Blood Grouping Reagent Anti-K (Anti-Kell)

For Indirect Antiglobulin Test

A Qualitative Test for Recognition of the K Antigen on Human Red Blood Cells

SUMMARY AND EXPLANATION

The K antigen is found on the erythrocytes of approximately 10% of the Caucasian population. The presence or absence of the K antigen on human red blood cells is determined by testing the red blood cells with Anti-K reagent. Agglutination of the red blood cells with Anti-K reagent is a positive test result, which indicates the presence of the K antigen on the red blood cells. Absence of agglutination is a negative test result, which indicates the K antigen is not demonstrable.

Anti-K reagent is useful for the selection of donors for patients who have become immunized to the K antigen as a result of transfusion or pregnancy. When used in conjunction with Anti-k reagent, it will also indicate the zygosity of the red blood cells being tested.

PRINCIPLE OF PROCEDURE

The procedure used with this reagent is based on the principle of agglutination. Normal human red blood cells, possessing antigens, will clump in the presence of antibody directed toward the antigens.

REAGENT

Blood Grouping Reagent Anti-K (Anti-Kell) for Indirect Antiglobulin Test, as supplied by Ortho-Clinical Diagnostics, Inc., is prepared from pools of human serum containing K antibodies.

This reagent contains sodium azide 0.1% as a preservative. Additional constituents include sodium chloride and bovine albumin. Use as furnished. FOR IN VITRO DIAGNOSTIC USE.

Warning: Contains sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azide. On disposal, flush with a large volume of water to prevent azide buildup.

MEETS FDA POTENCY REQUIREMENTS. Do not use beyond expiration date. Store at 2 to 8°C. May be at room temperature (20 to 30°C) while in use.

Turbidity due to the presence of soluble lipoproteins may occur. During the manufacturing process, a small amount of lipoproteins may remain in the product and become visible over time. These lipoproteins do not affect product performance.

Efforts should be made to prevent contamination of the product. Turbidity may indicate microbial contamination. Quality Control testing is required to confirm reactivity of the product prior to use.

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.

CONTROLS

It is recommended the reagent be tested on each day of use with appropriate positive and negative controls.

Positive control—red blood cells, preferably heterozygous, known to possess the K antigen.

Negative control—red blood cells known to lack the K antigen.

If a patient control run simultaneously with the test results in agglutination, the test result is unreliable. The control recommended for use with this product is isotonic saline or a 6% to 8% solution of bovine albumin in isotonic saline. If preferred, a direct antiglobulin test may be performed. Invalid positive test results may be obtained when testing with a reagent which requires an antiglobulin procedure if the blood tested is from a person with a positive direct antiglobulin test.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to specimen collection. Blood should be collected by approved techniques. The sample should be tested as soon as possible following collection. If a delay in testing should occur, the sample should be stored at 2 to 8°C.

Blood drawn into heparin or oxalate should be tested within two days. Clotted specimens or blood drawn into sodium citrate or EDTA should be tested within 14 days. Donor blood may be tested up to date of expiration.

PROCEDURE

Material Provided

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Required Supplementary Materials

1. Test tubes, 10 x 75 mm or 12 x 75 mm
2. Transfer pipettes
3. Incubator, 37°C
4. Centrifuge
5. Isotonic saline, 0.85-0.9% sodium chloride
6. Anti-human globulin containing anti-IgG (such as Anti-Human Globulin [Rabbit and Murine Monoclonal] BioClone® Anti-IgG, -C3d; polyspecific or ORTHO Anti-IgG)
7. Antiglobulin control cells (such as ORTHO Coombs Control)

Directions for Use

Test Procedure

1. Prepare a 3% to 5% suspension of red blood cells in isotonic saline.
2. To a test tube, add one drop of Anti-K reagent.
3. Using a transfer pipette, add one drop of the cell suspension to the test tube.
4. Mix well and incubate the tube at 37°C ± 1°C for 15 minutes.
5. After incubation, wash the cells three times with tubes full of isotonic saline. Decant completely after the last washing.
6. Add two drops of anti-human globulin containing anti-IgG or anti-IgG (refer to the respective directions for use).
7. Mix the contents of the tube gently and centrifuge immediately.
   Suggested centrifugation: approximately 15 ± 30 seconds at 3400 rpm (900-1000 rcf) or 1 minute at 1000 rpm (100-125 rcf).*
8. Resuspend the cells by gentle agitation and examine immediately for macroscopic agglutination.
9. To control all negative antiglobulin tests, add red blood cells sensitized with IgG antibody, e.g., ORTHO Coombs Control (see package insert for procedure).

RESULTS

Interpretation

1. If the test with Anti-K is positive and the patient control is negative, the red blood cells are designated K positive.
2. If both the test and patient control are negative, the red blood cells are designated K negative.
3. No valid conclusion can be reached when both the test and patient control are positive.

Stability of Final Reaction Mixture

All results must be interpreted immediately upon test completion.

ORTHO

LIMITATIONS OF PROCEDURE

1. Red cells demonstrating a positive direct antiglobulin test cannot be accurately tested for the K antigen with this reagent.
2. Aged red cells may yield weaker reactions than those obtained with fresh red cells.
3. Contaminated blood specimens and/or supplementary materials used in the procedure described may interfere with the test results.
4. Antibodies directed at low-frequency antigens may occur as unsuspected contaminants in blood grouping reagents. In addition, certain antigens (e.g., Bg, Sd^a) can be present in an exalted state on the red blood cells. These phenomena may be a source of rare false-positive reactions, which may occur with more than one lot of a given specificity. Since manufacturers commonly obtain raw material from the same sources, the same contaminating antibody may be present in products acquired from different manufacturers. It is not possible for any manufacturer to claim the absence of all contaminating antibodies, as red blood cells carrying antigens of low frequency or exalted antigens are not always available for testing. Suppressed or diminished expression of certain blood group antigens may conversely give rise to false-negative reactions. For these reasons, caution should always be exercised when assigning genotypes on the basis of test results.
5. The optimal saline wash solution for most antibodies is pH 7.0-7.2. Use of saline with a low pH may cause decreased sensitivity.
6. Delays in testing following the addition of anti-human globulin reagent may result in weakened reactivity. It is important to centrifuge tubes immediately after the addition of anti-IgG reagent and examine for agglutination immediately upon completion of centrifugation.

SPECIFIC PERFORMANCE CHARACTERISTICS

When properly stored and used according to the procedure described under Directions for Use, this reagent will agglutinate red cells which have the K antigen. The potency of this reagent meets FDA requirements. The reactivity of each lot is demonstrated in tests with the recommended procedure using cells exhibiting heterozygous (Kk) and homozygous (KK) antigen expressions from several donors. The specificity of each lot is shown by the recommended tube method using a panel of cells which lack the antigen against which the reagent is directed but contain as many other antigens having a frequency of 1% or greater as possible. Antibodies to Le^c, Le^d, Kp^a, Yt^b, Co^b, Mg and Wr^a are not excluded in routine specificity tests. However, tests for these antibodies are performed if test cells containing the corresponding antigen become available. Specificity test results submitted to the FDA for release of an individual lot of product will be furnished upon request.

Technical questions concerning this reagent should be directed to Customer Technical Support at 1-800-322-6374.

*The centrifugal force applied to cell/reagent mixtures should be the minimum required to produce a "button" of red cells and a clear supernate.

Overcentrifugation, i.e., the application of forces in excess of the minimum, causes the cells to adhere to the bottom of the test tube so that vigorous agitation is necessary before they can be resuspended. During such agitation, weak agglutination may be dispersed causing a positive reaction to be missed.

Undercentrifugation, i.e., the failure to apply forces necessary to cause the cells to form a "button" and a clear supernate, may result in a weak or negative reaction.

No one speed and time of centrifugation can be recommended which will cover the wide variety of centrifuges available; each laboratory must calibrate its own equipment and determine the time required at a given speed to achieve the desired result.

BIBLIOGRAPHY

Race RR, Sanger R. Blood groups in man, 6th ed. Oxford: Blackwell Scientific Publications. 1975:283.

Technical Manual. 14th ed. Bethesda, MD: American Association of Blood Banks, 2003.

SUMMARY OF REVISIONS

Section	Revision
REAGENT	Revised information regarding turbidity
PROCEDURE — Directions for Use	Deleted SIMWASH™ directions
PROCEDURE — Saline Wash Method	The title "Saline Wash Method" was deleted and replaced with the title "Test Procedure."
PROCEDURE — Test Procedure	Revised information in steps 7 and 8
RESULTS — Stability of Final Reaction Mixture	Added the word "immediately."
LIMITATIONS OF PROCEDURE	Added limitations 5 and 6
BIBLIOGRAPHY	AABB Technical Manual edition and date revised

Store at 2 to 8°C
Preservative: Sodium azide 0.1%
Turbidity or precipitation may indicate product alteration

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MEETS FDA POTENCY REQUIREMENTS

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CAUTION: HANDLE AS IF CAPABLE OF TRANSMITTING INFECTIOUS AGENTS

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