Medion Diagnostics

Reverse-Cyte®
Groups A_1 and B
Groups A_1, A_2 and B
Reagent Red Blood Cells 3 ± 1%

U.S.License No. **1740**

For confirmation of ABO blood grouping

For in vitro diagnostic use

Summary and Principle

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 Cells grouping.[2]

Anti-A and anti-B bind to Red Blood Cells possessing the corresponding antigenic determinants resulting in direct agglutination. Reverse-Cyte® Reagent Red Blood Cells are used to detect antibodies to human blood groups A and B.

Reagents

Reverse-Cyte® Reagent Red Blood Cells Groups A_1 and B or Groups A_1, A_2 and B: Rh phenotype cde (rr) human Red Blood Cells, 3 ± 1% suspensions in isotonic medium with added buffers (bicarbonate and phosphate) and preservatives (0.03% neomycin and 0.05% chloramphenicol). The suspending medium contains EDTA which may decrease complement mediated hemolysis. No U.S. standard of potency. Meets other FDA requirements. 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.

Resuspend by gentle inversion immediately prior to use. Reagent Red Blood Cells are ready to use. Washing Reagent Red Blood Cells prior to use removes EDTA-preservative solution. 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 diminish slightly during the dating period.

Caution: 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 48 hours prior to testing; however, serum may be frozen at –20 to –80°C and tested at a later time if necessary.

Procedures

Reagent provided

Reverse-Cyte® Reagent Red Blood Cells Groups A_1 and B or Groups A_1, A_2 and B, 3 ± 1%.

Material Required but Not Provided

1. Test tubes (12 x 75 mm or 10 x 75 mm)
2. Physiologic saline
3. Optical aid[3]
4. Centrifuge (calibrated for 1000 rcf* or 150 rcf*)
5. Pipets

Procedure Outline

Tube Test

1. Place 2 or more drops of test serum or plasma in each properly labeled tube.
2. Add 1 drop of each Reverse-Cyte® Reagent Red Blood Cell suspension to its labeled tube. Shake to mix.
3. Centrifuge for 15 – 20 seconds at approximately 1000 rcf* (1 minute at approximately 150 rcf*) or time appropriate to the calibration of the centrifuge.
4. Gently resuspend Red Blood Cells completely and examine immediately for agglutination. Grade and record results.

Note: Weak isoagglutinins may be enhanced by incubation before centrifugation. See Limitations of Procedure for more information.

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.

Known relatively weak positive serums should be tested with each Reverse-Cyte® suspension each day the Reagent Red Blood Cells are in 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

* rcf = 0.00001118 x rotation radius (cm) x rpm^2

grouping test. This testing may be conveniently performed using Blood Bank Ouality Assurance (BBQA II) Testing Reagents available from Medion Diagnostics. To ensure proper centrifugation, each individual centrifuge should be calibrated for the specific test procedure being performed. Red Blood Cells should be packed firmly, but negative control Red Blood Cells should resuspend easily.[3]

Results

Interpretation

Reaction with Test Serum				
Reagent Red Blood Cells Group A_1	Reagent Red Blood Cells Group A_2	Reagent Red Blood Cells Group B	Blood Group	Frequency in Cauc. Blacks
+	+	+	0**	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)

Limitations of Procedure

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.
False negative results may occur if

1. an expected isoagglutinin reacts poorly at room temperature. Very weak isoagglutinins of this kind may frequently be demonstrated by incubating the serum-Red Blood Cells mixtures for 15 minutes at a lower temperature (5–15°C) before centrifugation. If lower temperature incubation is used, an autocontrol and antibody screening Red Blood Cells (such as Search-Cyte® Reagent Red Blood Cells) should be tested in parallel to detect false positive reactions due to cold reacting auto- or alloantibodies.
2. neonatal serum is used since isoagglutinins usually are not demonstrable in infants until 3 months of age.
3. serum from the elderly is used since isoagglutinin activity may be reduced.
4. serum from patients with hypo-/agammaglobulinemia is used since it may not contain detectable ABO antibodies.

Discrepant results may occur if

1. 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. 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. 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. 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.

** See Limitations of Procedure
*** Other weak subgroups of A may substitute for A_2.

Specific Performance Characteristics

Each lot of Reverse-Cyte® 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. 10th ed. Blackwell Scientific Publications, 1997: Chapter 4.
2. Technical manual of the American Association of Blood Banks. 14th ed. 2002, Chapter 13.
3. Ibidem: Method 8.5, p. 765.

Warranty

This product is warranted to perform as described in its labeling and in the product literature, and Medion Diagnostics AG disclaims any implied warranty of merchantability or fitness for any other purpose, and in no event shall Medion Diagnostics AG be liable for any consequential damages arising out of the aforesaid express warranty.

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