Blood Grouping Reagents
Anti-A (Monoclonal)
Anti-B (Monoclonal)
Anti-A,B (Monoclonal)
Anti-D (Anti-RH1) (Monoclonal)
Control
Anti-Human Globulin
Anti-IgG (Rabbit) (Green)
Ortho BioVue® System
(Newborn Cassette)

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REF

100 cassettes 6901906

INTENDED USE

Qualitative test for recognition of the A, B and D (RH1) antigens on human red blood cells and for the detection of cell-bound IgG antibody in newborns

FOR IN VITRO DIAGNOSTIC USE

SUMMARY AND EXPLANATION

Blood Grouping

Testing with Anti-A, Anti-B and Anti-D is necessary to determine if red blood cells possess or lack A, B and/or D blood group antigens. Since neonates have poorly developed A and B antigens and lack naturally occurring ABO antibodies, the Anti-A,B reagent aids in the confirmation of the ABO group.

Direct Antiglobulin Testing

The direct antiglobulin test shows whether or not red blood cells have become sensitized in vivo and is useful in the diagnosis of hemolytic disease of the newborn (HDN).

PRINCIPLE OF PROCEDURE

The procedure used with these reagents is based on the principle of agglutination. Human red cells possessing antigens or sensitized with immunoglobulin will agglutinate in the presence of antibody directed toward the antigens. The Ortho BioVue System utilizes column agglutination technology, comprised of glass beads and reagent contained in a column. Upon addition of red blood cells and subsequent centrifugation of the cassette, agglutinated red blood cells are trapped by the glass beads and nonagglutinated red blood cells travel to the bottom of the column.

In the direct antiglobulin test, the density of the diluent allows the red blood cells to pass through the column but the less dense neutralizing serum proteins remain above the glass bead/diluent interface. Therefore, washing of the red cells is not necessary and they pass through the glass beads without neutralizing the Anti-IgG contained in the column.

REAGENTS

Ortho BioVue System Newborn cassettes are comprised of 6 columns containing a buffered solution with bovine albumin and macromolecular potentiators, as well as the preservatives 0.1% (w/v) sodium azide and 0.01M ethylenediaminetetraacetic acid (EDTA).

Product Code 6901906	**Component Description**
Column 1: Blood Grouping Reagent Anti-A	Anti-A murine (IgM) monoclonal antibody blend (clones MHO4 and 3D3) FD&C Blue No. 1
Column 2: Blood Grouping Reagent Anti-B	Anti-B murine (IgM) monoclonal antibody blend (clones NB10.5A5 and NB1.19) FD&C Yellow No. 5
Column 3: Blood Grouping Reagent Anti-A,B	Anti-A,B murine (IgM) monoclonal antibody blend (clones MHO4 and 3D3) (Anti-A) (clones NB10.5A5 and NB1.19) (Anti-B) Potentiator optimized for detection of subgroups of A and B
Column 4: Blood Grouping Reagent Anti-D (Anti-RH1)	Anti-D human (IgM) monoclonal antibody (clone D7B8)
Column 5: Control	Potentiator optimized for use as a control for blood group tests
Column 6: Anti-Human Globulin Anti-IgG (Rabbit) (Green)	Rabbit Anti-IgG FD&C Blue No. 1 and FD&C Yellow No. 5

STORAGE REQUIREMENT

Store cassettes upright at 2 to 25°C.

Do not store the cassettes in a self-defrosting refrigerator/freezer.

Do not store cassettes near any heat source (e.g., heat block, radiator, large instrumentation, refrigerator, freezer, etc., or any area receiving direct sunlight).

PRECAUTIONS

1. Handle all blood and materials in contact with blood as if capable of transmitting infectious agents. It is recommended that blood and materials in contact with blood be handled using established good laboratory practices.[1]
2. All blood products should be treated as potentially infectious. Source material from which this product was derived was found negative for hepatitis B surface antigen (HBsAg) and antibodies to hepatitis C virus (HCV) and human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2). No known test methods can offer assurance that products derived from human blood will not transmit infectious agents.
3. Some cassette components may be considered as hazardous or potentially infectious waste. Dispose of all materials according to applicable guidelines and regulations.[2]
4. Improper storage conditions will adversely affect product performance. CAUTION: Attached to the cassette tray is a temperature monitor label. If the red showing through on the circle of the label meets or exceeds the color standard in the corner of the label, the cassettes have been exposed to temperatures which can affect the performance of the reagents contained in the cassettes. False-negative results could occur with the use of these cassettes. Do not use the cassettes contained in the tray.

5. Do not use reagents beyond their labeled expiration date.
6. Freezing of the cassettes or evaporation of the liquid due to heat may interfere with free passage of unagglutinated red blood cells through the glass bead column.
7. Do not use cassettes that appear damaged (i.e., break in foil seal or break, crack or bubble in the column) or 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).
8. Use the Ortho BioVue System Centrifuge to provide the required centrifugation parameters for this system. Proper calibration of the centrifuge is essential to achieve accurate test results.
9. Use of a centrifuge with a lid-locking device is recommended.
10. Improper use of the liner assembly or dropping the cassette after the insertion of the liner could result in cross-contamination of reagents during pipetting.
11. Erroneous results may be obtained due to improper technique in performing any diagnostic test. The most common sources of such results are:
    - Use of red blood cell concentrations other than those described under Specimen Collection and Preparation section
    - Microbial contamination of supplementary materials used in the procedure
    - Use of specimens containing particulate matter (impedes the free flow of red blood cells through the column)
    - Use of severely hemolyzed samples (may interfere with reading reactions in the column)
12. In order to minimize the presence of bubbles with your Ortho BioVue cassettes, we recommend that if you normally store your cassettes in the refrigerator at 2 to 8°C you should equilibrate your cassettes at room temperature (20 to 25°C) for at least 96 hours prior to use.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient/donor is required prior to specimen collection. Blood should be collected by approved medical techniques. Samples collected with EDTA or without anticoagulant may be used.

Samples should be tested as soon as possible following collection. If a delay in testing occurs, samples should be stored at 2 to 8°C. Blood drawn into EDTA is preferred for the direct antiglobulin test. Blood drawn into EDTA or with no anticoagulant (clotted specimens) should be tested within seven days.

Red blood cells collected from the umbilical cord should be free from contamination (i.e., Wharton's jelly, tissue). If contamination is suspected, washing with isotonic saline may be necessary.

Red blood cell suspensions can be prepared using the following combinations of saline and packed red blood cells:

Saline Volume	Packed Red Blood Cell Volume[a]	Red Blood Cell Concentration
1 mL	40 µL	3%
1 mL	50 µL	4%
1 mL	65 µL	5%

[a] Blood samples centrifuged at 900 to 1000 x g for 5 minutes will result in a packed red blood cell concentration of approximately 80%. These specifications for centrifugation eliminate over-packing of red cells which may result in false-positive results. Data on file at Ortho-Clinical Diagnostics, Inc.

REAGENT PREPARATION

The Ortho BioVue System cassette is provided ready to use. Each column contains a single specificity of reagent suitable for one test. The cassette is heat-sealed with aluminum foil to preserve the integrity of the reagents. Upon opening of the foil seal, the cassettes should be used within one hour. Do not use the cassette if the liquid level in the column is at or below the top of the glass beads.

PROCEDURE

The procedure identified below is for manual BioVue cassette 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 to demonstrate compatibility of this product on automated systems.

Materials Provided

100 cassettes (Product Code 6901906)
(See Reagents section for component description.)

Materials Required But Not Provided

1. Ortho BioVue System Centrifuge
2. Isotonic saline
3. Micropipetter for delivery of 10 µL
4. Disposable pipette tips
5. Ortho BioVue System Work Rack (Product Code 707830)
6. Liner Assembly, BioVue (Product Code 4056)

Test Procedure

1. Prepare red cell suspensions according to Specimen Collection and Preparation section.
2. Allow the cassette and test sample to come to room temperature before use. Orient the cassette with the back label (bar code side) facing you. Label the cassette appropriately with sample identification.
3. Open the wells of the cassette using the liner assembly. Turn the cassette upside down and press down onto the liner. Slide the assembly out of the liner holder.
   NOTE: The cassette should be used within one hour after insertion of the liner.
4. Add 10 µL of the 3 to 5% red blood cell suspension to the reaction chambers of the cassette.
5. Centrifuge the cassette using the Ortho BioVue System Centrifuge.
   NOTE: Centrifugation should occur within 30 minutes of addition of the samples to the reaction chamber.
6. Read the front and back of the individual columns for agglutination upon test completion.
7. Record the reaction strength from the side with the stronger positive result.

Quality Control Procedures

Serological testing is necessary to recognize reagent deterioration. It is recommended that each lot of reagents be tested on each day of use with appropriate positive and negative controls according to approved standard operating procedures.

Positive Control for the Blood Grouping Reagents – Use red blood cells known to possess the antigen toward which the reagent antibody is directed. If possible, a heterozygous expression of the antigen should be used. Results should demonstrate agglutination represented by red blood cells retained in or on top of the glass bead column.

Positive Control for the IgG Reagent – Use red blood cells sensitized with IgG to test for Anti-IgG.

Negative Control for the Blood Grouping Reagents – Use red blood cells known to lack the antigen toward which the reagent antibody is directed. Results should demonstrate no agglutination of the red blood cells represented by a button of packed cells at the bottom of the column.

Negative Control for the IgG Reagent – Use unsensitized red blood cells such as reagent red blood cells for antibody screening.

Control Column – Use normal (unsensitized) red blood cells. Results should demonstrate no agglutination of the red blood cells represented by a button of packed cells at the bottom of the column.

INTERPRETATION OF RESULTS

Positive Result (+):	Agglutination of the red blood cells is a positive test result and indicates the presence of the corresponding antigen. Agglutination of red blood cells in the presence of Anti-IgG is a positive test result which indicates the presence of human IgG on the red blood cells.
Negative Result (-):	No agglutination of the red blood cells is a negative test result and indicates the corresponding antigen is not demonstrable. Absence of agglutination in the IgG column indicates there is no detectable IgG on the red blood cells.
Control Column:	If **any** degree of positive reactivity is observed in the Control Column, a valid interpretation of the blood group **cannot** be determined. Further investigation by the user is required to determine the serological basis for the reactivity of the Control.
4+ Reaction	Agglutinated red blood cells form a band at the top of the bead column.
3+ Reaction	Most agglutinated red blood cells are retained or trapped in the upper half of the bead column.
2+ Reaction	Agglutinated red blood cells are 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 red blood cells are retained or trapped 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	Most agglutinated red blood cells pass through and form a disrupted (not smooth) button at the bottom of the bead column. Small agglutinates are visible above the button.
0 Negative	All red blood cells pass through and form a smooth button at the bottom of the bead column.

Mixed cell populations may be detected by the Ortho BioVue System as agglutinated red blood cells at the top of the bead column and unagglutinated red blood cells at the bottom of the column. Detection limits may vary from those observed by other techniques.

LIMITATIONS OF THE PROCEDURE

1. The Test Procedure and Interpretation of Results must be followed closely to assure the accuracy of the test results. A laboratory that institutes the Ortho BioVue System should have a program that will train personnel on the proper use and handling of the product.
2. Due to antigen deterioration, aged red blood cells may exhibit weaker reactivity than fresh cells.
3. Enzyme-treated red blood cells should not be used with these reagents.
4. Red blood cells that appear to be D negative by this test method must be further tested for weak or partial D antigen by an acceptable test method when dictated by local requirements.
5. Good laboratory practice recommends that all weak Rh(D) positive typing results of 2+ or less be confirmed by an alternative method.[12,13]
6. Invalid test results due to spontaneous agglutination may occur on rare occasions with the Anti-D and Anti-A,B reagents when testing red blood cells heavily coated with antibodies.

7. Abnormal serum proteins or other contaminants in the test sample may cause red blood cells to aggregate, which may be interpreted as agglutination.
8. Components of complement on the red blood cell will not be detected.

EXPECTED RESULTS*

Blood Grouping

In clinical studies, the ABO and D (RH1) groupings for samples tested demonstrated the following distribution in the Ortho BioVue System:

Blood Group	Number of Samples Tested	Positive Samples	Frequency (%)
A	4253	1503	35.34
B	4253	524	12.32
AB	4253	203	4.77
O	4253	2023	47.57
D (RH1)	4231	3682	87.02

Ethnic backgrounds were available for 3264 (76.2%) of the samples tested. Of these samples, 61.6% were collected from persons of Caucasian background, 10.9% of African American background, 2.3% of Hispanic heritage, 0.9% of Oriental heritage, and 0.5% of American Indian, Saudi Arabian, Arabian, Asian Indian or Filipino heritage. Changes to the distribution will vary depending on the ethnic population under test. Of the entire test population, 497 samples were from neonates.

The results obtained for ABO grouping by the BioVue method gave 99.95 - 99.98% agreement when compared to tube test. There was 99.93% agreement between tube test and BioVue methods for the detection of D (RH1) antigen. Percent agreement indicates concordance between the two assays and does not indicate which method gave the correct results.

Direct Antiglobulin Test (DAT)

In clinical studies using 3% to 5% red cell suspensions in saline with the IgG column, the results obtained for DAT by the BioVue method gave 99.6% (248/249) agreement when compared to the licensed tube test. There was 100% (237/237) agreement between tube and BioVue methods for direct antiglobulin testing of cord blood samples. Percent agreement indicates concordance between the two assays only and does not indicate which method gave the correct result.

*Data on file at Ortho-Clinical Diagnostics, Inc.

SPECIFIC PERFORMANCE CHARACTERISTICS*

Blood Grouping

Blood Grouping Reagents Anti-A (Monoclonal), Anti-B (Monoclonal), Anti-A,B (Monoclonal) and Anti-D (Monoclonal), contained in the Ortho BioVue System cassette, have been tested and found to specifically agglutinate human red cells if the corresponding antigen is present, when used according to the recommended directions for use.[3,4] Anti-IgG reagent specifically agglutinates red cells sensitized with IgG.[5]

The **Anti-A reagent** can detect most examples of the weak subgroups of the A antigen (such as A_2, A_3 and A_x) and may detect previously unrecognized A antigen in a small percentage of group B individuals referred to as B(A)[6]. This reagent does not react with Tn polyagglutinable cells.

The **Anti-B reagent** can detect some examples of the weak subgroups of the B antigen (such as B_3, B_x and B_m). This reagent does not react with acquired B antigen or Tn polyagglutinable cells.

The **Anti-A,B reagent** detects all group A and B cells detected by the Anti-A and Anti-B reagents and is capable of detecting additional A and B subgroups not detected by the Anti-A and Anti-B reagents. The potentiator concentration used in the Anti-A,B reagent may nonspecifically agglutinate heavily sensitized red cells not agglutinated by Anti-A and/or Anti-B reagent(s).

The **Anti-D reagent** can detect most examples of weak and partial D (including weak D types 1, 2, 3 and 4.0 and D categories II, III, IV, V, VII, DBT and R_o^{Har}). It did not react with 9 of 9 D category VI cells tested.[7] Positive Rh(D) reactions of 2+ or less may indicate a weak D phenotype or spontaneous agglutination. Retesting with an alternative method wil ensure the reactivity is due to the presence of the D-antigen and not due to spontaneous agglutination.[12,13] This reagent may exhibit different serological activity when compared to other Anti-D reagents.

Direct Antiglobulin Test[8-11]

The immunogen used to produce rabbit anti-human IgG is a gamma globulin fraction of human plasma. Testing for antibodies to immunoglobulin mu (μ) chains and light (κ and λ) chains is not performed but such antibodies may be present in this reagent.

Each lot of IgG cassettes is tested and shown to agglutinate red cells weakly sensitized with IgG. Unsensitized red cells and red cells sensitized in vitro with C3b and C4 are negative. This reagent may agglutinate IgM sensitized red cells.

*Data on file at Ortho-Clinical Diagnostics, Inc.

SUMMARY OF REVISIONS

Section	Revision
PRECAUTIONS	Deleted statement regarding MSDS sheets.
	Added statement regarding storage temperature.

BIBLIOGRAPHY / BIBLIOGRAPHIE / LITERATUR / BIBLIOGRAFIA / BIBLIOGRAFI / BIBLIOGRAFIE / LITTERATUR / ΒιβλιογραΦία / LITTERATURFÖRTECKNING

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KEY TO SYMBOLS / LÉGENDE DES SYMBOLES / SYMBOL-LEGENDE / CLAVE DE LOS SÍMBOLOS / LEGENDA DEI SIMBOLI / SIMBOLOGIA / FORKLARING TIL SYMBOLER / ΕΠΕΞΗΓΗΣΗ ΣΥΜΒΟΛΩΝ / FÖRKLARING TILL SYMBOLERNA

The following symbols may have been used in the labeling of this product. / Les symboles suivants ont pu être utilisés pour étiqueter ce produit. / Es ist möglich, dass die folgenden Symbole bei der Etikettierung dieses Produktes verwendet wurden. / Los siguientes símbolos pueden haber sido empleados en el etiquetado de este producto. / Nelle etichette di questo prodotto possono essere stati utilizzati i simboli seguenti. / Os seguintes símbolos podem ter sido utilizados no rótulo deste produto. / Følgende symboler ka være anvendt ved mærkningen af dette produkt. / Τα ακόλουθα σύμβολα ενδέχεται να έχουν χρησιμοποιηθεί στη σήμανση αυτού του. / Följande symboler kan ha använts vid märkningen av denna produkt.

Attention: See instructions for use /
Attention : Se référer aux instructions d'utilisation/
Wichtig: Siehe Gebrauchsanweisung /
Atención: Ver las instrucciones de uso /
Attenzione: Vedi le istruzioni per l'uso /
Atenção: Consulte as instruções de utilização /
Obs.: Se brugsanvisning /
Προσοχή: Βλέπετε οδηγίες χρήσης /
Obs! Se bruksanvisningen

LOT

Lot Number / Numéro de lot / Loscode /
Número de lote / Lotto numero / Número de lote /
Lot-nummer / Αριθμός παρτίδας / Lot-nummer

Use by/expiration date (CCYY-MM-DD) /
Utiliser avant/date d'expiration (AAAA-MM-JJ) /
Benutzen vor/Verfalldatum (JJJJ-MM-TT) /
Úsese antes de/Fecha de caducidad (SSAA-MM-DD) /
Data di scadenza (AAAA-MM-GG) /
Utilizar até/data de validade (SSAA-MM-DD) /
Anvendes senest/udløbsdato (ÅÅÅÅ-MM-DD) /
ημερομηνία λήξης (AAEE-MM-HH) /
Används före/Utgångsdatum (ÅÅÅÅ-MM-DD)

Store between / Conserver entre / Lagern zwischen /
Almacenar entre / Conservare ad una temperatura tra /
Armazenar entre / Opbevares mellem / Φύλαξη μεταξύ /
Förvaras mellan

IVD

For In Vitro Diagnostic Use / Pour Diagnostic in Vitro /
In-vitro-Diagnostikum / Para uso diagnóstico in vitro /
Per uso diagnostico in vitro / Para diagnósticos In Vitro /
Til diagnostisk brug in vitro / Για διαγνωστική χρήση
In Vitro / För diagnostisering in vitro

EC REP

Authorized Representative / Mandataire / Bevollmächtigeter /
Representante autorizado / Rappresentante autorizzato /
Representante Autorizado / Autoriseret repræsentant /
Εξουσιοδοτημένος Αντιπρόσωπος */ Auktoriserad representant*

REF

Product Code / Code produit / Artikelcode / Código del producto /
Codice prodotto / Código do Produto / Produktkode /
Κωδικός Προϊόντος / Produktkod

This end up / Haut / Diese Seite nach oben / Este extremo hacia arriba / Questa estremità in alto / Este Lado Para Cima /
Denne side op / Η Συσκευασία Πρέπει να Είναι Όρθια από Αυτήν την Πλευρά / Denna sida upp

Keep Dry / Conserver dans un endroit sec / Trocken aufbewahren /
Mantener seco / Conservare in luogo asciutto /
Mantenha em Ambiente Seco / Undgå fugt /
Διατηρήστε το Στεγνό / Förvaras torrt

Fragile, handle with care / Fragile, à manipuler avec précaution /
Zerbrechlich, mit Vorsicht behandeln / Frágil, manipular con precaución / Fragile, maneggiare con cura / Frágil, tratar com cuidado / Fragil, håndteres med forsigtighed /
Εύθραυστο, χειριστείτε με προσοχή / Ömtålig, hanteras varsamt

Cassette / Cassette / Kassette / Cassette / Cassetta / Cassete /
Kassette / Κασέτα / Kassett

EC REP

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