

Intended use and applications

Status: **IVD, CE mark.**

Intended use: Liquid reagents, ready to use. Chromogenic anti-Xa assay for the quantitative determination of the heparin cofactor activity of Antithrombin (AT) in human citrated plasma. Automatic or Manual method.

Diagnosis of congenital or acquired AT deficiencies.

Reagents and principle

Reagents:

R1: **Bovine FXa, liquid reagent, containing heparin.** 4 vials of 11ml.

R2: **FXa specific chromogenic substrate (SXa-11), liquid reagent.** 4 vials of 4ml.

Principle:

BIOPHEN AT (LRT) assay is a kinetics method based on the inhibition of Factor Xa (FXa), which is at a constant concentration and in excess, by AT in presence of heparin. The remaining FXa is then measured by its amydolytic activity on a FXa specific chromogenic substrate, which releases pNA. The amount of pNA generated is inversely proportional to the AT concentration present in the tested plasma.

The assay being insensitive to heparin, AT can be assayed on plasmas from heparin treated patients.

Heparin + AT → [AT Hep.]

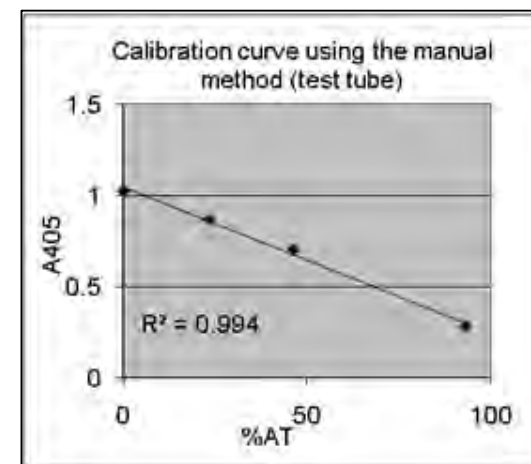
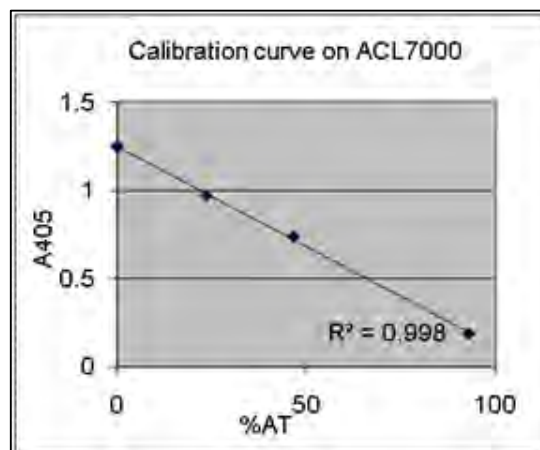
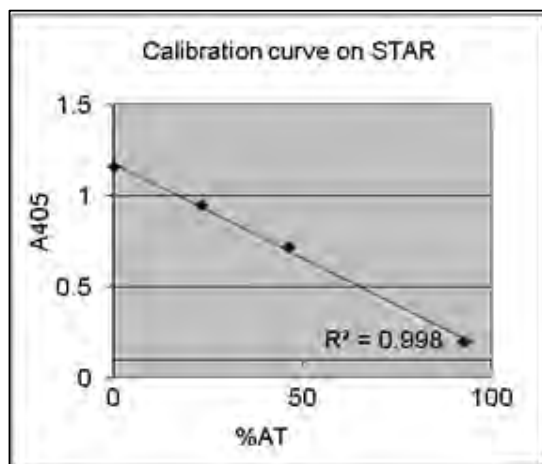
[AT Hep.] + [FXa (excess)] → [FXa-AT-Hep.] + [residual FXa]

[FXa (residual)] + SXa-11 → Peptide + pNA

Characteristics and advantages

- **Simple, rapid, ready to use liquid reagents:** total assay time < 3 min.
- Easy to use with **automated instrument** or basic equipment
- Tests per kit: ~4*50 (STAR), 4*75 with most of laboratory instruments, or 4*100 (microplate)
- **Associated calibrators and controls** validated against the International Standard for human (AT), plasma (NIBSC 93/768, SSC/ISTH lot 3).
- Wide linearity and dynamic range : 0 (to 10) to about 120% AT in human citrated plasma
- Assayed dilution 1:10 with physiological saline
- Detection threshold < 10% AT
- Specific, sensitive, reproducible.
- **Economical and Highly stable** (24 months at 2-8°C , > 7 days at RT (18-25°C).
- **Safe, optimized, standardized:** highly purified bovine FXa; inter lots correlation r2 = 0.95
- **No significant interference** of Heparin Cofactor II, α 2-macroglobulin or α 1-Antitrypsin (due to the principle of the test); of bilirubin < 0.1mg/ml, haemoglobin < 2.5mg/ml added to plasma.
- Caution: avoid any evaporation or contamination of reagents during use.

Calibration curves (example for lot 02203)



Form AH122
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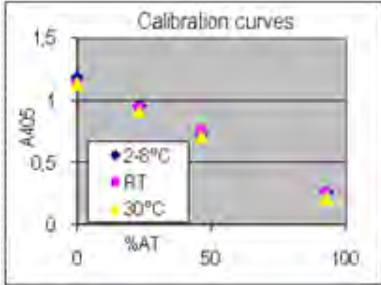
Example of recovery results (ACL7000)

Stability studies (2-8°C, RT(18-25°C), "Heath stress" (accelerated ageing) at 30°C):

Purified human AT and buffer (1), or a normal human plasma and a « AT poor » plasma at about 10% AT (2), were respectively mixed at various levels, to verify consistent recovery:

Reagents are stored at 2-8°C, or for 7 days at RT (18-25°C), or heated at 30°C for 3 weeks. They are then tested on calibration curve and controls.

	(1)	(2)
	Measured %AT	
C (purified or plasma AT)	122	79
C/2	65	41
C/4	29	26
0 (buffer or AT poor plasma)	2	11



Storage:	2-8°C	After 7 days at RT	After 3 weeks at 30°C
	Measured %AT		
Normal control	88	91	89
Abnormal control	38	36	36

Conclusion: Recovery is satisfying in both cases, over the dynamic range.

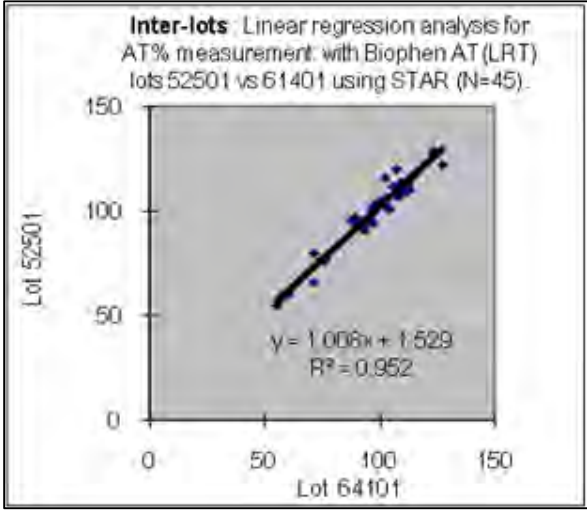
Conclusion: The calibration curves are not affected by the storage at 30°C for 3 weeks. Excellent recovery for controls. Performances are well preserved during the storage at 30°C, which allows shipping the reagents at RT.

Inter lots

Intra and inter-assay data (manual method: test tube, water bath at 37°C, and spectrophotometer)

Internal inter-lots comparison for AT measurement (IU/ml or %), using Biophen AT LRT device lots 52501 and 64101, and the STAR instrument, on N= 45 samples (6 high/low controls, 30 normals from French Blood bank, 7 VKA/dicumarol treated samples, 2 pathological pregnancies left from analysis).

INTRA-ASSAY : The two controls are tested 10 fold in the same series.
INTER-ASSAY : The two controls are tested 10 fold, in 10 independent series, each one being newly calibrated.
 Mean measured value (IU/ml), SD and CV% are reported:



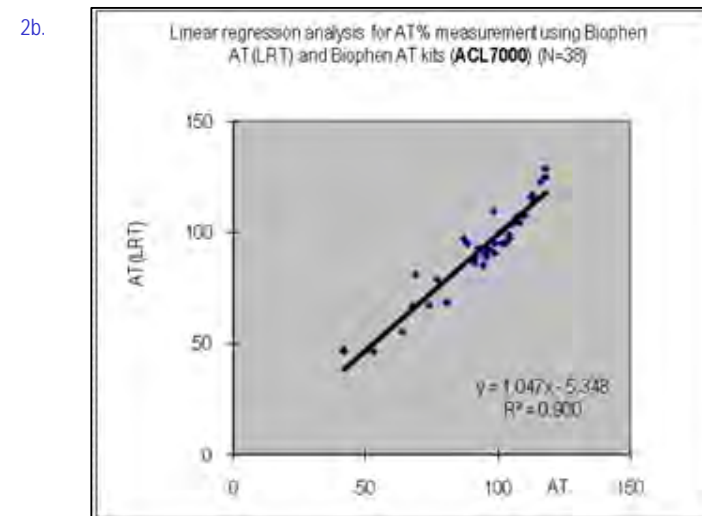
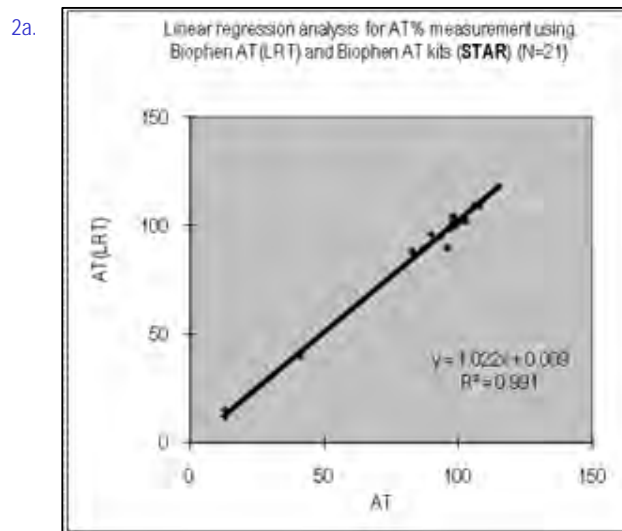
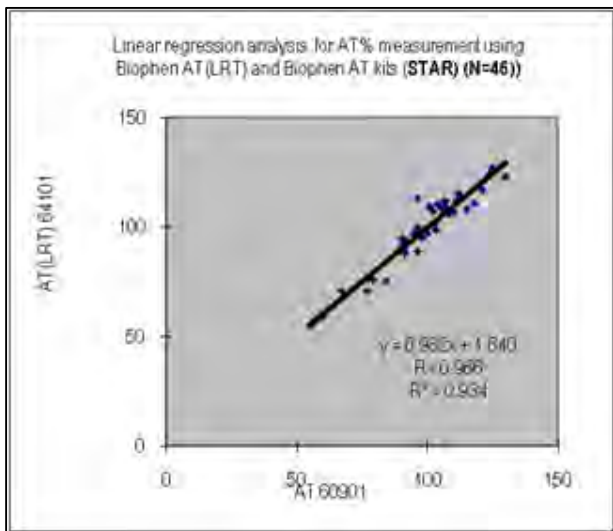
Sample	Intra assay (N=10)			Inter assay (N=10)		
	Mean measured IU/ml	SD	CV%	Mean measured IU/ml	SD	CV%
Normal Control	95	0.94	0.99	90	2.45	2.73
Abnormal control	57	0.79	1.38	56	0.32	0.57

Conclusion: results are well consistent from lot to lot.

Conclusion: In this evaluation using the water bath method, intra and inter assay CV% (on measured concentrations) are <3%.

Performance comparison with Biophen AT (CE, 510(k)) using the ACL7000 or STAR instrument:

- Internal comparison for AT% measurement using Biophen AT (lot 60901) or AT (LRT) device (lot 64101) (STAR), N=46 samples (high/low controls, 30 normals from French Blood bank, 9 VKA/dicumarol treated samples, 1 pathological pregnancy).
- Internal comparison for AT% measurement using Biophen AT (lot 43002) or AT (LRT) device (lot 52501):
 - STAR, N=21 samples (normals from French Blood bank, 8 VKA/dicumarol treated samples, and 2 plasmas with poor AT content).
 - ACL7000, N=38 samples (21 normals from French Blood bank, and plasmas addressed for thrombosis risk check up or for low AT suspicion).



Conclusion: good correlation between results obtained using Biophen AT and Biophen AT (LRT) kits, for AT% measurement in citrated plasma samples (range 12 to about 120%).
 (Note: r2 increases as expected with extended measuring range).

Measured AT% (N=21, STAR)	BIOPHEN AT lot 43002	BIOPHEN AT (LRT) lot 52501
Mean AT% [Median]	87 [97]	89 [100]
SD	28,4	29,2
Min-Max	13 - 115	12 - 118

Internal evaluation of Biophen Plasma Calibrator, Normal and Abnormal Control system for AT content, using the various HYPHEN kits available:

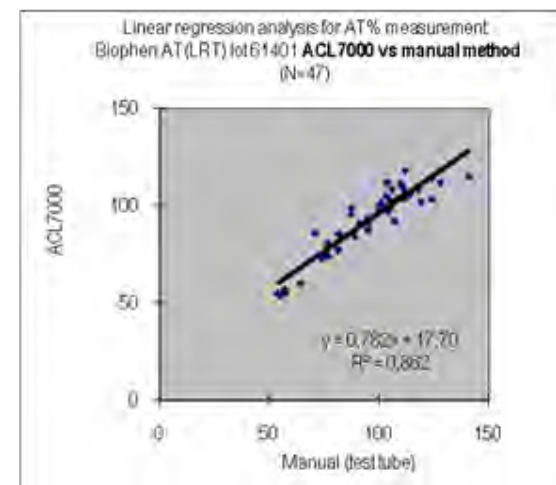
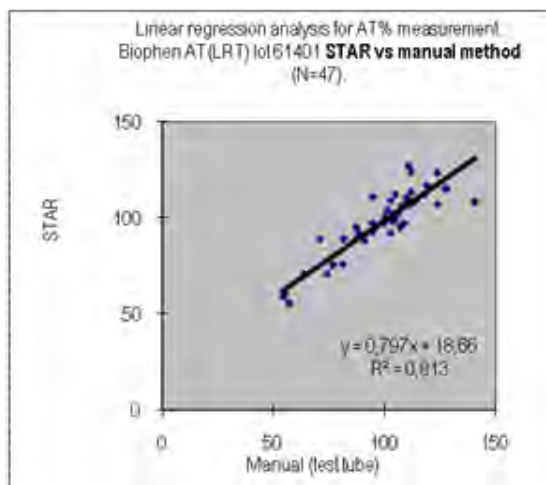
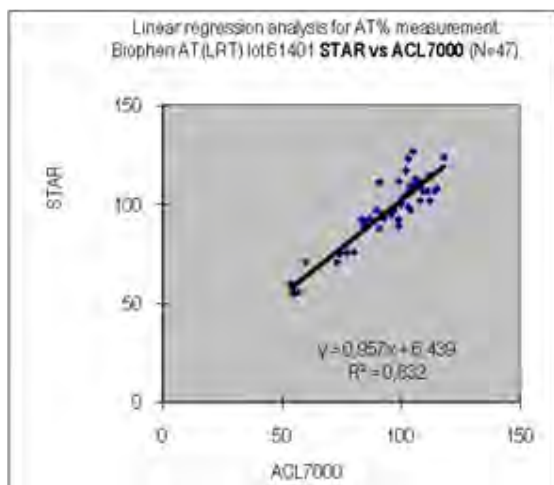
The samples were determined against the International Standard for human AT (plasma) (NIBSC) and the SSC/ISTH lot 3 standard. The manual method, STAR, and ACL7000 (when available) methods were used for each kit. At least 3 independent series (ie a total of at least 9 different vials) were analyzed with each kit. The results were as follows:

Kit used for determination:	Sample:		
	Plasma Calibrator	Normal control	Abnormal control
Biophen AT (# A221102/A221105)	91%	85%	37%
Liaphen AT (# A120002)	94%	92%	37%
Biophen AT anti-IIa (# A221122)	94%	88%	37%
Biophen AT (LRT) (#A221111)	92%	89%	38%
Mean of all methods:	93%	87%	37%

Conclusion: results are well consistent between the different methods and kits.

Inter instruments

Internal inter-instruments comparison (STAR; ACL7000; manual method using test tube, water bath at 37°C and spectrophotometer) on measured AT% using Biophen AT LRT device (lot 64101), on N=47 samples (7 high/low controls; 30 normals from French Blood Bank, 8 VKA/dicumarol treated patients' samples, 2 pathological pregnancies left from analysis) :



Conclusion: inter instruments consistency of the results is satisfying, and a low AT level is well detected whatever the method is.

QMS, Quality Control system and referential

This reagent is designed, manufactured, controlled and followed according to HYPHEN BioMed quality management system (based on ISO 9001 and ISO 13485), and according to the 98/79/CE directive from European parliament and council (October 27th, 1998) related to IVD medical devices.

A complete lot master file is recorded and validated in full compliance with Manufacturing and Quality Control SOP for each lot, as well as complete stability studies.

Homogeneous performance from lot to lot is ensured by using Internal Reference Standards for AT (plasma), accurately validated against the corresponding NIBSC International Standard, as well as by using various quality controls covering the dynamic range.

Related products

1. Biophen Plasma Calibrator, Normal and Abnormal Control Plasmas (#A222101/A223201/A223301) (CE, 510(k))
2. Biophen AT (#A221102/A221105) (CE, 510(k))
3. Biophen AT anti-IIa (#A221122)
4. Liaphen AT (#A120002)