Individuals using assistive technology may not be able to fully access the information contained in this file. For assistance, please call 800-835-4709 or 240-402-8010, extension 1. CBER Consumer Affairs Branch or send an e-mail to: ocod@fda.hhs.gov and include 508 Accommodation and the title of the document in the subject line of your e-mail.

3033116/118 (XX/202X)

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 and titration of antibodies 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 and for titration of antibodies 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.

REAGENTS

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.
- Once a vial has been used, it must be stored at the indicated storage temperature.
- To avoid contamination, close the caps on the vials when they are not in use. Ensure that the caps on the Reagent Red Blood Cell vials have not been swapped.
- If handled and stored appropriately, this product is stable from the time it is first opened until the indicated expiration date.
- **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 shelf-life.

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 shelf-life.
- 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.

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. The samples should be tested as soon as possible. Frozen samples stored up to 5 years at -20 °C or colder may be used after thawing. If the recipient has been pregnant or transfused within the previous three months samples stored at 2 - 8 °C should be used within 72 hours after collection.

MATERIALS

Materials Provided

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
To interpret the titration test results follow the IFU for DG Gel 8 cards.

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 foetus and 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

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

Please refer to the Instruction for Use of DG Gel 8 cards.

Associated instruments:

For Manual Method

- DG SPIN centrifuge
- DG Reader Net or DG Reader (optional)

For Fully Automated Methods

- Erytra Eflexis, Erytra or WADiana Compact

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 if

1. Serum from the elderly is used, since isoagglutinin activity may be reduced.
2. Serum from patients with hypo-/agammaglobulinemia is used, since it may not contain detectable ABO antibodies.
3. 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 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 0.8% 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.
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 shelf-life. 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 shelf-life.
- For manual method, the performance of the reagents was confirmed against FDA-licensed reagents in a comparison study where reagents were tested in parallel at different clinical sites. The estimated percent agreements and their lower limits of 95% one-side confidence interval for all sites combined are indicated on the table below.

Overall Statistical Analysis Results of the comparison study				
	Negative Agreement		Positive Agreement	
	Nº of samples	Percent Agreement (Lower 95% CI)	Nº of samples	Percent Agreement (Lower 95% CI)
Reverse ABO Group A_1	1,261	99.84% (99.50%)	1,775	99.72% (99.41%)
Reverse ABO Group B	530	99.62% (98.82%)	2,504	99.76% (99.53%)
Reverse ABO Group A_1,B	1,791	99.78% (99.49%)	4,279	99.74% (99.57%)
Reverse ABO Group A_2	1,295	99.69% (99.29%)	1,741	98.97% (98.47%)

- Percent of Agreement only indicates agreement between reagents and does not indicate which reagent gave the correct result(s).
- For further information about the performance data for manual method using DG Reader or DG Reader Net and for automated method, please refer to the Instruction for Use of the related instrument.
- For the antibody titration test, the performance of the reagent in the automatic technique was confirmed against the manual method in a comparison study. For further information about the performance of the automated method, please refer to the instruction for use of the related instrument.

IVD *In vitro* diagnostic medical device

LOT Batch code

Use by YYYY-MM-DD or YYYY-MM

Temperature limitation

Consult instructions for use

REF Catalog number


This way up


Fragile, handle with care


Keep dry


Manufacturer

BIBLIOGRAPHY

1. Mollison P.L., Blood Transfusion in Clinical Medicine. 12th ed. Blackwell Scientific Publications, 2014, Chapter 4.
2. Technical Manual of the American Association of Blood Banks. 19th ed. 2017, Chapter 10 and 17.

Manufactured by:
Medion Grifols Diagnostics AG
Bonnstrasse 9, CH-3186 Düdingen / Switzerland

Technical Service:
Emeryville, CA, USA 94608
Telephone (in U.S.): (800) 452-6877
Or: (510) 923-3757
Fax: (800) 462-3938
Email: service.americas@grifols.com

Date of last version: xxx 202x

SYMBOLS KEY

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