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BLOOD GROUPING REAGENTS

Anti-C (Monoclonal)
Anti-E (Monoclonal)
Anti-c̄ (Monoclonal)

IVD *In vitro* diagnostic medical device

American Red Cross

INTENDED USE

American Red Cross (Red Cross) Anti-C (Monoclonal), Anti-E (Monoclonal), and Anti-c̄ (Monoclonal) are used for the *in vitro* detection of the C (RH2), E (RH3), and c̄ (RH4) antigens respectively, on human red blood cells by direct agglutination using the manual tube testing method.

SUMMARY AND EXPLANATION

The Rhesus (Rh) blood group system contains more than 56 antigens or antigen complexes expressed on human red blood cells and is one of the most complex among the 44 human blood group systems.[1-3] The RHCE alleles encode the principal C/c̄ and E/ē antigens. More than 150 different RHCE alleles are known, and many are associated with altered or weakened expression of the principal antigens. Partial C antigens are well recognized, with the majority reported among people of African ethnicity.[3] The antibodies to the antigens C, E, and c̄ , may be associated with transfusion reactions and hemolytic disease of the fetus and newborn (HDFN) and therefore their presence or absence is important in pre-transfusion testing and the prediction of HDFN.

The frequencies of Rh antigens vary between different populations, as shown in Table 1:

Antigen Nomenclature		Prevalence[3]	
Antigen	ISBT	Whites	Blacks
C	RH2	68%	27%
E	RH3	29%	22%
c̄	RH4	80%	96%

PRINCIPLE OF PROCEDURE

When the Directions for Use (DFU) are followed, these reagents will detect the presence of the associated antigen by causing visible agglutination of red blood cells expressing the specific antigen (positive test) after incubation at 36ºC to 38ºC. The absence of the associated antigen is demonstrated by the lack of agglutination of the red blood cells (negative test), after incubation at 36ºC to 38ºC, within the limits of the procedure.

REAGENT

Red Cross Anti-C (Monoclonal), Anti-E (Monoclonal), and Anti-c̄ (Monoclonal) are prepared from monoclonal antibodies derived from cell culture supernatant from human/murine heterohybridoma cell lines (Anti-C MS-24, Anti-E MS-80/MS-258, and Anti-c̄ MS-33). The antibodies are immunoglobulin class IgM and provide a potent and specific reagent that meets the requirements of the Food and Drug Administration (FDA).

Each lot is optimized for tube testing and standardized for pH and total protein concentration in a buffered diluent containing macromolecular chemical potentiators. The bovine albumin component of these products is derived exclusively from United States sources of disease-free cattle, inspected, and certified by the U.S. Veterinary Services. This ruminant-based product is deemed to have low Transmissible Spongiform Encephalopathy (TSE) risk. The reagents contain sodium azide (0.1% final concentration) as a preservative.

These monoclonal antibodies are manufactured using intermediate products produced for the Red Cross in a shared manufacturing agreement with Millipore (UK) Ltd., Fleming Road, Kirkton Campus, EH547BN, Livingston, UK; U.S. License Number 1761.

Red Cross Anti-C (Monoclonal), Anti-E (Monoclonal), and Anti-c̄ (Monoclonal) are for *in vitro* diagnostic use and are supplied ready for use. Use as furnished, do not dilute.

Red Cross Monoclonal Blood Grouping Reagents meet FDA potency requirements.

CAUTION STATEMENTS
Rx only

CAUTION: All blood products should be treated as potentially infectious. Source material from which these products were derived was found negative when tested in accordance with current FDA required tests. No known test methods can offer assurance that products derived from hybridoma cell culture supernatant will not transmit infectious agents. The absence of murine virus has not been determined.

CAUTION: This Product Contains Natural Rubber Latex (Dropper Bulbs) Which May Cause Allergic Reactions.

WARNING: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If discarded into sinks, flush with a large volume of water to prevent azide build-up.

STORAGE

Store at 2ºC to 8ºC when not in use.

Do not use beyond the expiration date. The format for the expiration date is expressed as YYYY-MM-DD (year-month-day).

Do not use if turbid.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient or donor is required prior to specimen collection. Blood should be collected by an acceptable phlebotomy technique. Samples may be drawn into EDTA, CPD, CPDA-1, CPD with AS-1, CP2D with AS-3, and CPD with AS-5, or may be drawn without anticoagulant (non-barrier red top). Do not use samples drawn into tubes with neutral gel separators. Samples should be tested as soon as possible after collection. Samples should be stored at 2ºC to 8ºC when not required for testing.

Blood drawn into EDTA, and non-barrier red top (clotted) tubes should be tested less than or equal to 10 days from collection. Donor samples drawn into CPD, CPDA-1, CPD with AS-1, CP2D with AS-3, and CPD with AS-5 may be tested up to the expiration date of the donor unit.

MATERIALS

Materials provided:

1. Red Cross Anti-C (Monoclonal), Anti-E (Monoclonal) or Anti-$\bar{c}$ (Monoclonal)

Materials required but not supplied:

1. Test tubes, 10 x 75mm or 12 x 75mm
2. Test tube racks
3. Pipettes
4. Centrifuge calibrated for serological use
5. Isotonic Saline (pH 6.5 – 7.5)
6. Incubating equipment
7. Antigen positive and negative control cells
8. Timer
9. Optical aid (optional)

PROCEDURE

1. Prepare a 2-4% suspension of red blood cells washed at least once with isotonic saline.
2. Add 1 drop of Red Cross Anti-C (Monoclonal), Anti-E (Monoclonal) or Anti-$\bar{c}$ (Monoclonal) reagent to an appropriately labeled test tube.
3. Add 1 drop of the previously prepared 2-4% red blood cell suspension.
4. Mix well.
5. Incubate at 36ºC to 38ºC for 5 minutes.
6. Mix well and centrifuge the tube for 15 seconds at 3400 rpm (900-1000 rcf*) or equivalent, as indicated on the quality control calibration.
7. Resuspend the red blood cells by gentle agitation.
8. Read macroscopically for agglutination and record results. An optical aid may be used if desired.

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

STABILITY OF REACTION

Following centrifugation, all tube tests should be read/recorded without delay. Time delays may cause a dissociation of the antigen-positive complexes resulting in false negative or weaker than expected reactions.

QUALITY CONTROL

The reactivity of these Blood Grouping Reagents should be confirmed on each day of use by testing with known antigen-positive (preferably heterozygous expression) and with known antigen-negative red blood cells. Each reagent is acceptable for use if it reacts only with the antigen-positive red blood cells.

INTERPRETATION OF RESULTS

Positive (+) test result: visible agglutination of the red blood cells after the 37ºC incubation and centrifugation.

NOTE: Hemolysis, if obtained, should not be interpreted as a positive result since the conditions for complement activation due to a red cell antibody-antigen reaction do not exist.

Negative (-) test result: no agglutination of red blood cells after the 37ºC incubation and centrifugation.

LIMITATIONS

All serological tests have limitations. To maximize success in obtaining valid results, follow the DFU carefully.
Deviations from manufacturer's instructions without appropriate validation and controls may produce erroneous results.

Monoclonal human IgM Rh antibodies have been shown to possess anti-I/i cold agglutinin activity, particularly with cord cells or enzyme treated cells. This may be seen if the tests are incubated below the recommended temperature.[1]

Samples demonstrating a positive direct antiglobulin test, cold agglutinins, or rouleaux formation may show false positive results in testing with monoclonal antibodies. Results from such samples must be interpreted with caution. If false positive results are suspected, an appropriate control, for example 6% albumin solution, should be included in any investigatory process.

False positive or false negative test results may occur from bacterial or chemical contamination of test materials, improper incubation temperature or time, centrifugation errors, storage temperatures excursions, or omission of test reagents.

Red Cross Anti-E (Monoclonal) – Rarely, weak false positive results (≤ 1+) may be seen. Caution should be used when interpreting test results. In this case, testing is recommended with a different licensed Anti-E blood grouping reagent.

Weaker positive reactions may be obtained from samples with unusual Rh phenotypes.

Enzyme treated red blood cells may give false positive reactions with Red Cross Anti-E (Monoclonal) and Anti-$\bar{c}$ (Monoclonal) Blood Grouping Reagents. Use of enzyme treated cells with these reagents is not recommended.

The resuspension of the button in serological reactions in the tube test procedure must be carried out by using gentle agitation. Shaking too strongly may cause the agglutinates to be dispersed.

Suppressed or weakened expression of blood group antigens may give rise to false negative reactions or non-concordance with similar reagents and/or alternate methodologies. For example, antigens in persons with rare Rh genotypes may not be detected in direct agglutination using these reagents. Similarly, variants may give reactions that conflict with previous results that used polyclonal blood grouping reagents or reagents made with other cell line-derived monoclonal sources.

SPECIFIC PERFORMANCE CHARACTERISTICS

Red Cross Anti-C (Monoclonal), Anti-E (Monoclonal) and Anti-$\bar{c}$ (Monoclonal) have been manufactured to meet FDA potency requirements. Each lot is tested against a panel of antigen-positive red blood cells (heterozygous expression and/or weakened expression if possible) to ensure appropriate reactivity when used by the recommended test procedure in the DFU. The specificity of each lot is verified by the recommended tube testing method using a panel of well characterized red cells that lack the reagent antigen.

Performance Characteristics by manual tube method:

The performance of Red Cross Anti-C (Monoclonal), Anti-E (Monoclonal) and Anti-$\bar{c}$ (Monoclonal) was tested against FDA licensed reference reagents (comparators) in a multi-center field trial, representing blood collection establishments, transfusion services and/or immunohematology reference laboratories which tested donor, patient, and neonatal samples. The testing sites were selected to cover diverse geographic locations and patient/donor populations.

Field Comparison Study Results

Table 2

N=1533		Comparator Reagent			
		Positive	Negative		
Red Cross Anti-C Lot C-2	Positive	892	0	Positive Percent Agreement	100.00%
				PPA (95% 1-Sided LCI)	99.66%
	Negative	0	641	Negative Percent Agreement	100.00%
				NPA (95% 1-Sided LCI)	99.53%

Table 3

N=1533		Comparator Reagent			
		Positive	Negative		
Red Cross Anti-C Lot C-3	Positive	892	0	Positive Percent Agreement	100.00%
				PPA (95% 1-Sided LCI)	99.66%
	Negative	0	641	Negative Percent Agreement	100.00%
				NPA (95% 1-Sided LCI)	99.53%

Table 4

N=1679		Comparator Reagent			
		Positive	Negative		
Red Cross Anti-E Lot E-2	Positive	467	2	Positive Percent Agreement	100.00%
				PPA (95% 1-Sided LCI)	99.36%
	Negative	0	1210	Negative Percent Agreement	99.83%*
				NPA (95% 1-Sided LCI)	99.48%

* The NPA (Point Estimate) criterion was 99.83% due to the two (2) false positive results. The criterion was met for the 95% lower bound CI exceeding 99% for both the PPA and NPA.

Table 5

N=1679		Comparator Reagent			
		Positive	Negative		
Red Cross Anti-E Lot E-3	Positive	467	3	Positive Percent Agreement	100.00%
				PPA (95% 1-Sided LCI)	99.36%
	Negative	0	1209	Negative Percent Agreement	99.75%*
				NPA (95% 1-Sided LCI)	99.36%

* The NPA (Point Estimate) criterion was 99.75% due to the three (3) false positive results. The criterion was met for the 95% lower bound CI exceeding 99% for both the PPA and NPA.

Table 6

N=1606		Comparator Reagent			
		Positive	Negative		
Red Cross Anti-c̄ Lot LC-2	Positive	1090	0	Positive Percent Agreement	99.91%
				PPA (95% 1-Sided LCI)	99.57%
	Negative	1*	515	Negative Percent Agreement	100.00%
				NPA (95% 1-Sided LCI)	99.42%

**Resolved in favor of the investigational reagent. Resolved PPA 100.00% (99.73 95% 1-sided LCI).

Table 7

excee		Comparator Reagent			
		Positive	Negative		
Red Cross Anti-c̄ Lot LC-3	Positive	1090	0	Positive Percent Agreement	99.91%
				PPA (95% 1-Sided LCI)	99.57%
	Negative	1*	515	Negative Percent Agreement	100.00%
				NPA (95% 1-Sided LCI)	99.42%

*Resolved in favor of the investigational reagent. Resolved PPA 100.00% (99.73 95% 1-sided LCI).

Precision Study Results

As part of the performance evaluation, a precision and reproducibility study was performed using multiple operators at multiple sites on 5 non-consecutive days over a 20-day period. Testing confirmed repeatable and reproducible results within each run, run-to-run, day-to-day, operator-to-operator, and site-to-site. There were no discordant results.

A lot-to-lot study was also performed using three lots of each specificity on 5 non-consecutive days over a 20-day period which confirmed repeatable and reproducible results within each test run, run-to-run, and day-to-day. There were no discordant results.

Specificity tests submitted to the FDA for release of an individual lot of product will be furnished upon request.

For technical questions, contact the American Red Cross Diagnostic Manufacturing Division at 1-800-882-3737.

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