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CE 0483

Instruction for use

Rev. 002 / 2021-07

Description	REF
Anti-C^w MS-110 (monoclonal) 2 ml	32102
Anti-C^w MS-110 (monoclonal) 5 ml	32105

IN-VITRO-DIAGNOSTIKUM

SUMMARY

Anti-Cw ist part of the Rh System which contains more than 40 antigenes and complexes. The frequency of Cw varies between populations, it is very rare in people of African or Asian descent, in Caucasians, the overall frequency is 2.6%, but can be as high as 8% in some European populations. Cells positive for Cw are almost always C positive, but in rare cases Cw is accompanied bei c instead of C.
Anti-Cw has been implicated in transfusion reactions and in morbus haemolyticus neonatorum.

INTENDED USE

Anti-Cw monoclonal (IgM) is used for the specific, qualitative detection of the corresponding antigen on erythrocytes and is suitable for slide, spot plate, microtiter plate and tube tests.
The test methods given are based on the principle of haemagglutination. After adding erythrocytes to the test reagents, a specific antigen-antibody reaction takes place if the corresponding antigen is present on the erythrocytes. This reaction can be recognized visually by the agglutination of the erythrocytes. If there is no agglutination, this indicates a negative result and, considering the limitations of the test methods, indicates the absence of the corresponding antigen.

PRODUCT INFORMATION

The monoclonal Anti-C^w reagent is harvested from human hybridoma cells.The antibodies are diluted in a buffer containing 0.9% sodium chloride, bovine albumin (no stabiliser), EDTA and reagents that facilitate the resuspension of the cell pellet after centrifugation. Preservative: < 0.1% sodium azide.
The anti-A and anti-B test sera are dyed blue and yellow, respectively, for easy identification.
These antisera have been optimized for use without further dilution or additions.
The lot number and expiry date are detailed on the tube label.

STORAGE

Store the test reagents at 2°C-8°C until the expiry date detailed on the product label. After opening a product for the first time, tightly close again and store at 2°C-8°C.

SPECIMEN COLLECTION AND PREPARATION

Blood specimens for C^w typing should be collected aseptically in EDTA or citrate tubes. The specimen should be tested as soon as possible following collection. If a delay in testing should occur, store the specimen at 2°C-8°C. Specimens displaying haemolysis or microbial contamination should not be tested with this reagent as this may result in false positive or false negative results.
All blood samples should be washed twice with a 0.9% NaCl solution before being tested by the tube, spot plate or microplate technique. When using the slide technique, prepare a 35-45% suspension of test erythrocytes (whole blood); when using the spot plate technique, use either whole blood or prepare a 10% suspension of test erythrocytes in a 0.9% NaCl solution.

WARNINGS AND PRECAUTIONS

1. These reagents are intended for *in vitro* diagnostic laboratory use only.
2. These reagents are designed for use by authorized operators trained in serological techniques.
3. These reagents are not intended for self-application.
4. Do not use these reagents past the expiration date.
5. Discard the contents of damaged vials.
6. The reagent content < 0.1% Sodium azide.
7. The reagents have been filtered through a 0.2µm capsule to reduce the bio-burden.
8. Once a vial has been opened the contents should remain viable up until the expiry date. Discard the contents if turbidity or contamination occur after opening.
9. CE-Immundiagnostika GmbH cannot guarantee that products derived from human or animal sources are free from infectious agents. Care must be taken in the use and disposal of each vial and its contents.
10. Wear protective clothing such as, for instance, a gown and disposable gloves when using these products

DISPOSAL OF REAGENT AND DEALING WITH SPILLAGES

For information on disposal of the reagent and decontamination of a spillage site, see the Material Safety Data Sheet, available on request from CE-Immundiagnostika GmbH.

CONTROLS AND ADVICE

1. Positive and negative control erythrocytes shall be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected reactions.
2. Since these reagents do not contain macromolecular potentiators, it is very unlikely that false positive or false negative reactions are caused with IgG coated cells.
3. Weak antigens may not be identified. It is advisable to re-test ambiguous results using the tube technique.
4. One volume is approximately 35-45µl when using the vial dropper provided.
5. The reading and interpretation of results must be carried out by properly trained and qualified personnel in accordance with the requirements of the country where the reagents are in use.
6. These reagents should be used only according to these instructions for use.

REQUIRED MATERIAL AND REAGENTS

- 0.9% NaCl solution
- Glass test tubes
- Test tube holder
- Test tube centrifuge
- Volumetric pipettes
- Microplate, plate shaker
- Spot plate
- Glass microscope slide
- Applicator stick
- Positive and negative control erythrocytes
- Chronometer

RECOMMENDED TECHNIQUES

A. TUBE TECHNIQUE

1. Prepare a 2-4% suspension of erythrocytes in a 0.9% NaCl solution.
2. Place 1 volume of antiserum and 1 volume of test suspension of test erythrocytes in a labelled test tube.
3. Mix thoroughly and centrifuge straight away at 400g for 1 minute (at 1,500 rcf or for a suitable alternative time and force).

4. Read the result immediately: Gently agitate the tube to dislodge the erythrocyte pellet from the bottom of the test tube and read macroscopically for agglutination; record result.

B. MICROPLATE TECHNIQUE

Preparation of the microplates:
Microplates made by different manufacturers / suppliers have different static properties that may give rise to non-specific red blood cell and protein reactions. It is advisable to pre-treat unused microplates prior to use to reduce the build-up of red blood cells to a minimum. We recommend using “U” wells made from plastic material.

1. Place 1 volume of 22% bovine albumin (BSA) in the appropriate wells.
2. Mix thoroughly, gently agitating or using a microplate shaker, to ensure that wells are covered evenly.
3. Incubate at room temperature (18-25°C) for no less than 10 and no more than 15 minutes.
4. Drain the BSA and discard the content of the wells in a suitable waste disposal container.
5. Flush the microplate at least 10 times with tap water.
6. Then rinse the microplate twice with distilled or deionized water.
7. Tilt and dab the microplate to remove excess water.
8. Allow the microplate to dry prior to use.

Alternative techniques may be used provided that they have been validated by the user.

Procedure:

1. Prepare a 2 - 4% suspension of test erythrocytes in a 0.9% NaCl solution. (Recommendation: 2% suspension)
2. Using the vial dropper, place 30µl of the appropriate antiserum into the marked wells of the microplate.
3. Add 30µl of the previously prepared suspension of test erythrocytes to the microplate.
4. Mix for 30 seconds, either manually or using a shaker.
5. Centrifuge the microplate for 1 minute at 400g (at 1,500 rcf or for a suitable alternative time and force).
6. Briefly agitate the microplate, using a shaker, if necessary.

Record the result and the reaction intensity, testing positive and negative control erythrocytes in parallel. Reading devices, if used, must have been validated. Using additional visual expedients such as, for instance, mirrors or magnifying glasses may facilitate reading the results.

C. SLIDE TECHNIQUE

1. Using whole blood, prepare a 35-45% suspension of test erythrocytes.
2. Place 1 volume of antiserum and one volume of whole blood on a slide.
3. Using a clean applicator stick, thoroughly mix both volumes over an area of about 20x40mm.
4. Slowly move the slide back and forth.
5. Read macroscopically after no more than 2 minutes and record.
6. Incorrect handling or excessive incubation time may lead to drying-induced artefacts, and the test must be considered invalid.

D. SPOT PLATE TECHNIQUE

1. Using whole blood, prepare a 35-45% suspension of test erythrocytes or a 10% suspension of test erythrocytes in a 0.9% NaCl solution.
2. Place 1 volume of antiserum + 1 volume of suspension of test erythrocytes on a spot plate.
3. Using a clean applicator stick, mix both volumes thoroughly.
4. Incubate at RT for 5-10 minutes.
5. Read macroscopically and record.

Incorrect handling or excessive incubation time may lead to drying-induced artefacts.

INTERPRETATION OF TEST RESULTS

1. **Positive:** Agglutination of the test erythrocytes indicates, within accepted limitations of test procedure (see below), the presence of the appropriate AB0 antigen on the test erythrocytes.
2. **Negative:** No agglutination of the test erythrocytes indicates, within accepted limitations of test procedure (see below), the absence of the appropriate AB0 antigen on the test erythrocytes.

Discrepancies: If the results obtained for antigens on the test erythrocytes do not correlate with other antigens testing detected, further investigation is required.

LIMITATIONS

1. Stored blood may give weaker reactions than fresh blood.
2. False positive or false negative results may also occur due to:
   • Contamination of test materials
   • Improper storage, erythrocyte concentration, incubation time or temperature
   • Improper or excessive centrifugation
   • Deviation from the recommended techniques
3. When using the spot plate technique and whole blood specimens, rouleaux formation may occasionally occur that resembles weak agglutination and may be interpreted as a false positive reaction. This phenomenon is due to non-immunological causes. Rouleaux formation can be expected in heparin blood and in patients treated with plasma expanders (e. g. dextran) as well as in patients with plasmacytoma (high protein content, altered protein composition), oncological disorders (abnormal hemogram) and impaired coagulation. Blood specimens from these patients must always be tested using the tube technique, as this phenomenon can generally not be verified when using a suspension of erythrocytes. Blood specimens from patients suffering from certain disorders may show false positive/false negative reactions. Cord blood specimens contaminated with Wharton’s jelly may show false positive reactions
4. **According to the Hemotherapy Guideline, Chapter 4.4.8, 2017, two different test reagents must always be used to determine the Cw antigen.**

STABILITY OF THE REACTIONS

1. Read all tube and microplate tests straight after centrifugation.
2. Slide tests should be interpreted within 2 minutes to ensure specificity and to avoid the possibility a negative result may be incorrectly interpreted as positive due to drying of the reagent.
3. Tests must be considered invalid if they have been performed at temperatures other than those recommended.

SPECIFIC PERFORMANCE CHARAKTERISTICS

The performance evaluation is analogous to the Common Technical Specifications (CTS: Decision of the EU Commission of February 3rd, 2009).

1. Antisera were tested using all recommended methods prior to release.
2. Each LOT monoclonal Anti-Cw is tested according to the requirements of the Common Technical Specifications on In Vitro Diagnostic Medical Devices and meets the requirements.
3. The specificity of monoclonal antibodies is demonstrated using panels of antigen-negative erythrocytes.
4. In the quality control, erythrocytes or whole blood washed twice in 0.9% saline solution are used.
5. Tested on over 500 samples with sensitivity and specificity > 99%

DISCLAIMER

1. The user is responsible for the performance of the reagent by any method other than those mentioned in the Recommended Techniques.
2. Any deviations from the method recommended should be validated prior to use.

BIBLIOGRAPHY

1. Brecher M.E.(2002), ed. Technical manual. 14th ed. Bethesda MD: American association of blodd Banks.
2. Issit P.D. and Antsee D.J. (1998), Applied Blood Group serology, 4th. Edition, Montgomery Scientific Publications, Chapter 12.
3. Daniels G. (1995), Human Blood Groups, Blackwell Science Ltd., Chapter 5
4. Human Blood Groups, by Geoff Daniels, 1st Ed., Blackwell Science, Oxford 1995.
5. HMSO, Guidelines for Blood Transfusion Services., 2nd Ed., 1994.

TABLE OF SYMBOLS

LOT	Lot number	IVD	For *in vitro* diagnostic use only
REF	Catalogue number	2 °C 8 °C	Store between +2°C to +8°C
	Expiry date		Manufacturer
	Consult instructions for use (insert)		

CATALOGUE NUMBERS

REF	Menge
32102 Anti-C^{w} MS-110	1 x 1 x 2 ml 5 x 1 x 2 ml 10 x 1 x 2 ml 50 x 1 x 2 ml
32105 Anti-C^{w} MS-110	1 x 1 x 5 ml 5 x 1 x 5 ml 10 x 1 x 5 ml 50 x 1 x 5 ml