INNOVANCE® VWF Ac

Revision bar indicates update to previous version.

CE 0197

Intended Use

INNOVANCE® VWF Ac is an in vitro diagnostic reagent for the quantitative, WHO-standardized determination of von Willebrand factor (VWF) GPIb-binding activity as aid to diagnosis and monitoring of congenital or acquired VWF deficiencies in patients with bleeding disorders or at risk for VWF deficiency in human sodium citrated plasma by means of automated turbidimetric methods.

Summary and Explanation

Von Willebrand Disease (VWD) is the most frequent congenital human bleeding disorder and is caused by a reduction or dysfunction of von Willebrand factor (VWF). VWF is a multimeric, high-molecular glycoprotein involved in primary hemostasis, supporting platelet adhesion and aggregation via binding to the platelet glycoprotein Ib (GPIb) receptor under shear stress at the site of injury. Furthermore, VWF is the specific carrier protein of coagulation factor VIII (FVIII), protecting FVIII against inactivation and rapid clearance.

VWD is an autosomal inherited disorder of three different types: type 1 (partial quantitative deficiency of VWF), type 2 (qualitative VWF defects) and type 3 (almost complete deficiency of VWF). VWD type 2 can be further sub-typed into 2A, 2B, and 2M based on their different multimeric pattern and/or altered platelet affinity. Type 2N is characterized by a reduced FVIII-binding activity with otherwise normal functionality. These variants are differentiated on the basis of a series of laboratory tests for VWF activity (e.g. GPIb-binding or ristocetin cofactor assay, collagen binding assay), VWF antigen, platelet function testing (PFA Systems), FVIII activity, platelet count, and VWF multimer analysis[1–9].

Similar clinical and laboratory findings as with congenital VWD may be seen in acquired VWS, a rare but probably underestimated bleeding disorder[10,11]. In patients with aortic stenosis the high molecular multimers are reduced to shear stress, causing an acquired type 2A VWF deficiency, which predisposes patients to bleeding. Such defects also develop with left ventricular assist devices, valve dysfunction, cardiomyopathy or congenital heart defects[12].

The determination of VWF activity in plasma is indicated in the following cases:

- clarifying the cause of a prolonged APTT,
- diagnosing congenital or acquired VWF deficiency states,
- monitoring VWF in patients under therapy (e.g. Desmopressin or VWF concentrates),
- distinguishing between functional defects and quantitative deficiencies (in conjunction with immunochemical methods).

On the other hand, elevated levels of VWF have been reported in response to stress, inflammation and endothelial lesions, and have been related to thrombotic complications such as venous thromboembolism and myocardial infarction[13,14]. Such increases of VWF levels may obscure the laboratory diagnosis of mild VWD.

Principles of the Procedure

The assay principle makes use of the binding of VWF to its receptor Glycoprotein Ib (GPIb). GPIb is the main VWF receptor on platelets. Polystyrene particles are coated with an antibody against GPIb. Recombinant GPIb (two gain-of-function mutations included) is added and binds to the

antibody as well as to the VWF of the sample. Due to the gain-of-function mutations, VWF binding to GPIb does not require ristocetin (VWF:GPIbM)[4]. This VWF binding induces a particle agglutination which can be measured as an increase in extinction by turbidimetric measurements.

Reagents

Note: INNOVANCE® VWF Ac can be used on automated coagulation analyzers. Sysmex provides Reference Guides (Application Sheets) for several coagulation analyzers. The Reference Guides (Application Sheets) contain analyzer/assay specific handling and performance information which may differ from that provided in these Instructions for Use. In this case, the information contained in the Reference Guides (Application Sheets) supersedes the information in these Instructions for Use. Please also consult the instruction manual of the instrument manufacturer!

Reagent	Description	Storage	Stability
INNOVANCE® VWF Ac			
REAGENT I	Ready to use liquid containing: • sucrose • polystyrene particles coated with anti-GPIb monoclonal antibodies, mouse (2.2 g/L) • Amphotericin B • Gentamicin sulfate • Buffer	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	2–8 °C: once opened, 4 weeks[a]; <–20 °C: once opened, 6 months[a,b] Do not refreeze!
REAGENT II	Ready to use liquid containing: • saline buffer • heterophilic blocking reagent • polyvinylpyrrolidone • Detergent • Preservative: • Sodium azide (<1 g/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	2–8 °C: once opened, 4 weeks[a]; <–20 °C: once opened, 6 months[a,b] Do not refreeze!
REAGENT III	Ready to use liquid containing: • saline buffer • recombinant GPIb (<80 mg/L) • Amphotericin B • Gentamicin sulfate	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	2–8 °C: once opened, 12 weeks[a]; <–20 °C: once opened, 6 months[a,b] Do not refreeze!

[a] closed vial

[b] once-frozen reagents at 2 to 8 °C: 2 weeks

On-board stability

Information regarding on-board stability is specified in the Reference Guides (Application Sheets) for the different coagulation analyzers.

Warnings and Precautions

For *in-vitro* diagnostic use only.

For laboratory professional use.

According to EU regulation 2017/746, any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State through your local distribution representative in which the user and/or patient is established.

Safety data sheets (MSDS/SDS) available upon request.

CAUTION! POTENTIAL BIOHAZARD
INNOVANCE VWF Ac REAGENT I, INNOVANCE VWF Ac REAGENT III
Each donor or donor unit was tested and found to be negative for human immunodeficiency virus (HIV) 1 and 2, hepatitis B virus (HBV) and hepatitis C virus (HCV) using either tests that are CE marked or FDA approved for this purpose. Because no known test can offer complete assurance of the absence of infectious agents, all human derived products should be handled with appropriate caution.

Caution
INNOVANCE VWF Ac REAGENT II
This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

Contains sodium azide as a preservative. Sodium azide can react with copper or lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides. Disposal into drain systems must be in compliance with prevailing regulatory requirements.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with all government requirements.

Summary of Safety and Performance (SSP) is available in the European database on medical devices (see Eudamed public website: https://ec.europa.eu/tools/eudamed). In case Eudamed is not available, SSP can be delivered by the manufacturer on request.

Preparing Reagents

The INNOVANCE® VWF Ac test kit components are liquid. To ensure homogeneity, gently swirl the reagents shortly before use – **do not shake!** Avoid foam formation.

Specimen Collection and Handling

Specimen type: Platelet poor citrated human plasma.

Collecting the Specimen

To obtain plasma, carefully mix 1 part sodium citrate solution (0.11 mol/L; 3.2 %) with 9 parts venous blood, avoiding the formation of foam. An evacuated tube system or syringe may be used. Centrifuge the blood tube as soon as possible for no less than 15 minutes at ≥1 500 × g. Plasma can be stored on the cells or it can be removed from the cellular component and stored in unopened tube at room temperature.

Please refer to CLSI guideline H21-A5[15] for further details. The manufacturer's instructions for the sampling equipment must also be observed.

Preparation of Frozen Samples

- Freeze plasma within 8 hours of blood collection at ≤ −20 °C.
- Thaw frozen plasma within 10 minutes at 37 °C, homogenize by carefully mixing without foam formation. Then carry out the VWF Ac determination within 24 hours. Do not use re-freezed samples.

Storing the Specimen

Stability of the samples:

15 to 25 °C	24 hours (fresh and once frozen)
≤ −20 °C	12 months

Please refer to CLSI guideline H21-A5 for details[15].

Procedure

Materials Provided

REF	Contents		
OPHL03	INNOVANCE® VWF Ac		
	INNOVANCE® VWF Ac reagent I INNOVANCE VWF Ac REAGENT I	3 ×	2.0 mL
	INNOVANCE® VWF Ac reagent II INNOVANCE VWF Ac REAGENT II	3 ×	3.5 mL
	INNOVANCE® VWF Ac reagent III INNOVANCE VWF Ac REAGENT III	1 ×	2.5 mL

Materials Required but not Provided

Refer also to the Reference Guide (Application Sheets).

Item	Description
REF ORKE41	CONTROL N, Control Plasma N
REF OUPZ17	CONTROL P, Control Plasma P
REF B4234-25	OV BUFFER, Dade® Owren's Veronal Buffer
REF B4265-37	CA SYSTEM BUFFER, Dade® CA System Buffer
REF ORKL17	STANDARD PLASMA, Standard Human Plasma
Coagulation analyzers[c], such as:	• Automated Blood Coagulation Analyzer CA-600 series (CA-600 series) • AUTOMATED BLOOD COAGULATION ANALYZER CS-2500 (CS-2500 System) • AUTOMATED BLOOD COAGULATION ANALYZER CS-5100 (CS-5100 System)

[c] Availability of analyzers may vary by country.

Please note that the applications on other analyzers can be validated by the instrument manufacturer in accordance with the requirements of the REGULATION (EU) 2017/746 under their responsibility as long as the intended purpose and performance are not modified.

Test Procedure

Evaluation
Analysis takes place automatically in the coagulation analyzer.

Performing Calibration

A standard curve is generated by automatic determination of different dilutions of STANDARD PLASMA and OV BUFFER. The standard curve must be re-generated if there is a change in the instrument or in the lot of INNOVANCE® VWF Ac used, or if control results are out of the acceptance range.

Internal Quality Control

Normal range: CONTROL N
Pathological range: CONTROL P

If it is necessary to use a control in the lower measuring range, reconstituted CONTROL P can be diluted 1:3 with OV BUFFER. The expected value is then equal to 1/3 of the indicated value as stated in the lot-specific Table of Assigned Values for CONTROL P.

Two levels of quality control material (normal and pathological range) have to be measured at start of the test run, with each calibration, upon reagent vial changes and at least every eight hours on each day of testing. Each laboratory should determine its own quality control range, either by means of the target values and ranges provided by the manufacturer of the controls or by means of control values determined in the laboratory. If the measured control value lies outside of the pre-determined range, then the reagents, the standard curve and the coagulation analyzer should be checked. Do not report patient results until the problem has been identified, corrected and documented.

Results

The INNOVANCE® VWF Ac assay is automatically carried out by coagulation analyzers. Assay calibration is performed with STANDARD PLASMA which is calibrated against the VWF:GPIbM value of the International Standard for Blood Coagulation FVIII and Von Willebrand Factor in Plasma. The results are reported as % of Norm.

100 % of Norm = 1 IU/mL.

Limitations

The potential interference by bilirubin, hemoglobin and lipids are described in the analyzer specific Reference Guides (Application Sheets).

Turbidity and particles in the samples may interfere with the assay. Therefore, samples containing particles must be centrifuged prior to testing. Lipemic or turbid samples which cannot be clarified by centrifugation (10 minutes at approximately 15000 × g) must be excluded from the assay.

Patient samples may contain heterophilic antibodies (e.g. human anti-mouse antibodies (HAMA) and rheumatoid factors) or paraproteins that could react in turbidimetric assays using mouse monoclonal antibodies to give falsely elevated or depressed results[16,17]. This assay has been designed to minimize interference from heterophilic antibodies by addition of a blocking reagent. No interference of rheumatoid factors (RF) was observed up to 940 IU/mL. Nevertheless, complete elimination of this interference from all patient specimens cannot be guaranteed.

The manufacturer has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. Please note that the applications on other analyzers can be validated by the instrument manufacturer in accordance with the requirements of the REGULATION (EU) 2017/746 under their responsibility as long as the intended purpose and performance are not modified. User defined modifications are not supported by the manufacturer as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in Application Sheets or these Instructions for Use.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

Expected Values

Fresh plasma specimens obtained from apparently healthy donors were tested using the INNOVANCE® VWF Ac assay on the BCS®/BCS® XP System with the following results:

BCS®/BCS® XP	n	2.5th–97th percentile [% of Norm]
blood group 0	129	46.3–145.6
blood group non-0	134	61.4–179.1
blood group independent	263	47.8–173.2

The blood group independent reference range is calculated from pooled sample results (49 % blood group 0 and 51 % blood group non-0). Other system specific results are given in the respective Reference Guides (Application Sheets). No statistically significant difference was observed between fresh and frozen samples.

Reference intervals vary from laboratory to laboratory depending on the population served and the technique, method, equipment and reagent lot used. Therefore, each laboratory must establish its own reference intervals or verify them whenever one or more of the aforementioned variables are changed.

Performance Characteristics

Measuring Range

The measuring range depends on the individual application of the assay due to instrument related conditions. Application specific performance data is listed in the respective Reference Guides of the instruments.

Calibrated range on BCS®/BCS® XP System: 4 to 150 % of Norm. Samples with unknown VWF level should first be measured by using the test protocol covering the range between 15 and 150 % VWF. If the result is below 15 %, the instrument will automatically measure the sample again by using the test protocol for the low range. Samples initially above the calibration range are diluted by the instrument resulting in a measuring range up to 600 % of Norm.

Precision

Precision studies were conducted with the BCS®/BCS® XP System, as described in the CLSI Guideline EP5-A2 (20 days, 80 aliquots), using CONTROL N and CONTROL P as well as three pathological plasma pools.

Other system specific results are given in the respective Reference Guides (Application Sheets).

Sample	Mean [% of Norm]	Repeatability SD [% VWF]	Repeatability CV [%]	Within-Device/Lab Precision SD [% VWF]	Within-Device/Lab Precision CV [%]
CONTROL N	89.0	1.5	1.7	2.6	2.9
CONTROL P	31.2	0.7	2.2	0.7	2.4
Pathological plasma pool 1	21.8	0.7	3.2	0.7	3.2
Pathological plasma pool 2	11.2	0.1	1.1	0.2	1.8
Pathological plasma pool 3	563.0	14.8	2.6	27.4	4.9

The reproducibility was assessed by the manufacturer for vWF activity with INNOVANCE® VWF Ac based on proficiency testing information in 2019/2020. The overall reproducibility median CV% was found to be <10 % including lot, instrument, laboratory and operator variability factors.

Limit of Detection

The Limit of Detection (LoD) for the INNOVANCE® VWF Ac method on a BCS®/BCS® XP analyzer is 2.2 %, determined consistent with CLSI guideline EP17-A and with false positives (alpha) and false negatives (beta) less than 5 %, based upon 240 determinations with 120 blank and 120 low level samples. The Limit of Blank (LoB) is 1.1 %. LoD is the lowest concentration that can be detected reliably. LoB is the highest concentration that is likely to be observed for a blank sample.

Antigen Excess

The INNOVANCE® VWF Ac application on the BCS®/BCS® XP shows no high dose hook effect up to 900 % VWF.

Method Comparison

In a comparison of INNOVANCE® VWF Ac with BC VWF REAGENT using a BCS®/BCS® XP System, 352 samples were determined in the range from 4 to 600 % of Norm. The coefficient of correlation was 0.99 (y-axis intercept: 2.1 % of Norm; slope 0.94). Other system specific results are given in the respective Reference Guides (Application Sheets).

Technical Assistance

For customer support, contact your local technical support provider or distributor.

Current Version of Application Sheets

INNOVANCE® VWF Ac can be used in combination with various automated coagulation analyzers. Sysmex provides Reference Guides/Application Sheets for the coagulation analyzers listed in section "Materials Required but not Provided", page 4 under the dedicated link below:

sysmex-ifu.com/ag

As the manufacturer continuously monitors the product performance and safety, the users are required to ensure that they work with the correct revision of the instructions for the product lots in use. Please periodically review the availability of new electronic labeling revisions to ensure safe use of the product.

The IFU version number is visible on each product box label. Sysmex ensures that all products lots bearing the same IFU version number are compatible with the electronic labeling provided via sysmex-ifu.com.

References

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16. CLSI. Immunoassay interference by endogenous antibodies; Approved Guideline. CLSI document **I/LA30-A** [ISBN]. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA; 2008.
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Definition of Symbols

The following symbols may appear on the product labeling:

	Do not reuse		Use By
LOT	Batch Code	REF	Catalogue Number
	Caution		Manufacturer
EC REP	Authorized representative in the European Community	CH REP	Authorized representative in Switzerland
	Contains sufficient for <n> tests		Biological Risks
IVD	*In Vitro* Diagnostic Medical Device		Temperature Limitation
	Consult instruction for Use		Non-sterile
CE	CE marking of conformity	CE 0197	CE marking of conformity with notified body ID number. Notified body ID number can vary.
CONTENTS	Contents	→	Reconstitution volume
LEVEL	Level		Keep away from sunlight and heat
WARNING	Warning	DANGER	Danger
RxOnly	Prescription device (US only)	UDI	Device Identification (UDI) barcode
REACH xx/xx/xx	REACH Authorization Number		

Legal Information

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