LIAPHEN™ vWF: Ag

REF 120206

R1 4 x 5 mL, R2 4 x 6 mL

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

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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 an automated method. Reagents are in the liquid presentation, ready to use.

SUMMARY AND EXPLANATION:

Technical:

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, by complexing to Factor VIII, extends its half-life into the bloodstream.
Ultra-large multimers are proteolytically cleaved by ADAMTS13 into 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].

Clinical:

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 **Reaction Buffer**, liquid form.
4 vials of 5 mL.
R2 **Latex**, liquid form.
4 vials of 6 mL.

Reagents R1 and R2 contain BSA and small amounts of sodium azide (0.9 g/L).

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of animal origin. Users of reagents of these types must exercise extreme care in full compliance with safety precautions in the manipulation of these biological materials as if they were 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.
- Aging studies show that the reagents can be shipped at room temperature without degradation.
- This device of *in vitro* diagnostic use is intended for professional use in the laboratory.

REAGENT PREPARATION:

R1 R2 Reagent is ready to use; homogenize by gentle inversion while avoiding formation of foam and load it directly on the analyzer following application guide instruction.

STORAGE AND STABILITY:

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.

R1 R2 Reagent stability after opening, free from any contamination or evaporation, and stored closed, is of:

- **4 weeks** at 2-8°C.
- **2 weeks** at room temperature (18-25°C).
- **Do not freeze.**
- **Stability on board of the analyzer: see the specific application.**

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- Imidazole Buffer (AR021B/AR021K/AR021L/AR021M/AR021N), 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:

- Spectrophotometer or automatic analyzer for immuno-turbidimetric assays.
- Calibrated pipettes; silicon glass or plastic test tubes.

SPECIMEN COLLECTION AND PREPARATION:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture. Discard the first tube.
Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5[5] guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references[5,6].

PROCEDURE:

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

Assay method:

1. Reconstitute the reference preparation or plasma calibrator, and plasma controls as indicated in the specific instructions or according to internal practice.
Program the calibration concentrations from 0 to 150% vWF:Ag (0-20-75-150% vWF: Ag in Imidazole buffer), the **4:15** dilution corresponding to the indicated "C" concentration of vWF:Ag for the commercial calibrator.

2. Program the specimens and controls dilution in Imidazole buffer, as described in the table below:

Specimens	References	Dilution
Controls	**223201 / 223301**	**4:15**
Specimens to test	**NA**	**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 quickly. The exact calibrator and control concentrations for each batch are indicated on the flyer provided with the kit.

3. As an example, the below table shows the schema for CS-series application. Dispense the following to the reaction cuvettes incubated at **37°C** (directly managed by the analyzer):

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**, 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.

For high concentrations (between 150% and 1600%), we recommend performing a pre-dilution in Imidazole buffer (the measured concentration should then be multiplied by the "pre-dilution" factor).

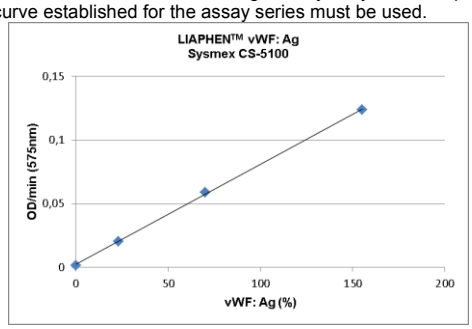
For an automated method, application guides are available on request. See specific application guide and specific precautions for each analyzer.

CALIBRATION:

The LIAPHEN™ vWF: Ag assay can be calibrated for the assay of vWF antigen in human plasma. The calibrator covering the calibration range is available from HYPHEN BioMed (see the REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED paragraph) and can be used to establish the calibration curve.

- The calibration range is about 0 to 150% (on Sysmex CS-5100).

The calibration curve shown below 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 established, 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 acceptance range for the method.
Each laboratory must define its acceptance 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.
- If other dilutions are used, the level obtained should be multiplied by the additional dilution factor used.
- The results should be interpreted according to the patient's clinical and biological condition.

LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully.
- 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.
- 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 guide for the analyzer used (no significant effect is observed on Sysmex CS-5100 for vWF concentrations up to 1600%).

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].

PERFORMANCES:

- The lower analyzer detection limit depends on the analytical system used (<1% on Sysmex CS-5100).
- The measuring range depends on the analytical system used (about 3 to 1600% of vWF: Ag on Sysmex CS-series with redilution, the test being linear from 10 to 170% without redilution).
- Performance studies were conducted internally on Sysmex CS-5100. Performance was assessed using laboratory controls over a 5-day period, 2 series per day and 3 repetitions within each series for a control level. The following results were obtained:

Control	Intra assay				Inter assays			
	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

- Correlation with reference method (vWF :Ag (Siemens) vs LIAPHEN™ vWF:Ag on Sysmex CS-5100) :
  n = 73 y = 1.032x+0.49 r = 0.998

- Interferences:
  No interference, on the analyzer Sysmex CS-5100 was observed with the molecules and up to following concentrations:

Hemoglobin	1000 mg/dL	**Heparin (UFH/LMWH)**	2/2 IU/mL
Bilirubin (Free)	60 mg/dL	**Rivaroxaban**	400 ng/mL
Bilirubin (Conjugated)	60 mg/dL	**Apixaban**	400 ng/mL
Intralipids	1000 mg/dL	**Dabigatran**	400 ng/mL
Rheumatoid factor	750 IU/mL		

Also refer to the specific application guide of the analyzer used.

REFERENCES:

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3. Schwameis M. *et al*, vWF excess and ADAMTS13 deficiency: a unifying pathomechanism linking inflammation to thrombosis in DIC, malaria, and TTP. Thrombosis and Haemostasis. 2015.
4. Farkas P. *et al*, Complement activation, inflammation and relative ADAMTS13 deficiency in secondary thrombotic microangiopathies. Immunobiology. 2017.
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.
7. Gill JC. *et al.* The Effect of ABO group on the diagnosis of von Willebrand disease. Blood. 1987.

SYMBOLS:

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

Changes compared to the previous version.