TRANSFUSION MEDICINE

INSTRUCTIONS FOR USE

BLOOD GROUPING REAGENT
ORTHO™ Sera Anti-D (IAT) (Anti-RH1)

REF 6904493

Intended Use

For *in vitro* diagnostic use only
For Indirect Antiglobulin Test (IAT) by Column Agglutination Technology

The Anti-D (IAT) reagent is for the qualitative *in vitro* detection of human RhD positive blood group status by the indirect antiglobulin test.

Summary and Explanation

First described in 1939, the RhD antigen is surpassed in importance only by the antigens of the ABO blood group system. Transfusion of RhD positive blood to a RhD negative recipient or failure to administer prophylactic anti-D to a RhD negative woman can result in the production of anti-D. Consequently, establishing the correct RhD group is fundamental to safe transfusion practice and perinatal practice. Certain individuals exhibit a quantitative reduction in the expression of their RhD antigen and are categorized as weak D. Others display a qualitative variation in RhD antigen expression and are referred to as partial RhD. Weak D individuals may also be partial RhD.[1-5]

Principles of Procedure

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

Reagents

Anti-D is supplied as one reagent.

- 1 vial containing 5 mL of human monoclonal antibodies of type IgM/IgG (cell lines LDM3/ESD1) containing 0.1% (w/v) sodium azide and bovine material.

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.
Replace cap and return to storage 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.
- Clotted, hemolyzed, grossly icteric or contaminated blood specimens should not be used.
- 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 stored in citrate anticoagulant may be tested until the expiry date of the donation.
- Specimens should not be exposed to extreme heat.
- Red blood cells that are direct antiglobulin positive should not be used in the indirect antiglobulin procedure.

Precautions

Do not use if turbid.
Do not dilute.
Use product as supplied.
Do not freeze.
Do not use beyond the expiry date.
This reagent contains 0.1% (w/v) sodium azide.
Handle and dispose of reagents as potentially infectious.
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.** **Harmful to aquatic life with long lasting effects. Avoid release to the environment. Dispose of contents/container in accordance with local/regional/national/international regulations.**

CAUTION:	**Source material from which this product is derived was found non-reactive for HBsAg, Anti-HIV 1/2 and Anti-HCV. No known test methods can offer complete assurance that products derived from human blood will not transmit infectious disease. 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.**

Procedure

General Information

This reagent has been standardized for use by the technique described below. When using methods other than those described in this IFU, the procedures provided by the manufacturer of those methods must be followed. Approved validation procedures must be performed. We advise that regulatory agencies be consulted to determine specific validation requirements. When using automated instruments, follow the procedures that are contained in the operator's manual provided by the device manufacturer.

Materials Provided

ORTHO™ Sera Anti-D (IAT)

Additional Materials Required but not Provided

- Isotonic saline
- Reagent red blood cells suitable for the control of Anti-D (IAT)
- Anti-Human Globulin Anti IgG Ortho BioVue® System Cassettes
- Micropipetters for delivery of 10 µL, 40 µL and 50 µL
- Ortho BioVue® System Heatblock and Ortho BioVue® System Centrifuge OR ORTHO™ Workstation
- ORTHO Optix™ Reader

Test Procedure

Indirect Antiglobulin Test

1. Prepare a 0.8% or 3-5% red cell suspension from patient or donor cells, using isotonic saline.
2. Allow the cassette and reagent to come to 20-25 °C before use.
3. Label the cassette appropriately with a sample identifier.
4. Add 40 µL of the reagent antisera to the appropriate reaction chamber(s) of the opened cassette.

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

5. Add 50 µL of 0.8% red cell suspension or add 10 µL of 3-5% red cell suspension to the appropriate reaction chamber(s) of the cassette.

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

6. 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.

7. Incubate at 37 °C for 15 minutes.
8. Immediately centrifuge the cassette using the Ortho BioVue® System Centrifuge or ORTHO™ Workstation.
9. Read the front and back of the individual columns for agglutination upon test completion.
10. 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 bead column.

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

Reaction Grading Guide	
4+ Reaction	Agglutinated cells form a band at the top of the bead column.
3+ Reaction	Most agglutinated cells remain in the upper half of the bead column.
2+ Reaction	Agglutinated cells observed throughout the length of the bead column. A small button of cells may also be visible at the bottom of the bead column.
1+ Reaction	Most agglutinated cells remain in the lower half of the bead column. A button of cells will also be visible at the bottom of the bead column.
0.5+ Reaction	Half cell button at the bottom of the bead column is disrupted (not smooth). Small agglutinates are visible above the button.
0 Negative	All cells pass through the beads and form a button at the bottom of the bead column.

CAUTION: **Clots, particulates or other artifacts may cause some red blood cells to be trapped at the top of the bead column that may cause an anomalous result in a negative test (refer to Limitations of the Procedure, item 5).**

NOTE: Refer to ORTHO™ BioVue® System Cassettes Visual Reference Guide (J39791), Ortho Clinical Diagnostics.[6]

Stability of Results

Test results should be read, interpreted and recorded upon completion of 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.

Limitations of the Procedure

1. Strict adherence to the procedures and use of recommended equipment is essential.
2. 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.
3. Suppressed or weak expression of blood group antigens may give rise to false-negative reactions.
4. Anomalous results may be caused by the following:
    - Fibrin or particulate matter
    - Red blood cells sticking to the sides of the reaction chamber
    - DAT positive red cells
    - Do not use cassettes 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 glass beads) or exhibit discoloration (due to bacterial contamination, which can cause false reactions).
    - Loss of fluid in the cassette column may cause (weak) false positive results.
    - J reactions may occasionally be observed with high red cell concentrations. J reactions may also be observed if during centrifugation the cassettes are 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 bead column 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.
5. 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.

Performance Characteristics

The blend of antibodies in this blood grouping reagent will directly agglutinate D positive red cells and may directly agglutinate red blood cells from most weak D and partial RhD including DVI.

In addition the reagent will detect most partial D, and most weak D by IAT and, therefore, is suitable for RhD grouping of donor samples.

DHAR red blood cells have not been tested using this reagent, and therefore its reactivity with this cell type has not been determined

Expected Results

In performance evaluation studies (data on file at Alba Bioscience Limited); blood samples were tested with ORTHO™ Sera Anti-D (IAT) by Ortho BioVue® System Column Agglutination Technology (CAT) as follows:

Reagent	Number Tested*	CAT Red Cell Suspension	Concordance**	Positive Samples	
				N	Frequency (%)
Anti-D (IAT)	1217	0.8%	100%	990	81
	100	3-5%			

* Total number of distinct samples tested with Anti-D (IAT) is 1217, as 100 samples were tested using both 0.8% and 3-5% red cell suspensions.

** Concordance indicates agreement between the ORTHO™ Sera Anti-D (IAT) Human Monoclonal IgM/IgG and comparator reagents only and does not indicate which reagents gave the correct results.

Results were evaluated against comparable CE marked products using the appropriate Low Ionic Strength (LIS) and/or Normal Ionic Strength (NIS) methods for the comparators.

The testing included the following minimum numbers of samples: donor and patient samples, a minimum of 10% each; neonatal samples, minimum of 2%; samples of ABO blood group A or B, minimum of 40%. The total proportion of group O samples tested was 44%. The test outcomes were representative of typical antigen frequency, based on the UK population as the testing was performed at sites in the UK.[5]

Specific Performance Characteristics

Prior to release, each lot of ORTHO™ Sera Anti-D (IAT) blood grouping reagent has been tested manually using the Ortho BioVue® System and when used according to the recommended instructions for use, found to specifically agglutinate human red cells with the corresponding antigen.

The ORTHO™ Sera Anti-D (IAT) reagent reacts with cells expressing the RhD antigen.

For additional information or technical support, contact Ortho Care™ Technical Solutions Center.

Bibliography

1. McCann-Carter M.C., Bruce M., Shaw E.M., Thorpe S.J., Sweeney G.M., Armstrong S.S., James, K. (1993). The production and evaluation of two human monoclonal anti-D antibodies. *Transfusion Medicine* **3**:187-194.
2. Nantes. (1996). Third International Workshop and Symposium on Monoclonal Antibodies and Related Antigens. Transfusion Clinique et Biologique. 3(6).
3. Stratton F. (1946). A new RhD allelomorph, Nature (Lond) 158:25.
4. Tipett P., Sanger R. (1962/1977). Observations on subdivisions of the Rh antigen D. Vox Sang 7:9 and ARZTL Lab 23:476-480.
5. Essential Guide to Blood Groups (2013), 3rd Edition, Daniels, G and Bromilow, I, Blackwell Publishing Ltd.
6. ORTHO™ BioVue® System Cassettes Visual Reference Guide (J39791), 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	IVD	Keep Dry	
Batch Code or Lot Number	LOT	Upper Limit of Temperature		This end up	
Serial Number	SN	Lower Limit of Temperature		Do Not Use if Damaged	
Catalog Number or Product Code	REF	Temperature Limitation		Cassettes/Cards	
Date of Manufacture		Consult instructions for use		Concentration	CONC
Manufacturer		Biological Risks		Der Grüne Punkt (the Green Dot). Manufacturer follows certain packaging material waste disposal management regulations.	
Authorized Representative in the European Community	EC REP				

Summary of Revisions

Date of Revision	Version	Section	Description of Technical Changes*
2023-04-17	9.0	Back page	EC-REP address updated
2022-03-30	8.0	Back page	Change of EC-REP Removed obsolete Alba branding Minor formatting
2020-05-05	7.0	Additional Materials Required but Not Provided	Added ORTHO Optix™ Reader
2019-09-19	6.0	Back Page	Added EC Representative address
2019-05-08	5.0	Back Page	Notified Body number changed from 0843 (UL) to 1434 (PCBC)
2018-06-28	4.0	Precautions	Sodium azide caution statement expanded.
		Interpretation of Results and Bibliography	Changed Interpretation Guide to Visual Reference Guide J39791.
		Glossary of Symbols	Caution symbol removed.
		Last page	Manufacturer address revised. Changed from © Ortho-Clinical Diagnostics, Inc. to © Ortho Clinical Diagnostics.
2016-09-27	3.0	Performance Characteristics	Clarification on performance claims. Changed OCD Customer Technical Support to Ortho Care™ Technical Solutions Center.
2015-07-30	2.0	N/A	General formatting updates for consistency.
		Intended Use	Clarified that the reagent is for the detection of RhD positive blood group status.
		Summary and Explanation	Clarified that establishing the correct RhD group is also fundamental to perinatal practice. Updated bibliographic references.
		Reagents	Added instruction to not dilute the reagents.
		Storage Requirements	Revised storage requirements.
		Specimen Collection	Added two guidelines.
		Precautions	Added two precautions. Removed R&S statement per effective SDS publication.

Date of Revision	Version	Section	Description of Technical Changes*
		Procedure: Additional Materials Required but not Provided	Added ORTHO™ Workstation.
		Procedure: Test Procedure	Step 2: Added temperature range. Step 5: Added Caution. Step 8: Added ORTHO™ Workstation.
		Interpretation of Results	Expanded descriptions of negative and positive results. Added chart of interpretations. Added Caution and Note.
		Limitations of the Procedure	Step 1: New step. Step 5: Moved from Step 4 into separate step.
		Performance Characteristics: Expected Results	Details added for the number of samples tested. Added specific reagents to the description of concordance.
		Bibliography	Updated references.
		Glossary of Symbols	Removed symbols which no longer apply to this product's labeling.
		Back Page	Updated OCD logo.
2013-03-05	1.0	N/A	Initial version 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.

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