Revised September 2009
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Blood Grouping Reagents
Anti-C (Anti-RH2) (Monoclonal)
Anti-E (Anti-RH3) (Monoclonal)
Anti-$\bar{c}$ (Anti-RH4) (Monoclonal)
Anti-e (Anti-RH5) (Monoclonal)
Anti-K (Anti-K1) (Monoclonal)
Control
Ortho BioVue® System
(Rh/K Cassette)

REF

400 cassettes 707280
100 cassettes 707250

INTENDED USE

Qualitative test for recognition of the C (RH2), E (RH3), $\bar{c}$ (RH4), e (RH5) and K (K1) antigens on human red blood cells

FOR IN VITRO DIAGNOSTIC USE

SUMMARY AND EXPLANATION

The C, E, $\bar{c}$ and e antigens are part of the Rh-hr blood group system. The K (K1) antigen is part of the Kell blood group system. Antibodies to these antigens are capable of causing red blood cell destruction and may result in hemolytic disease of the newborn (HDN) and transfusion reactions. Red cells may be tested for C, E, $\bar{c}$, e and K for several purposes including antibody identification, selection of compatible blood for transfusion and management of HDN.

PRINCIPLE OF PROCEDURE

The procedure used with these reagents is based on the principle of agglutination. Normal human red cells, possessing antigens, will agglutinate in the presence of antibody directed toward the antigen. 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.

REAGENTS

Ortho BioVue System Rh/K 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 Codes 707280 and 707250	**Component Description**
Column 1: Blood Grouping Reagent Anti-C (Anti-RH2)	Anti-C (IgM) monoclonal antibody (clone MS24)
Column 2: Blood Grouping Reagent Anti-E (Anti-RH3)	Anti-E (IgM) monoclonal antibody (clone C2)
Column 3: Blood Grouping Reagent Anti-$\bar{c}$ (Anti-RH4)	Anti-$\bar{c}$ (IgM) monoclonal antibody (clone MS42)
Column 4: Blood Grouping Reagent Anti-e (Anti-RH5)	Anti-e (IgM) monoclonal antibody (clones MS16, MS21 and MS63)
Column 5: Blood Grouping Reagent Anti-K (Anti-K1)	Anti-K1 (IgM) monoclonal antibody (clone MS56)
Column 6: Control	Potentiator optimized for use as a control for blood group tests

STORAGE REQUIREMENT

Store cassettes upright at 2 to 25°C.

Do not store 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. Some cassette components may be considered as hazardous or potentially infectious waste. Dispose of all materials according to applicable guidelines and regulations.[2]
3. Do not use reagents beyond their labeled expiration date.
4. 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.

5. 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).
6. 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.
7. 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.
8. 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)
9. 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 anticoagulant 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. Clotted specimens or blood drawn into EDTA, heparin or sodium citrate should be tested within seven days. Donor blood may be tested up to the date of expiration.

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%
1 mL	10 µL	0.8%
0.8 mL	10 µL	1.0%

[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

400 cassettes (Product Code 707280)
100 cassettes (Product Code 707250)
(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, 40 µL and 50 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 a 3% to 5% OR
   • 40 µL of a 1.0% OR
   • 50 µL of a 0.8% 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 – 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.

Negative Control – 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.

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.
Negative Result (-):	No agglutination of the red blood cells is a negative test result and indicates the corresponding antigen is not demonstrable.
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. Invalid test results due to spontaneous agglutination may occur on rare occasions with these reagents when testing red blood cells heavily coated with antibodies.
5. Abnormal serum proteins in the test sample may cause red blood cells to aggregate, which may be interpreted as agglutination.
6. Plasma expanders have been shown to interfere with some blood bank tests. Data are not available concerning interference using the Ortho BioVue System. Problem-solving techniques should be used if interference is observed.
7. The Anti-E clone (C2) used in the Anti-E reagent does not detect the E^w antigen.

EXPECTED RESULTS*

In clinical studies, the C (RH2), E (RH3), $\bar{c}$ (RH4), e (RH5) and K (K1) groupings for samples tested demonstrated the following distribution in the Ortho BioVue System:

Blood Group	Number of Samples Tested	Positive Samples	Frequency (%)
C (RH2)	4224	2779	65.79
E (RH3)	4191	1351	32.24
$\bar{c}$ (RH4)	4251	3641	85.65
e (RH5)	4251	4138	97.34
K (K1)	4199	277	6.6

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.

The results obtained for detection of C and $\bar{c}$ antigens by the BioVue method gave 99.41% and 99.95% agreement, respectively, when compared to tube test. There was 98.63% agreement between tube test and BioVue methods for the detection of E antigen, 100.00% agreement for e antigen and 99.52% for detection of K (K1) antigen. Percent agreement indicates concordance between the two assays only and does not indicate which method gave the correct results.

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

SPECIFIC PERFORMANCE CHARACTERISTICS*

Blood Grouping Reagents Anti-C (Monoclonal), Anti-E (Monoclonal), Anti-$\bar{c}$ (Monoclonal), Anti-e (Monoclonal) and Anti-K (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]

The **Anti-C reagent** reacts with cells expressing the C (RH2) antigen. Cells expressing weak C, identified using a polyclonal anti-C reagent, were positive with this reagent. Cells tested included examples identified as C^w, C^x, Rz, Rh32, Rh35, r^G and r'^s. This reagent was negative with cells lacking the C antigen including $R_1{}^wr$ and DC^w- (Rh-deletion) red cells that were negative using a polyclonal anti-C reagent.

The **Anti-E reagent** reacts with cells expressing the E (RH3) antigen. It detected one example of E^T and two cells expressing weak E identified using a polyclonal anti-E reagent. This reagent does not react with cells expressing the E^w (RH11) antigen.

The **Anti-$\bar{c}$ reagent** reacts with cells expressing the $\bar{c}$ (RH4) antigen. It was positive with Rh26, LOCR and Dc- (Rh-deletion) red cells that were also positive with a polyclonal anti-$\bar{c}$.

The **Anti-e reagent** reacts with cells expressing the e (RH5) antigen. It reacted with multiple examples of cells expressing partial e antigen ($e+hr^{S-}$ and $e+hr^{B-}$) and cells with depressed expression of e (Rh32, Rh33 and Rh35).

The **Anti-K reagent** reacts with the K (K1) antigen. When tested with red cells expressing other Kell system antigens, it reacted with all K+ cells including K+k+, K+k- and K+K17 as well as one example of K+ Rh null. It did not react with the following K negative red cells: K-k+, Js(a+b-), Kp(a+b-), K11, K12, K14, K19, K22, K_0 and McLeod.

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

SUMMARY OF REVISIONS

Section	Revision
REAGENTS	(French version only) Changed "l'acide de sodium" to "l'azide de sodium."

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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