

BLOOD GROUPING REAGENTS

Anti-C (RH2)	REF 210527
Anti-c (RH4)	REF 210528
Anti-E (RH3)	REF 210529
Anti-e (RH5)	REF 210530
Anti-K (KEL1)	REF 210533

(Human/Murine Monoclonal IgM)

For tube technique

- For *In Vitro* Diagnostic Use
- Meets FDA potency requirements
- Discard if turbid
- Preservative: 0.09% (w/v) sodium azide, 0.02% sodium arsenite

INTENDED USE

These reagents are designed to determine the presence of blood group antigens C (RH2), E (RH3), c (RH4), e (RH5) and K (KEL1) on the surface of human red blood cells by manual method.

SUMMARY AND EXPLANATION

Rh blood group system

After the A and B antigens of the ABO blood group system and after the D antigen, the other most important antigens in the Rh system are C, E, c and e. These antigens are not as immunogenic as D, but may cause rapid destruction of red blood cells in the presence of the corresponding antibody.

Antigen D is by far the most immunogenic of the Rh antigens. All the antibodies against all Rh antigens should be considered to have the potential to cause hemolytic transfusion reactions (HTRs) and hemolytic disease of the newborn (HDN).

Positive results indicate the presence of the antigen, while negative results indicate the absence of the antigen on the red blood cells. It is significant to identify the presence of these antigens when selecting blood for transfusion to patients with these antibodies.

Table 1 lists the five most common Rh antigens, the Wiener nomenclature and the approximate frequency of each antigen in the Caucasian population.

Table 2 lists the most common patterns of reactions obtained and the most common genotypes.

Table 1

Fisher-Race	Weiner	Caucasian %
D	Rh ₀	85
C	rh'	70
E	rh''	30
c	hr'	80
e	hr''	98

Table 2

Anti-D	Anti-C	Anti-E	Anti-c	Anti-e	Wiener	Fisher-Race
+	+	0	+	+	R ¹ r	CDe/cde
+	+	0	0	+	R ¹ R ¹	CDe/CDe
0	0	0	+	+	rr	cde/cde
+	+	+	+	+	R ¹ R ²	CDe/cDE
+	0	+	+	+	R ² r	cDE/cde
+	0	+	+	0	R ² R ²	cDE/cDE

+	0	0	+	+	R ⁰ r	cDe/cde
0	+	0	+	+	r'r	Cde/cde
0	0	+	+	+	r ⁰ r	cdE/cde

Kell blood group system

The most frequently encountered antibody in the Kell system is anti-K. The K (KEL1) antigen is strongly immunogenic, and anti-K is frequently found in the sera of transfused patients. A positive test indicates the presence of the K antigen, while a negative test indicates the absence of the K antigen on the red blood cells. Approximately 90% of donors are K negative. It is significant to identify the K antigen when selecting blood for transfusion to patients with anti-K.

PRINCIPLE OF THE TEST

The manual technique employed in a tube, utilizes the principle of hemagglutination. Test red blood cells bearing an antigen agglutinate in the presence of the reagent containing the corresponding antibody using the direct hemagglutination method.

REAGENTS

All the reagents contain sodium azide (0.09%). The Anti-C (RH2) (REF 210527), Anti-c (RH4) (REF 210528) and Anti-e (RH5) (REF 210530) contain sodium arsenite (0.02%). All reagents contain bovine materials. Any bovine materials used in the manufacture of these products are sourced from donor animals that have been inspected and certified by Veterinary Service inspectors to be disease free.

The reagents are produced by DIAGAST from monoclonal antibodies derived from the *in vitro* culture supernatant of human/murine heterohybridomas.

These reagents are provided with calibrated droppers.

Code	Product Designation	Packaging
210527	Anti-C (RH2)	4 x 5 mL
210528	Anti-c (RH4)	4 x 5 mL
210529	Anti-E (RH3)	4 x 5 mL
210530	Anti-e (RH5)	4 x 5 mL
210533	Anti-K (KEL1)	4 x 5 mL

WARNINGS AND PRECAUTIONS

- These reagents contain sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive compounds. If discarded into a sink, flush with a large volume of water to prevent azide build-up. Handle and dispose of reagents as potentially infectious, in accordance with local, state, and national laws.
- Use proper Personal Protective Equipment according to local SOPs or guidelines.
- All materials that have come into contact with the samples are to be handled as potentially infectious products.
- Special protective measures and conditions for disposal and disinfection should be implemented in accordance with local regulations.
- For *In Vitro* Diagnostic Use
- Do not use beyond expiration date.
- Do not use damaged or leaking reagents.
- Do not use if turbid.
- Do not dilute.
- The absence of all viruses has not been determined in these reagents.
- These reagents have components (Dropper bulb) containing dry natural rubber which may cause allergic reactions.

- These reagents contain material of human or animal origin and may transmit infectious agents and should be handled with extreme caution.

CAUTION: ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. SOURCE MATERIAL FROM WHICH THIS PRODUCT WAS DERIVED WAS FOUND NEGATIVE WHEN TESTED FOR HIV, HBV AND HCV. NO KNOWN TEST METHODS CAN OFFER ASSURANCE THAT PRODUCTS DERIVED FROM HUMAN BLOOD WILL NOT TRANSMIT INFECTIOUS AGENTS.

STORAGE AND STABILITY

- Store reagents at 2°C to 8°C when not use. Do not freeze.
- Do not use beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to the specimen collection.

The blood samples collected following standard blood sampling guidelines in EDTA, heparin or sodium citrate anticoagulant should be stored at 2-8°C.

They should be tested as follows:

- Clotted specimens or blood drawn into sodium citrate or EDTA should be tested within 7 days.
- Blood drawn into heparin should be tested within 2 days.

Red blood cells from bags collected in ACD, ACD with AS-1, CPD, CPD with AS-1, CPDA-1, CP2D and CP2D with AS-3 can also be used up to 7 days after the expiration date indicated on the label of the bag.

Do not use blood specimens that exhibit contamination.

MATERIALS

Material provided:

- Anti-C (RH2) (REF 210527): Monoclonal antibodies blend. Anti-C IgM human/murine clone P3X25513G8 and clone MS24.
- Anti-c (RH4) (REF 210528): Monoclonal antibody. Anti-c IgM human/murine clone 951.
- Anti-E (RH3) (REF 210529): Monoclonal antibody. Anti-E IgM human/murine clone 906.
- Anti-e (RH5) (REF 210530): Monoclonal antibodies blend. Anti-e IgM human/murine clone P3GD512 and clone MS63.
- Anti-K (KEL1) (REF 210533): Monoclonal antibody. Anti-K IgM human/murine clone MS56.

Clones MS24, MS63 and MS56 are produced using intermediate products produced for DIAGAST in a shared manufacturing agreement with Millipore (UK) Ltd., 9 Fleming Road, Kirkton Campus, EH547BN, Livingston, UK; FFMU License Number 1761.

Material required but not provided:

- Test tubes, tube rack.
- Pasteur pipettes (drop volume 40 to 50 µl) or Automatic pipettes with adjustable precision.
- Centrifuge of relative force from 100 to 1200 rcf.
- Timer.
- Isotonic saline solution (0.9% NaCl).
- Positive control blood samples of guaranteed phenotype are required carrying the corresponding heterozygous antigen and similarly for a negative control, blood samples should be used which lack the antigen corresponding to the reagent used.

TEST PROCEDURE

Direct method in a tube at room temperature

1. In a test tube, prepare a 3-5% red blood cell suspension in isotonic saline solution.
2. Using the vial dropper, transfer 1 drop of reagent to a test tube.
3. Add 1 drop or 50 µL of erythrocyte suspension.
4. Shake to mix, then centrifuge at 1000 rcf for 15 seconds or use a time and speed appropriate to the calibration of the centrifuge.

5. Gently swirl the test tube to detach the erythrocyte pellet, observe macroscopically to detect the appearance of any agglutinates.
6. Read and record the reaction immediately. It is recommended grading positive reactions.

RESULTS

Positive Result: If there is agglutination (the red blood cells form one or several clump(s)), the reaction is positive and the antigen or at least one of the antigens corresponding to the reagent used is present on the tested red blood cells.

Negative Result: If there is no agglutination (the red blood cells reform a homogeneous suspension), the reaction is negative and the antigen is not present on the tested red blood cells.

Interpretation: The reaction can only be interpreted if the analytical system has been validated with control samples of guaranteed phenotype.

QUALITY CONTROLS

The use of samples of guaranteed phenotyping as control samples allows the user to detect anomalies with (handling, reagents, apparatus and the environment) and to implement corrective actions as required.

Known sample controls should be run in parallel on each day of use.

- a sample possessing the antigen corresponding to the antibody in the reagent used,
- a sample devoid of the antigen corresponding to the antibody in the reagent used.

If an unexpected control result is obtained, a complete assessment of the reagents and material used should be made.

LIMITATIONS OF THE PROCEDURE

- These reagents are not to be used in a method not described in this Instructions For Use.
- It is recommended to use the calibrated dropper provided in the vial to dispense a reagent drop.
- The reactions are to be read immediately after centrifuging and resuspending.
- False positive or false negative can occur due to improper centrifugation.
- It is imperative to work with clean apparatus and uncontaminated products (bacterial or other contamination).
- Strict compliance with the following is required:
 - storage conditions,
 - equipment calibration is recommended.
- No reagent can guarantee the detection of all the antigenic profiles rare, weak or variants.

SPECIFIC PERFORMANCE CHARACTERISTICS

- These reagents meet FDA potency requirements for Blood Grouping Reagents to be used in tube technique.
- Every lot of each product is tested to assure reliable reactivity and specificity in use in accordance with FDA requirements.
- The tests conducted on particular phenotypes, while satisfactory, cannot ensure recognition of all weak or variant subjects, due to the variability of antigen motifs.
- 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.

Table 3. Overall Statistical Analysis results of the comparison study

Reagent	N° of samples	Negative Percent Agreement (Lower 95% CI)	N° of samples	Positive Percent Agreement (Lower 95% CI)
Anti-C	800	99.88% 99.41%	1080	100% 99.72%
Anti-E	1408	100% 99.79%	472	100% 99.37%

Reagent	N° of samples	Negative Percent Agreement (Lower 95% CI)	N° of samples	Positive Percent Agreement (Lower 95% CI)
Anti-c	327	100% 99.09%	1553	100% 99.81%
Anti-e	79	97.47% 92.24%*	1891	99.95% 99.75%
Anti-K	3314	100% 99.91%	306	100% 99.03%

*Lower value for the Negative Percent Agreement lower confidence bound was obtained due to the limited quantity of e negative samples because of the high frequency of the e antigen in the United States population.

Percent of Agreement only indicates agreement between the DIAGAST reagents and the FDA-licensed reagents and does not indicate which reagent gave the correct result(s).

BIBLIOGRAPHY

- Technical Manual. 20th ed. Bethesda, MD: American Association of Blood Banks, 2020.
- Standards for Blood Banks and Transfusion Services. 32nd ed. Bethesda, MD: American Association of Blood Banks, 2020
- Mannessier L., Horbez C., Broly H. Caractérisation et validation d'un anticorps monoclonal humain anti-C (Characterization and validation of human monoclonal anti-C) Rev. Fr. Transfus. Hémobiol., 1991, 34, 403-408

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SYMBOLS KEY

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

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