LIAPHEN™ vWF: Ag

REF 120206

R1 4 x 5 mL, R2 4 x 6 mL

IVD

Immuno-turbidimetric method for vWF: Ag,
with ready to use liquid reagents.

HYPHEN BioMed

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English, last revision: 04-2017

INTENDED USE:

LIAPHEN™ vWF: Ag kit is an immunoturbidimetric assay for *in vitro* quantitative determination of von Willebrand Factor Antigen (vWF: Ag) on human citrated plasma, using a manual or automated method. Reagents are in the liquid presentation, ready to use.

SUMMARY AND EXPLANATION:

vWF is a multimeric protein produced in endothelial cells and megakaryocytes. It circulates in blood as multimers ranging from 500 to more than 20,000 kDa.
vWF mediates platelet adhesion to subendothelium of the damaged blood vessel and serves as a carrier for Factor VIII, extending its half-life into the bloodstream.
Ultra-large multimers are proteolytically cleaved by ADAMTS13 into smaller and less active vWF forms. The biological function of vWF depends largely on the size of its multimers. Larger multimers are more likely to bind to platelets and collagen, and to promote platelet adhesion in circulating blood[1,2].

vWF functional or quantitative deficiency leads to von Willebrand disease (vWD), which can be divided into 3 groups:

- Type 1: vWD is characterized by a partial quantitative deficit of vWF (most frequently).
- Type 2: vWD is characterized by an abnormal vWF adhesion activity. It is divided into 4 subtypes: 2A, 2B, 2M and 2N, depending on the multimers functional abnormality.
- Type 3: vWD is characterized by a severe quantitative deficit of vWF.

vWF deficiencies can be associated to different other pathologies, thus constituting an acquired von Willebrand disease.
When vascular endothelium is affected, the vWF concentration can be increased in relation to inflammatory processes [3,4].

PRINCIPLE:

LIAPHEN™ vWF:Ag is an immunoturbidimetric method, based on antigen-antibody reaction: vWF antigen of the sample reacts with Latex particles sensitized with rabbit anti-vWF polyclonal antibodies, leading to latex particles agglutination. This agglutination can be directly detected by a change of absorbance. The absorbance change is directly proportional to the amount of vWF:Ag in the sample.

REAGENTS:

R1 **Reagent 1:** Reaction Buffer, liquid form. Contains BSA.
4 vials of 5 mL.
R2 **Reagent 2:** Latex, liquid form. Contains BSA.
4 vials of 6 mL.

Reagents contain small amounts of sodium azide (0.9 g/L), see WARNINGS AND PRECAUTIONS.

WARNINGS AND PRECAUTIONS:

- Biological products must be handled with all necessary precautions and considered as being potentially infectious.
- In contact with lead or copper pipes, sodium azide can generate explosive compounds.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits. Do not mix reagents from different kit batches when performing an assay; they are optimized for each batch of kits.
- Handle the reagents with care to avoid contamination during use. If possible, avoid reagent evaporation during use by limiting the liquid-air exchange surface. Evaporation reduces the reagent's stability in the analyzer.
- To preserve reagent stability, seal the vials after use with their respective caps.
- Aging studies, conducted over a 3-weeks period at 30°C, show that the reagents can be shipped at room temperature over a short period of time, without degradation.
- The bovine plasma used to prepare the BSA has been tested by recorded methods and is certified free of infectious agents, in particular the causative agent of bovine spongiform encephalitis.
- For *in vitro* diagnostic use.

REAGENT PREPARATION AND STABILITY:

R1 **Reagent 1**: **Reaction Buffer**
Clear vial. Ready to use. Allow to stabilize for 30 minutes at room temperature (18-25°C), before use.
Homogenize the reagent prior to use.
Reagent stability, excluding any contamination or evaporation, and stored in the original vial, is of:

- **4 weeks** at 2-8°C.
- **2 weeks** at room temperature (18-25°C).
- **Do not freeze.**

R2 **Reagent 2**: **Latex**
Clear vial. Ready to use. Allow to stabilize for 30 minutes at room temperature (18-25°C), before use.
Homogenize the reagent prior to use.
Reagent stability, excluding any contamination or evaporation, and stored in the original vial, is of:

- **4 weeks** at 2-8°C.
- **2 weeks** at room temperature (18-25°C).
- **Do not freeze.**

STORAGE CONDITIONS:

Unopened reagents should be stored at 2-8°C in their original packaging. Under these conditions, they can be used until the expiry date printed on the kit.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- Imidazole Buffer (AR021A/AR021K/AR021L), as diluent.
- Specific calibrators and controls with known titration of vWF: Ag, whose traceability is related to the International Standard of NIBSC for vWF: Ag in plasma, such as:

Product Name	Reference
BIOPHEN™ Plasma Calibrator	222101
BIOPHEN™ Normal Control Plasma	223201
BIOPHEN™ Abnormal Control Plasma	223301

Materials:

- Automatic instrument for immunoturbidimetric assays.
- Calibrated pipettes.

SPECIMEN COLLECTION AND PREPARATION:

Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5 guidelines for further information concerning specimen collection, handling and storage)[5].

- Specimens:

Human plasma obtained from anticoagulated blood (trisodium citrate).

- Collection:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M) by clean venipuncture. Discard the first tube.

- Centrifugation:

Within 2 hours, use a laboratory-validated method to obtain platelet-poor plasma, for example at least 15 minutes at 2500g at room temperature (18-25°C) and allow the plasma to settle in a plastic tube.

- Plasma storage[6]:
  - 4 hours at room temperature (18-25°C).
  - 24 months at -20°C.
  - 24 months at -70°C.

Frozen plasma specimens should be thawed rapidly at 37°C, then shaken thoroughly and tested immediately. Resuspend any precipitate by shaking vigorously immediately after thawing and before use.

PROCEDURE:

The kit can be used for kinetics methods, automated or manual methods. Perform the test at **37°C** and the turbidimetry is measured **at 575nm** (other wavelengths can be used, preferentially between 540 and 800nm).

Automated methods:
Applications for the various analyzers are available on request. **See the specific application and specific precautions for each analyzer.**

Assay method:
1. Reconstitute the reference preparation or plasmatic calibrator and plasmatic controls (2 recommended levels at about 40 and 100% of vWF: Ag) as indicated in the specific instructions for use or according to the internal practice.
Prepare the calibration points in the 0 to 150% range (0-20-75-150% vWF: Ag in Imidazole buffer).

2. Dilute the specimens, calibrators and controls in Imidazole buffer, as described in the table below:

Specimens	Predilution	Dilution
Calibrators	**No**	**4/15**
Controls	**No**	**4/15**
Specimens to test	**Complementary dilution factor if necessary to be in the 10-150% range.**	**4/15**

Establish the calibration curve and test it with the quality controls. If stored at room temperature (18-25°C), test the diluted specimens within 2 hours.

3. Dispense at **37°C**:

Reagents	Volume
Calibrators, specimens or controls diluted in Imidazole buffer	30 µL
R1 **Reaction buffer**	60 µL
Incubate at **37°C** for **130 sec**.	
R2 **Latex**	100 µL
Mix and measure the optical density continuously (between 20 and 50 sec) at **575 nm**, while incubating at 37°C.	

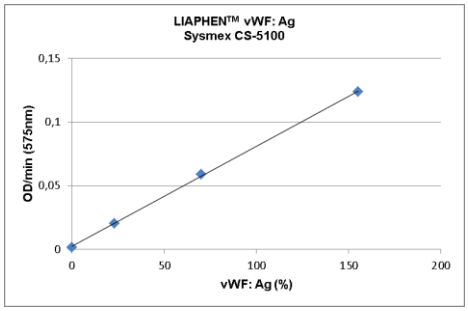
If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

CALIBRATION:

LIAPHEN™ vWF: Ag assay can be calibrated for the assay of vWF: Ag antigen in human plasma.
Using a linear scale:

- The test is linear from 10 to 170% of vWF: Ag on Sysmex CS-5100 (at the standard dilution).

The calibration curve shown below, obtained on Sysmex CS-5100, is given by way of example only. The calibration curve established for the assay series must be used.



QUALITY CONTROL:

The use of quality controls serves to validate method compliance, along with between-test assay homogeneity for a given batch of reagents.
Include the quality controls with each series, as per good laboratory practice, in order to validate the test. A new calibration curve should be defined, preferably for each test series, and at least for each new reagent batch, or after analyzer maintenance, or when the measured quality control values fall outside the acceptable range for the method.
Each laboratory must define its acceptable ranges and verify the expected performance in its analytical system.

RESULTS:

- On the Sysmex CS-series analyzer, the calibration curve is obtained in lin-lin scale, with the OD 575 nm along the Y-axis and the vWF: Ag concentration, expressed as %, along the X-axis.
- The concentration of vWF: Ag in the test specimen is directly inferred from the calibration curve, when the standard dilution is used.
- Results are expressed in % of vWF: Ag.
- The results should be interpreted according to the patient's clinical and biological condition.
- If other dilutions are used, the obtained concentration is the measured concentration multiplied by the complementary dilution factor used (on Sysmex CS-series, the analytical measuring range can be extended from 3 to around 1600% of vWF: Ag).

LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully. The laboratory is responsible for validating any changes made to these instructions for use.
- Any reagent presenting an unusual appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- Any plasma displaying a coagulum or showing signs of contamination must be rejected.
- Rheumatoid Factor and heterophilic antibodies may interfere in the assay by giving abnormally high vWF:Ag values.
- For the possible influence of Hook effect, refer to the specific application for the analyzer used (no significant effect is observed on Sysmex CS-5100 for vWF concentrations until 1600%).
- For the possible influence of interferences, refer to specific application for the analyzer used (no significant effect is observed on Sysmex CS-5100 for heparins concentrations up to 2 IU/mL, bilirubin concentration up to 60 mg/dL, hemoglobin and intralipids concentration up to 1000 mg/dL, by plasma overload tests).

EXPECTED VALUES:

The reference range was measured on healthy adult patients (n=120) on Sysmex CS-5100 (central 90%, 95th percentile) between 62 and 169% of vWF: Ag.
However, each laboratory has to establish its own normal interval.
Blood group of the donor, especially O-type, as other factors like age, sex and pregnancy, may influence vWF:Ag concentration in plasma[7].

PERFORMANCE:

- The lower analyzer detection limit depends on the analytical system used (<1% on Sysmex CS-5100).
- On Sysmex CS-series, the measuring range is of between 3 and 600% of vWF: Ag (for samples >600%, a complementary redilution can be used).
- Performance studies were conducted internally on 3 batches of reagent using a Sysmex CS-5100. Performance was assessed using laboratory controls over a 5-days period, 2 series per day and 3 repetitions within each series for a control level. The following results were obtained:

Control	Intra-series				Inter-series			
	n	Mean%	CV%	SD	n	Mean%	CV%	SD
Normal	40	102.8	2.2	2.3	30	103.4	2.2	2.3
Pathological	40	39.8	4.6	1.8	30	39.2	2.6	1.0

REFERENCES:

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3. Schwameis *et al*, vWF excess and ADAMTS13 deficiency: a unifying pathomechanism linking inflammation to thrombosis in DIC, malaria, and TTP. Thrombosis and Haemostasis, 113.3/2015.
4. Farkas *et al*, Complement activation, inflammation and relative ADAMTS13 deficiency in secondary thrombotic microangiopathies. Immunobiology, 2017, 222:119-127.
5. CLSI Document H21-A5 : "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". Fifth Edition, 28, 5, 2008.
6. Woodhams B. *et al.*, Stability of coagulation proteins in frozen plasma. Blood coagulation and Fibrinolysis. 2001.
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SYMBOLS:

Symbols used and signs listed in the ISO 15223-1 standard, see Symbol definitions document.