommended instructions for use, found to specifically agglutinate human red blood cells with the corresponding antigen.

The ORTHO™ Sera Anti-N (Murine Monoclonal) (IgG) reagent reacts with cells expressing the N antigen and meets FDA potency requirements.

For additional information or technical support, contact Ortho Care™ Technical Solutions Center at 1-800-421-3311.

Bibliography

1. Daniels, G. (2012). Human Blood Groups, 3rd ed. Oxford; Malden MA: Blackwell Science.
2. Reid, M.E., Lomas-Francis, C. and Olsson, M.L. (2012). The Blood Group Antigen Facts Book, 3rd ed. London, Academic Press.
3. Klein, H.G., Anstee, D.J. (2005) Mollison's Blood Transfusion in Clinical Medicine, 11th ed. Oxford; Malden MA: Blackwell Publishing.
4. Roback, J.D., Combs, M.R., Grossman, B.J., Hillyer, C.D., eds. (2011) Technical Manual, 17th ed. Bethesda, MD: AABB.
5. Daniels, G. and Bromilow, I. (2012). Essential Guide to Blood Groups. 2nd Edition, Blackwell Publishing Ltd.
6. ID-Micro Typing System™ Interpretation Guide (6902201), Ortho Clinical Diagnostics.

Glossary of Symbols

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

Do Not Reuse	Contains Sufficient for "n" Tests	Fragile, Handle with Care.
Use by or Expiration Date (Year-Month-Day)	*In vitro* Diagnostic Medical Device	Keep Dry
LOT Batch Code or Lot Number	Upper Limit of Temperature	This end up
SN Serial Number	Lower Limit of Temperature	Do Not Use if Damaged
REF Catalog Number or Product Code	Temperature Limitation	Cassettes/Cards
Date of Manufacture	Consult instructions for use	CONC Concentration
Manufacturer	Biological Risks	Der Grüne Punkt (the Green Dot). Manufacturer follows certain packaging material waste disposal management regulations.
EC REP Authorized Representative in the European Community		

Summary of Revisions

Date of Revision	Version	Section	Description of Technical Changes*
2019-01-30	3.0	Materials Required but not Provided	Addition of ORTHO VISION® Analyzer.
		Test Procedure	Updated statement regarding using instruments.
		Quality Control	Addition of statement regarding use of 0.8% red cells as a control when used directly from the vial.
		Limitations of the Procedure	Changed limitation #9 to: Donor/Patient red blood cells must be diluted with isotonic saline, before adding the red blood cells to the microtube.
		Performance Characteristics Comparator Study Results	Addition of migration study data for ORTHO VISION® Analyzer.
		Performance Characteristics Expected Results	Product name aligned throughout (changed from Anti-N Murine Monoclonal to Anti-N (Murine Monoclonal) (IgG).
		Specific Performance Characteristics	Product name aligned throughout (changed from Anti-N Murine Monoclonal to Anti-N (Murine Monoclonal) (IgG).
2018-08-01	2.0	Front page	Added Intended for Use in the United States to the header and US to the footer of the document.
		Specific Performance Characteristics	Changed OCD Customer Technical Support to Ortho Care™ Technical Solutions Center.
		Bibliography	Removed 'J' from Interpretation Guide publication number.
		Glossary of Symbols	Serious Health Hazards and Caution symbols removed.
		Back page	Manufacturer's address updated. Changed from © Ortho-Clinical Diagnostics, Inc. to © Ortho Clinical Diagnostics.
2015-07-20	1.0		Initial version of Instructions for Use.

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