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# LIAPHEN™ AT

CE
IVD

REF 120002 R1 2 x 2 mL, R2 2 x 10 mL
REF 120008 R1 2 x 1,5 mL, R2 2 x 5 mL

Immuno-turbidimetric method for Antithrombin, with ready to use liquid reagents.

English, last revision: 10-2023

## INTENDED USE:

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

## SUMMARY AND EXPLANATION:

***Technical:***

AT is the major physiological coagulation inhibitor. It inhibits coagulation serine esterases, especially Thrombin, Factors Xa (FXa) and IXa, AT regulates coagulation pathway and prevents from thrombosis. When complexed to heparin, AT becomes a potent and fast acting inhibitor of coagulation serine esterases[1].

***Clinical:***

Spontaneous thromboembolic diseases are observed in presence of congenital AT deficiencies, which are classed in 4 groups[2,3,4].
The AT concentration is decreased in neonates, and various contexts such as pregnancy, liver disease, DIC,…[4]
Functional assay[4] associated with an immunoassay allows to characterize the deficiency type.

## PRINCIPLE:

LIAPHEN™ AT is an immunoturbidimetric method, based on antigen-antibody reaction: AT:Ag of the sample reacts with Latex particles sensitized with goat anti-human AT 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 AT:Ag in the sample.

## REAGENTS:

R1 **Latex**, liquid form. Contains BSA.
R2 **Reaction buffer**, Hepes NaCl Buffer, liquid form.

REF 120002 ➔ R1 **2 vials of 2 mL.**
R2 **2 vials of 10 mL.**

REF 120008 ➔ R1 **2 vials of 1,5 mL.**
R2 **2 vials of 5 mL.**

## 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.
- 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.
*For manual method, allow to stabilize for 30 minutes at room temperature (18-25°C), homogenize before use.*

## 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:

- **6 months** at 2-8°C.
- **7 days** 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.
- Physiological Saline (0.9% NaCl).
- Specific calibrator and controls with known AT:Ag titration, such as:

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

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

***Materials:***

- Spectrophotometer or automatic instrument for immuno-turbidimetric assays.
- Stopwatch; Calibrated pipettes; plastic test cuvettes for spectrophotometer.

## 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 methods, automated or manual methods. Perform the test at **37°C** and the turbidimetry is measured at **620nm** (other wavelengths can be used, preferentially between 450 and 700nm).

**For an automated method, application guides are available on request. See specific application guide and specific precautions for each analyzer.**
**Manual dilution of the latex is not necessary for automated methods.**

**Assay method:**

1. Reconstitute the reference preparation or plasma calibrator, and plasma controls as indicated in the specific instructions or according to internal practice.

For a calibrator with a known AT:Ag concentration (C%) and a working dilution of **1:15**, the **150%** level (under assay conditions) is obtained by diluting this calibrator by the following factor: **10x(C)/100 ie D = C (in %) : 10**.
The calibration curve can also be established by using a pool of citrated normal plasmas (at least 30 normal individuals, men and women, aged between 18 and 55 years, with no known treatments or diseases), which, by definition, has a AT:Ag titer of **100%**. The assay includes a **1:15** plasma dilution, which by definition, represents the 100% AT:Ag level. The **1:10** dilution in physiological saline represents **150%** AT:Ag.

Prepare **2 mL** of the **1:10** normal plasma pool dilution, or a **1:D (D=C:10)** dilution of the calibrator (i.e. **C1**) corresponding to 150% AT:Ag. Prepare the following calibration curve by serial dilutions in physiological saline, as described in the following table in order to prepare the calibration curve:

| Standard | C1 | C2 | C3 | C4 | C5 | C6 |
|---|---|---|---|---|---|---|
| %AT:Ag | 150 | 100 | 75 | 50 | 25 | 0 |
| Volume standard | 2000 µL | 600 µL of C1 | 500 µL of C1 | 500 µL of C2 | 500 µL of C4 | 0 µl |
| Volume physiological saline | 0 µL | 300 µL | 500 µL | 500 µL | 500 µL | 1000 µL |

**For the manual method, a calibration curve must be performed for each test series.**

2. Dilute the specimens and controls in physiological saline, as described in the table below (manual method):

| Specimens | Reference | Dilution |
|---|---|---|
| BIOPHEN™ Normal Control Plasma | 223201 | **1:15** |
| BIOPHEN™ Abnormal Control Plasma | 223301 | **1:15** |
| Specimens | n.a | **1:15** |

For purified AT assays, the appropriate dilution must be done in physiological saline with 1% BSA (final concentration from about 0.5 to 10 µg/mL).

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. Dispense the following to a plastic tube incubated at **37°C**:

| **Prepare just before use the appropriate volume of R1 diluted in R2 (see below)** | |
|---|---|
| | **Volume** |
| Specimen, calibrator or controls diluted in physiological saline | 100 µL |
| **R1 Latex diluted at 1:5** in **R2** pre-incubated at **37°C and homogenized before use** | 400 µL |
| Mix and incubate **at 37°C** for **exactly 15 minutes**, **then immediately after:** | |
| **Mix** and read the absorbance at **620nm** against the physiological saline**. Respect the same overall reaction time for each sample.** | |

Create a plasma blank if sample is icteric, lipaemic, haemolysed, or if its color differs from the standard plasmas.

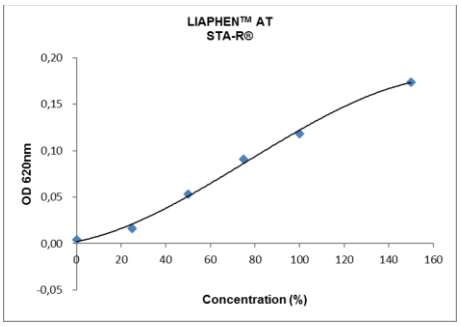
If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

## CALIBRATION:

LIAPHEN™ AT assay can be calibrated for the assay of AT (antigen). The plasma 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 STA-R®).

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:

- For the manual endpoint method, plot the calibration curve lin-lin, with the OD 620nm along the Y-axis and the AT:Ag concentration, expressed as %, along the X-axis by choosing the most suitable interpolation mode.
- The concentration of AT (%) 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.
- The presence of rheumatoid factor may interfere in the assay and result in an overestimation of AT:Ag level.
- For the possible influence of Hook effect, refer to the specific application guide for the analyzer used (no significant effect is observed on STA-R® for AT:Ag concentrations up to 200%).

## EXPECTED VALUES:

The reference range was measured on healthy adult subjects (n=100) on STA-R® (Central 90%, 95th percentile) between 86 and 124 % of AT:Ag.
Each laboratory has to determine its own normal range.

## PERFORMANCES:

- The measuring range depends on the analytical system used (about 11 to 150% of AT:Ag on STA-R®-series).
- Specificity: AT deficient plasma measured <5%.
- Performance studies were conducted internally on STA-R®. Performance was assessed using laboratory controls over a 10 series and 2 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 |
| High | 10 | 102 | 1.9 | 1.9 | 10 | 102 | 4.4 | 4.5 |
| Middle | 10 | 63 | 1.5 | 1.0 | 10 | 63 | 4.0 | 2.5 |
| Low | 10 | 23 | 3.0 | 0.7 | 10 | 23 | 7.4 | 1.7 |

- Correlation with reference method (Berichrom AT vs LIAPHEN™ AT on BCS-XP):
  n = 62  y = 1.04x - 1.47  r = 0.987
- Interferences:
  No interference, on the analyzer STA-R® was observed with the molecules and up to following concentrations:

| | | | |
|---|---|---|---|
| **Heparins (UFH / LMWH)** | 2 IU/mL | **Hemoglobin** | 500 mg/dL |
| **Bilirubin** | 60 mg/dL | **Intralipids (Triglycerids equivalent)** | 2000 mg/dL |

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

## REFERENCES:


1. Mann K.G. Biochemistry and Physiology of blood coagulation. Thrombosis and Haemostasis. 1999.
2. Patnaik M.M. and Moll S. Inherited antithrombin deficiency: a review. Haemophilia. 2008.
3. Amiral J and Seghatchian J. Revisiting antithrombin in health and disease, congenital deficiencies and genetic variants, and laboratory studies on α and β forms. Transfus Apher Sci. 2018.
4. Khor B. and Van Cott E.M. Laboratory tests for antithrombin deficiency. American Journal of Hematology. 2010.
5. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008.
6. Mauge L. and Alhenc-Gelas M. Stabilité pré-analytique des paramètres de la coagulation: revue des données disponibles. Ann Biol Clin. 2014.


## SYMBOLS:

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

*Changes compared to the previous version.*