Reverse-Cyte® A_1, B
Reverse-Cyte® A_1, A_2, B
Reagent Red Blood Cells 0.8±0.1%

U.S. License No. 1740

For confirmation of ABO blood grouping in gel techniques — For *in vitro* diagnostic use

INTENDED USE

Reverse-Cyte® A_1, B 0.8% and Reverse-Cyte® A_1, A_2 and B 0.8% Reagent Red Blood Cells are for the confirmation of ABO Blood grouping in gel techniques.

For use with the DG Gel 8 System.

SUMMARY AND EXPLANATION

Red blood cells (forward) ABO blood grouping is performed using reagent anti-A, -B and -A,B. As a confirmatory procedure, red blood cells of known ABO phenotypes are used for serum (reverse) grouping to demonstrate the presence or absence of anti-A and anti-B in human serum. Anti-A and anti-B are naturally occurring; they are nearly always present in serum when red blood cells lack the corresponding antigen.[1,2] Thus, serum grouping may be used to confirm results obtained in red blood cell grouping.[2]

PRINICIPLE OF THE TEST

Anti-A and anti-B bind to red blood cells possessing the corresponding antigenic determinants, resulting in direct agglutination. Reverse-Cyte® 0.8% Reagent Red Blood Cells is utilized in the gel technique to detect antibodies to human blood groups A and B.

REAGENT

Reverse-Cyte® A_1 and B or A_1, A_2 and B: Rh phenotype cde (rr) human red blood cells, 0.8 ± 0.1% suspensions in isotonic medium with added buffers (bicarbonate and phosphate) and preservatives (neomycin 0.010% (w/v) and chloramphenicol 0.017% (w/v)). The suspending medium contains EDTA which may decrease complement mediated hemolysis. No U.S. standard of potency.

STORAGE AND STABILITY

- The expiration date of each lot is no longer than 61 days from the collection date of red blood cells from any donor in the lot.
- Store at 2-8 ˚C.
- **Do not freeze.**

Indication of deterioration: Notable hemolysis (which may be caused by microbial contamination or improper handling), darkening of Reagent Red Blood Cells or spontaneous clumping. The reactivity of the product may decrease slightly during the dating period.

PRECAUTIONS

- For *in vitro* diagnostic use.
- Use of plasma may result in false positive due to fibrin clot formation.
- Do not use beyond expiration date. Reactivity of the product may decrease slightly during the dating period.
- 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.
- The pipette of the vial contains natural rubber latex, which may cause allergic reactions.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to specimen collection. Serum from freshly clotted blood is preferred. Plasma can be used, but caution should be exercised as false positives may occur due to fibrin clot formation. Plasma from donor blood collected in anticoagulants such as CPDA-1 or CPD may be tested up to the expiration date of the unit. For optimum test results, unpreserved serum or plasma should be stored at 2-8 °C no longer than 72 hours prior

to testing; however, serum may be frozen and stored up to 5 years at -20 °C or colder and tested at a later time if necessary.

MATERIALS

Materials Provided

Reverse-Cyte® 0.8% Reagent Red Blood Cells A_1 and B, 2x10 ml, cat. no. 213660
Reverse-Cyte® 0.8% Reagent Red Blood Cells A_1, A_2 and B, 3x10 ml, cat. no. 213662

Materials Required but Not Provided

DG Gel® 8 Neutral cards (Diagnostic Grifols, S.A.)
Centrifuge for DG Gel cards (DG Spin, Diagnostic Grifols, S.A.)

PROCEDURE

Both the reagent and the samples to be tested must be brought to room temperature (20-25 °C) prior to testing.

Carefully resuspend Reverse-Cyte® 0.8% Reagent Red Blood Cells by gentle inversion immediately prior to use. Reagent Red Blood Cells are ready-to-use.

Follow the procedure outlined in the DG Gel 8 System's instructions for use.

QUALITY CONTROL

Interpret both the serum and red blood cells ABO groupings. Any discrepancies must be resolved.[2]

Always use room temperature (20-25 °C) for these procedures; do not incubate tests at 37 °C.

A known negative and a known positive control with weak reacting antibodies should be run in parallel on each day of use

In addition, parallel testing with group O screening red blood cells will alert the technologist to the presence of unexpected antibodies or to other factors that may cause discrepant results in the reverse grouping test.

RESULTS

Interpretation

Reaction with Test Serum / Plasma					
Reagent Red Blood Cells Group A_1	Reagent Red Blood Cells Group A_2	Reagent Red Blood Cells Group B	Blood Group	Frequency (%) in	
				Caucasians	Blacks
+	+	+	O*	45	49
-	-	+	A	40	27
+	+	-	B	11	20
-	-	-	AB	4	4
+	-	+	Probably A_2 with anti-A_1**		
+	-	-	Probably A_2B with anti-A_1**		

\+ = Agglutination (positive reaction)
– = No agglutination (negative reaction)
* See limitations of procedure
** Other weak subgroups of A may substitute for A_2

LIMITATIONS OF PROCEDURE

1. As in all serological tests, such factors as contaminated materials, improper incubation time or temperature, improper centrifugation, or improper examination for agglutination may give rise to false test results.
2. If the expected reactions fail to appear, repeat the test with an incubation at 20-25 °C for 10 minutes. If lower temperature is used, an autocontrol and antibody screening red blood cells (such as Search-Cyte® 0.8% Reagent Red Blood Cells) should be tested in parallel to detect false positive reactions due to cold reacting auto- or alloantibodies.
3. Reactions varying from the given table have to be confirmed by further testing before a definite result is established.
4. Hemolysis of the A or B cells usually indicates presence of high titers of isoagglutinins and often also presence of immune A and B antibodies. The latter may cause hemolytic disease of the newborn due to ABO incompatibility.
5. If poor anti-coagulated plasma or incompletely clotted serum is used, fibrin residues may trap non-agglutinated red blood cells at the top of the gel, appearing as a pinkish or reddish layer, but the negative reaction can be interpreted as such. It is recommended to reclot the serum and repeat the test.[2]
6. Reverse-Cyte® 0.8% may be used as blood group compatible cells in antibody identification, especially for cold autoantibodies.
7. False positive or false negative results can occur due to contamination of test materials, improper reaction temperature, improper storage of materials, improper centrifugation, omission of test reagents, and/or certain disease states.
8. Any modifications of the test procedures described in this instruction for use require validation by the user.

False negative results may occur under the following conditions:

1. If serum from the elderly is used, since isoagglutinin activity may be reduced.
2. If serum from patients with hypo-/agammaglobulinemia is used, since it may not contain detectable ABO antibodies.
3. If plasma is used, complement-dependent hemolytic reactions may not be detected.
4. Samples of newborns up to the age of 4-6 months, patients with immunodeficiencies or with highly diluted antibodies due to plasma exchange procedures, may present low or non-existent levels of isoagglutinins.

False positive results may occur under the following conditions:

1. If the unexpected antibody anti-A_1 is present in a blood group A_2 or A_2B -individual (frequency approximately 1-2% in A_2 bloods, 22-25% in A_2B bloods). To resolve the problem, test the serum sample with group A_2 red blood cells.
2. If unexpected antibodies, such as anti-Lewis, anti-P_1, anti-M, etc., are present. Confirm by testing serum sample with antibody screening red blood cells (such as Search-Cyte® Reagent Red Blood Cells). Then identify antibodies by using an antibody identification panel (such as Data-Cyte® Plus Reagent Red Blood Cells). Resolve ABO grouping problem by testing serum with single donor A and B red blood cells negative for the antigen(s) corresponding to the unexpected antibodies.
3. If serum contains cold autoagglutinins (such as anti-I or anti-H) having sufficient activity at room temperature to produce agglutination. Such reactions can be clarified by:
   a. Testing the serum with autologous Red Blood Cells.
   b. Testing the serum with groups A, B and O cord cells.
4. If neonatal serums are used, since these may contain IgG anti-A and/or anti-B passively acquired from maternal serum.
5. In rare cases, the test serum contains an antibody directed at one of the components of the reagent diluent.
6. The formation of "rouleaux", caused by an excess of protein in the serum or the presence of abnormal proteins, drugs, plasma expanders, etc., may cause false positive reactions.[2]
7. Lipids, bilirubin, hemolytic samples, and rheumatic diseases may interfere with the test results.

SPECIFIC PERFORMANCE CHARACTERISTICS

Each lot of Reverse-Cyte® 0.8% Reagent Red Blood Cells is carefully prepared to permit detection of ABO isoagglutinins when used as outlined in these procedures.

Direct antiglobulin tests are negative on all red blood cells.

As with all red blood cells, the reactivity of the product may decrease during the dating period. The rate at which antigen reactivity is lost is partially dependent upon individual donor characteristics that are neither controlled nor predictable by the manufacturer. However, if properly stored when not in use, the reagent can be expected to perform as described throughout its dating.

BIBLIOGRAPHY

1. Mollison P.L., Blood Transfusion in Clinical Medicine. 11th ed. Blackwell Scientific Publications, 2005, Chapter 4.
2. Technical Manual of the American Association of Blood Banks. 17th ed. 2011, Chapter 12.

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SYMBOLS KEY

One or more of these symbols may have been used in the labeling/packaging of this product.

"symbol" *In vitro* diagnostic medical device
"symbol" Batch code
"symbol" Use by YYYY-MM-DD or YYYY-MM
"symbol" Temperature limitation
"symbol" Consult instructions for use
"symbol" Catalog number
"symbol" This way up
"symbol" Fragile, handle with care
"symbol" Keep dry