# von Willebrand Factor Activity - 0020004700

**ENGLISH - Insert revision 01/2005**

CE

## Intended use

Automated latex enhanced immunoassay for the quantitative determination of von Willebrand Factor Activity (VWF Activity) in human citrated plasma on IL Coagulation Systems.

## Summary and principle

The diagnosis of von Willebrand Disease (VWD), probably the most common congenital bleeding disorder, requires a number of special tests at the laboratory level. The measurement and comparison of von Willebrand Factor Antigen (VWF:Ag), VWF Activity and Factor VIII (FVIII) levels in plasma aid in the differentiation of quantitative defects (type 1 or type 3) or qualitative defect (type 2) of VWF and therefore to diagnose the different types of VWD.[1-2]
When an extremely low or undetectable level of VWF:Ag is obtained, a type 3 VWD could be expected. If a moderate or even normal result is obtained, VWF Activity and FVIII assays must be performed and compared with the VWF:Ag result. If all three values are within the normal range, VWD and Hemophilia A may be excluded. If at least one parameter is abnormally low, it is necessary to calculate the ratios VWF Activity/VWF:Ag and FVIII/VWF:Ag. If both ratios are close to 1 (some authors suggest 0.7 as cut-off), a VWD type 1 may be diagnosed.[1-2]
When the VWF/Activity/VWF:Ag ratio is low (0.7 is also the suggested cut-off), types 2A, 2B or 2M may be diagnosed. These subtypes are characterized by its abnormal multimeric pattern and/or its altered platelet affinity.[1-2] Additional laboratory tests as RIPA (Ristocetin Induced Platelet Aggregation), multimeric analysis and binding assays are required in order to be able to distinguish the different subtypes.[2]
When the FVIII/VWF:Ag ratio is low (0.7 is also the suggested cut-off), a type 2N or Hemophilia A may be diagnosed and a FVIII binding assay is necessary to discriminate among them.
The VWF Activity kit is a latex particle enhanced immunoturbidimetric assay to quantify VWF Activity in plasma. The activity of VWF is determined by measuring the increase of turbidity produced by the agglutination of the latex reagent. A specific anti-VWF monoclonal antibody adsorbed onto the latex reagent, directed against the platelet binding site of VWF (Glycoprotein Ib receptor), reacts with the VWF of patient plasma. The degree of agglutination is directly proportional to the activity of VWF in the sample and is determined by measuring the decrease of transmitted light caused by the aggregates.

## Composition

The **von Willebrand Factor Activity** kit consists of:

R **Latex Reagent** (Cat. No. 0020004710): 2 vials x 4.5 mL of a lyophilized suspension of polystyrene latex particles coated with purified anti-VWF mouse monoclonal antibody directed against a functional epitope of VWF, containing bovine serum albumin, stabilizers and preservative.

B **Buffer** (Cat. No. 0020004720): 2 vials x 4.5 mL of Tris buffer containing bovine serum albumin, stabilizers and preservative.

**PRECAUTIONS AND WARNINGS:**
Avoid contact with skin and eyes (S 24/25). Do not empty into drains (S 29). Wear suitable protective clothing (S 36).
The buffer contains less than 0.1% sodium azide that may form explosive azides in metal plumbing. Use proper disposal procedures.

This product is For *in vitro* Diagnostic Use.

## Preparation

**Buffer:** The reagent is ready for use.
**Latex Reagent**: Dissolve the contents of each vial by pouring the entire contents of one vial of Buffer into one vial of Latex Reagent. Replace the stopper and swirl gently for a minimum of 20 seconds to completely dissolve the lyophilized latex. Make sure of the complete reconstitution of the product. It must appear as a homogenous and slightly milky suspension. Keep the reagent at 15-25°C for 30 minutes and invert to mix before use. Do not shake.

Note: Avoid foam formation when homogenizing reconstituted reagents. Bubbles on top of the liquids may interfere with the instruments liquid sensors.

## Reagent storage and stability

Unopened reagents are stable until the expiration date shown on the vial when stored at 2-8°C.
**Latex Reagent -** Stability after reconstitution: 1 month at 2-8 °C in the original vial, 3 days at 15°C on the ACL® 8000/9000/10000, 1 day at 15°C on the ACL Futura®/ACL Advance Systems or 5 days at 15°C on the ACL TOP™.
Do not freeze.

For optimal stability remove reagents from the system and store them at 2-8°C in the original vial.

## Instrument/test procedures

Refer to the appropriate IL instrument's Operator's Manual and/or Application Manual for the complete assay procedure instructions.

## Specimen collection and preparation

Nine parts of freshly drawn venous blood are collected into one part of trisodium citrate. Refer to NCCLS Document H21-A4 for further instructions on specimen collection, handling and storage.[3]
Frozen plasma samples should be rapidly thawed at 37°C while gently mixing before testing. After thawing the assay must be performed within 2 hours.

## Additional reagents and control plasmas

The following are not supplied with the kit and must be purchased separately.

| | Americas and Pacific Rim Cat. No. | Europe Cat. No. |
|---|---|---|
| Calibration Plasma | 0020000000 | 0008467300 |
| Normal Control | 0020003120 | 0020003110 |
| Special Test Control Level 1 | 0020010100 | 0020010100 |
| Special Test Control Level 2 | 0020010200 | 0020010200 |
| Factor diluent | 0009757600 | 0009757600 |

## Quality control

Normal and abnormal controls are recommended for a complete quality control program.[4] Normal Control and Special Test Control Level 1 and 2 are designed for this program. Each laboratory should establish its own mean and standard deviation and should establish a quality control program to monitor laboratory testing. Controls should be analyzed at least once every 8 hour shift in accordance with good laboratory practice. Refer to the instrument's Operator's Manual for additional information. Refer to Westgard *et al* for identification and resolution of out-of-control situtations.[5]

## Results

VWF Activity results are reported in % normality. Refer to the instrument's Operator's Manual for additional information. The assay results should be used with other information, including the clinical context, in forming a diagnosis.

## Limitations/interfering substances

VWF Activity results on ACL 8000/9000/10000 are not affected by bilirubin up to 3.8 mg/dL and lipids up to 265 mg/dL.
VWF Activity results on ACL Futura/ACL Advance Systems are not affected by bilirubin up to 3.6 mg/dL and lipids up to 929 mg/dL.
The presence of rheumatoid factor may produce an overestimation of the test result. Hemolyzed and turbid samples should not be assayed.
VWF Activity results on ACL TOP are not affected by hemoglobin up to 70 mg/dL, bilirubin up to 4.2 mg/dL, triglycerides up to 1020 mg/dL and Rheumatoid Factor up to 200 IU/mL.

## Expected values

A normal range study was performed using the VWF Activity kit.

| Blood ABO Type[6] | N | ACL 8000/9000/10000 | N | ACL Futura/ACL Advance |
|---|---|---|---|---|
| O | 122 | 38.0 - 125.2 (% VWF Activity) | 120 | 40.8 - 138.8 (% VWF Activity) |
| A + B + AB | 126 | 49.2 - 169.7 (% VWF Activity) | 123 | 58.1 - 175.5 (% VWF Activity) |

In a separate study using 266 individual blood bank donor samples, the following results were obtained:

| Blood ABO Type[6] | N | ACL TOP |
|---|---|---|
| O | 132 | 40.3 - 125.9 (% VWF Activity) |
| A + B + AB | 134 | 48.8 - 163.4 (% VWF Activity) |

Note: VWF is an acute-phase reactant and stress, pregnancy and other situations affect its plasma concentration and therefore it might be advisable to repeat the analysis from another blood sample obtained on a different day.[1]

Ranges were calculated as recommended by the International Federation of Clinical Chemistry (IFCC).[7] These results were obtained using a specific lot of reagent. Due to many variables, which may affect results, each laboratory should establish its own VWF Activity normal range.

## Performance characteristics

**Precision:**
Within run and total (run to run and day to day) precision was assessed over multiple runs.

| ACL 8000/9000/10000 | Mean (% VWF Activity) | CV% (Within run) | CV% (Total) |
|---|---|---|---|
| Normal Control | 79.6 | 2.7 | 4.9 |
| Special Test Control Level 1 | 49.8 | 4.6 | 9.0 |
| Special Test Control Level 2 | 27.7 | 7.5 | 8.7 |
| **ACL Futura/ACL Advance** | **Mean (% VWF Activity)** | **CV% (Within run)** | **CV% (Total)** |
| Normal Control | 92.1 | 4.1 | 5.6 |
| Special Test Control Level 1 | 64.1 | 4.8 | 6.5 |
| Special Test Control Level 2 | 36.6 | 6.5 | 8.3 |
| **ACL TOP** | **Mean (% VWF Activity)** | **CV% (Within run)** | **CV% (Total)** |
| Normal Control | 102.4 | 2.6 | 3.4 |
| Special Test Control Level 1 | 68.4 | 3.5 | 6.3 |
| Special Test Control Level 2 | 30.3 | 6.5 | 8.0 |

**Correlation:**

| System | Slope | Intercept | r | Comparative method |
|---|---|---|---|---|
| ACL 8000/9000/10000 | 0.832 | 4.782 | 0.972 | VWF Activity EIA |
| ACL Futura/ACL Advance | 0.954 | 3.854 | 0.958 | VWF Activity EIA |
| ACL TOP | 0.988 | 2.288 | 0.995 | VWF Activity on ACL Advance |

An additional clinical study (n=114), comparing this VWF Activity kit on the ACL 9000 to a Ristocetin cofactor activity (VWF:RCo) assay on an automated optical coagulometer, resulted in an r of 0.950 and a slope of 0.841.

The precision and correlation results were obtained using specific lots of reagents and controls.

**Detection limit:**

| System | |
|---|---|
| ACL 8000/9000/10000 | 12 (% VWF Activity) |
| ACL Futura/ACL Advance | 1.3 (% VWF Activity) |
| ACL TOP | 3.2 (% VWF Activity) |

**Linearity:**

| System | Regular test (% VWF Activity) | Rerun test (% VWF Activity) |
|---|---|---|
| ACL 8000/9000/10000 | 21 - 200 | - |
| ACL Futura/ACL Advance | 19 - 120 | 40 - 240 |
| ACL TOP | 19 - 130 | 57 - 390 |

If the linear range (Rerun test) is exceeded, then samples should be manually diluted 1:4 with Factor diluent (100 µL of sample + 300 µL of Factor diluent) on the ACL 8000/9000/10000 and ACL Futura/ACL Advance or 1:10 with Factor diluent (50 µL of sample + 450 µL of Factor diluent) on the ACL TOP and reassayed in the standard test. The printed results must be multiplied by 4 or 10 to correct for the dilution.

The assay does not show prozone effect (i.e. antigen excess) up to 700% on ACL 8000/9000/10000, up to 350% on ACL Futura/ACL Advance and up to 1000% on the ACL TOP.

Instrumentation Laboratory

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## Bibliography / Literatur / Bibliografía / Bibliographie / Bibliografia /Bibliografia / Litteratur / Litteraturförteckning / Βιβλιογραφία


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## Symbols used / Verwendete Symbole / Símbolos utilizados / Symboles utilisés / Simboli impiegati / Símbolos utilizados / Anvendte symboler / Använda Symboler / Χρησιμοποιηθέντα σύμβολα

| IVD | LOT | | | | CONTROL | | | |
|---|---|---|---|---|---|---|---|---|
| *In vitro* diagnostic medical device | Batch code | Use by | Temperature limitation | Consult instructions for use | Control | Biological risks | Manufacturer | Authorised representative |
| *In-vitro* Diagnostikum | Chargen-Bezeichnung | Verwendbar bis | Festgelegte Temperatur | Beilage beachten | Kontrollen | Biologisches Risiko | Hergestellt von | Bevollmächtigter |
| De uso diagnóstico *in vitro* | Identificación número de lote | Caducidad | Temperatura de Almacenamiento | Consultar la metódica | Control | Riesgo biológico | Fabricado por | Representante autorizado |
| Dispositif mèdical de diagnostic *in vitro* | Désignation du lot | Utilisable jusqu'à | Températures limites de conservation | Lire le mode d'emploi | Contrôle | Risque biologique | Fabricant | Mandataire |
| Per uso diagnostico *in vitro* | Numero del lotto | Da utilizzare prima del | Limiti di temperatura | Vedere istruzioni per l'uso | Controllo | Rischio biologico | Prodotto da | Rappresentanza autorizzata |
| Dispositivo médico para utilização em diagnóstico *in vitro* | Número de lote | Data límite de utilização | Límite de temperatura | Consultar as instruções de utilização | Controlo | Risco biológico | Fabricado por | Representante autorizado |
| "in vitro" diagnostisk udstyr | Batch nr. | Anvendelse | Temperatur begrænsninger | Se vejledning for anvendelse | Kontrol | Miljø oplysninger | Producent | Leverandør |
| *In vitro* diagnostisk medicinsk produkt | Tillverkningskod | Användning | Temperatur gräns | Ta del av instruktionen före användning | Kontroll | Biologiska risker | Tillverkare | Auktoriserad representant |
| Προϊόν για διαγνωστική χρήση *In vitro* | Αρ. Παρτίδας | Χρήση έως | Περιορισμοί θερμοκρασίας | Συμβουλευτήτε τις οδηγίες χρήσης | Υλικό ποιοτικού ελέγχου | Βιολογικοί κίνδυνοι | Κατασκευαστής | Εξουσιοδοτημένος αντιπρόσωπος |