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CE

MediRox

Instructions for Use [EN]

MRX Green vWF Antigen

REF K5052

For *In vitro* Diagnostic Use.

1 Intended use

Latex immunoassay for quantitative determination of von Willebrand Factor (vWF) Antigen in citrated human plasma as an aid in diagnosis of von Willebrand Disease (vWD). Intended to be used by professional laboratory personnel using coagulation analysers with turbidimetric detection in the 500 – 700 nm wavelength range.

2 Background and principle of method

vWF is a multimeric glycoprotein that plays an important role in both primary and secondary hemostasis as it mediates platelet adhesion and aggregation at sites of vascular injury, and stabilises Factor VIII in the circulation protecting it from degradation.[1-3]

vWD is the most common hereditary bleeding disorder caused by either a quantitative (type 1 and type 3) or a qualitative (type 2) defect of vWF.[4, 5] To distinguish between the different types of vWD several different laboratory tests, including vWF Antigen, are needed.[6]

MRX Green vWF Antigen consists of vWF Antigen specific monoclonal antibodies coupled to sub-micron sized polystyrene particles. When the reagent is exposed to a plasma sample containing vWF, the particles will agglutinate, giving rise to increased light-scattering. When exposed to the appropriate wavelength of light, the increase in measured turbidity, or light-scattering, is proportional to the amount of vWF in the sample.

3 Components

MRX Green vWF Antigen consists of:

- Latex Reagent: 3 × 3.5 mL polystyrene particles, coated with monoclonal antibodies, suspended in buffer with stabilisers and preservatives.
- Reaction Buffer: 3 × 3.5 mL containing buffer, Heterophilic Blocking Reagent (HBR), and preservatives.

4 Warnings and precautions

Wear suitable clothing for protection. Avoid contact with skin and eyes. Do not empty into drains. Waste must be disposed of in accordance with local regulations.

The Latex Reagent contains Bovine Serum Albumin. The animals were approved by veterinarians by ante- and post-mortem inspections. However, as no method can offer complete assurance, this material should be handled as potentially infectious.

The Latex Reagent and Reaction Buffer contain sodium azide (less than 0.1%) and 2-methylisothiazol-3(2H)-one (less than 0.0015%) to prevent microbial growth; use proper disposal procedures.

EUH208: Contains 2-methylisothiazol-3(2H)-one. May produce an allergic reaction.

EUH210: Safety data sheet available on request.

5 Preparation

- Latex Reagent: Ready to use. As the microparticles will settle during storage, swirl the vial gently a few times every day before use to ensure a homogenous suspension. Do not shake.
- Reaction Buffer: Ready to use. Swirl the vial gently a few times before use.

6 Storage and stability

- Latex Reagent: Store at 2 - 8 °C. Do not freeze. After opening, stable for 10 weeks at 2 - 8 °C in the closed original vial, provided no contamination occurs. On-board stability: 4 days on Sysmex CS-2100i.
- Reaction Buffer: Store at 2 - 8 °C. Do not freeze. After opening, stable for 10 weeks at 2 - 8 °C in the closed original vial, provided no contamination occurs. On-board stability: 4 days on Sysmex CS-2100i.

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7 Specimen collection and preparation

Venous blood is collected in 3.2% sodium citrate at a ratio of 9 parts blood to 1 part anticoagulant (1:10 ratio). The ratio is critical. Trauma or stasis during blood sampling should be avoided. Inverse immediately after sampling. The presence of any clots in a specimen is a cause for rejection. Centrifuge to produce platelet-poor plasma and use for analysis. Refer to CLSI guideline H21-A5 for further instructions on specimen collection, handling and storage.[7]

8 Procedure

For each instrument, refer to its operator's manual and to the instrument-specific application sheet.

9 Material required but not provided

Coagulation analyser capable of turbidimetric detection in the 500 - 700 nm wavelength range, pipettes and the following:

Calibrator	REF
MRX vWF Antigen Calibrator	K5053

Control material	REF
MRX Specialty Normal Control	K5021
MRX Specialty Abnormal Control	K5022

Solutions	REF
Phosphate buffered saline (PBS) for dilution, e.g. MRX PBS Diluent	K5047
Deionised water for reconstitution e.g. MRX Laboratory Water	K5036

10 Quality control

To maintain consistent assay results, it is recommended that control plasmas are assayed at regular intervals. MRX Specialty Controls (K5021/K5022) are recommended for MRX Green vWF Antigen. Each laboratory should establish a control range to determine the allowable variation in the day-to-day performance of the test, as well as appropriate intervals for analysing controls in accordance with good laboratory practice. Recalibration is suggested, as a minimum, whenever control plasmas are not within the acceptable range and each time a new batch of reagent is used.

11 Results

The results are reported in % vWF Antigen, where 100% corresponds to 1 IU/mL vWF Antigen of the WHO 6th International Standard (NIBSC code 07/316).

Samples that are reported above the measuring range should be manually diluted and re-analysed. No result outside the measuring range should be used in forming a diagnosis or for patient management.

12 Expected values

Normal vWF Antigen levels are typically in the 50 - 200% range.[8] Individuals with blood group O have approximately 25% lower levels of vWF.[9] Other factors such as age, stress, pregnancy, chronic diseases and inflammation can increase vWF levels.[10]

Results from vWF Antigen concentrations determination in plasma samples from 123 healthy blood donors using MRX Green vWF Antigen are presented below. The analysis was performed using a Sysmex CS-2500 instrument.

No of samples	2.5th – 97.5th percentile
123	54 - 164% vWF-Ag

Due to inter-laboratory variation, each laboratory should set its own reference interval.

13 Limitations and interfering substances

MRX Green vWF Antigen is a latex immunoassay and, as for all antibody-based assays, there is a risk that certain rare mutations might affect the result. Be aware of this risk and pay special attention to unexpected results. The results should be used together with other clinical and diagnostic information in forming a diagnosis and for patient management. The recommendation from the International Society of Thrombosis and Haemostasis (ISTH) is to analyse vWF antigen in conjunction with platelet-dependent vWF activity and factor VIII coagulant activity in blood testing for vWD.[11]

Turbid or opalescent plasma may cause erratic results and should be interpreted with caution: dilute the sample and re-assay. MRX Green vWF Antigen is insensitive to the following substances on Sysmex CS-instrument series:

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Interfering substance	Tolerance
Conjugated Bilirubin	Up to 40 mg/dL
Unconjugated Bilirubin	Up to 40 mg/dL
Haemoglobin	Up to 1000 mg/dL
Triglycerides	Up to 900 mg/dL
Unfractionated heparin	Up to 100 U/dL
Low molecular weight heparin	Up to 330 U/dL

Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain anti-mouse antibodies (HAMA), which may cause over-estimation of vWF Antigen concentrations. The presence of rheumatoid factor may also result in falsely elevated vWF Antigen concentrations. The reaction buffer includes HBR that reduces unspecific reactions, but users should be aware that there still is a possibility of over-estimated vWF Antigen levels for samples with HAMA or rheumatoid factor.

14 Analytical performance characteristics

The following performance data was obtained with a Sysmex CS-2500 instrument, whereas reproducibility was tested on both Sysmex CS-2100i and CS-2500. Performance will depend on the instrument used.

MRX Green vWF Antigen has a measuring range of 5 - 180% vWF Antigen. There is no prozone effect below 600% vWF Antigen.

Method comparison performed using Passing-Bablok:

Predicate Device (x)	System (y)	Regression	R
Siemens vWF Ag	MRX Green vWF Antigen	y = 1.00x + 2.68	0.97

Precision:

Sample	Repeatability		Reproducibility	
	% vWF-Ag	CV %	% vWF-Ag	CV %
Level 1	18	1.7	18	3.0
Level 2	38	1.7	38	3.9
Level 3	102	1.6	99	4.4

15 Reporting of incidents

Any serious incidents that occur in relation to this device shall be reported to Nordic Biomarker as well as the national competent authority in which the user is established.

16 Additional information

A paper copy of these Instructions for Use is available on request. Contact your local distributor.
The instrument-specific application sheet is available from your local distributor.

17 References

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4. LEEBEEK, Frank WG; EIKENBOOM, Jeroen CJ. Von Willebrand's disease. *New England Journal of Medicine*, 2016, 375.21: 2067-2080.
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6. DE JONG, Annika; EIKENBOOM, Jeroen. Developments in the diagnostic procedures for von Willebrand disease. *Journal of Thrombosis and Haemostasis*, 2016, 14.3: 449-460.
7. CLSI. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline – Fifth Edition. CLSI document H21-A5. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
8. NG, Christopher; MOTTO, David G.; DI PAOLA, Jorge. Diagnostic approach to von Willebrand disease. *Blood, The Journal of the American Society of Hematology*, 2015, 125.13: 2029-2037.
9. WARD, Soracha E.; O'SULLIVAN, Jamie M.; O'DONNELL, James S. The relationship between ABO blood group, von Willebrand factor, and primary hemostasis. *Blood*, 2020, 136.25: 2864-2874.
10. HOFFMAN, Ronald, et al. *Hematology: basic principles and practice*. Elsevier Health Sciences, 2013.
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18 Definition of symbols

	Manufacturer	 nordicbiomarker.com/IFU	Consult electronic instructions for use
	CE mark		Use-by date
	In vitro diagnostic medical device		Temperature limit
	Catalogue number		Biological risks
	Batch code		Contains biological material of animal origin

19 Revision history

Version	Changes to previous version
3.0	Section 13, Limitations and interfering substances: Addition of reference to ISTH guidelines on diagnosis of vWD.