Antithrombin

Antithrombin (formerly known as antithrombin III, AT) is the major physiological coagulation inhibitor. It inhibits activated serine proteases, especially thrombin, factors Xa (FXa), IXa (FIXa), XIa (FXIa) and XIIa (FXIIa). It regulates the coagulation pathway and prevents thrombosis. The inhibitory capability is potentiated by heparin. When complexed to heparin, AT becomes a potent and fast acting inhibitor, especially thrombin and FXa.

Reference values:

plasma: 70 – 125 %

Clinical significance:

congenital deficiency: rare (0,02 % of population)

acquired deficiency: disseminated intravascular coagulation (DIC), sepsis
deep vein thrombosis (DVT)
liver disease, nephrotic syndrome
pulmonary embolism, stroke
hypoproteinemia
heparin therapy
oral contraceptive therapy
pregnancy
neonates during the first few days of life

increased level: acute hepatitis, cholestasis
kidney transplant
vitamin K deficiency
warfarin anticoagulation therapy

CHROMOGENIC ASSAY FOR THE QUANTITATIVE DETERMINATION OF ANTITHROMBIN ACTIVITY IN PLASMA

PRINCIPLE OF THE METHOD

This two-stage method is based on the ability of plasma to inhibit FXa in the presence of heparin. FXa in excess at a constant concentration is added to a diluted plasma containing AT in the presence of heparin. After the initial incubation ***(stage 1)*** the remaining FXa is determined with a FXa specific chromogenic substrate ***(stage 2)***. This residual FXa activity is inversely proportional to the AT concentration in the sample.

stage 1

AT + heparin ⟶ [AT - heparin]

[AT - heparin] + FXa (excess) ⟶ [AT - heparin - FXa] + FXa (remaining)

stage 2

FXa (remaining) + SXa-11 ⟶ peptide + pNA (p-nitroaniline)
yellow

MATERIALS AND INSTRUMENTS

The BIOPHEN AT (LRT) kit, tubes, an automatic pipette, a cuvette, a spectrophotometer SPEKOL 1300

CHEMICALS

reagent 1 (R1) – Bovine FXa (bovine FXa, heparin, sodium azide; pH 7,85)

reagent 2 (R2) – SXa-11 (chromogenic substrate specific for FXa)

plasma calibrator (BIOPHEN Plasma Calibrator Ref 222101)

citric acid (2 %)

physiological saline (0,9 % NaCl)

blood plasma diluted 1:10 with physiological saline

Before use let the reagents R1 a R2 stabilise for 30 minutes at the laboratory temperature.

CALIBRATION

1. Make the calibration curve by diluting of the plasma calibrator with physiological saline in the proportions shown in table.

activity (% AT)	$V_{plasma\ calibrator}$ (µl)	$V_{physiological\ saline}$ (µl)
0	0	500
25	125	375
50	250	250
100	500	0

2. Write the measured values into the table.
3. Draw the graph of the dependence of absorbance values obtained for each AT calibration standard on the amount of AT in %.

PROCEDURE

REFERENCE SOLUTION (BLANK)

1. Pipette the solutions into the labelled test tube according to the table.

physiological saline	**500 µl**
citric acid	**500 µl**
Mix and incubate at 37 °C for 120 seconds, then introduce:	
reagent (R2) - preincubation at 37 °C	**100 µl**
Mix and incubate at 37 °C for 90 seconds, then introduce:	
reagent (R1) - preincubation at 37 °C	**300 µl**
sample (blood plasma)	**50 µl**
Mix.	

TEST SAMPLE

2. Pipette the solutions into the labelled test tube according to the table.

sample (blood plasma)	**50 µl**
reagent (R1) - preincubation at 37 °C	**300 µl**
Mix and incubate at 37 °C for 90 seconds, then introduce:	
reagent (R2) - preincubation at 37 °C	**100 µl**
Mix and incubate at 37 °C for 120 seconds. *Stop the reaction by introducing:*	
citric acid	**500 µl**
Mix and then introduce:	
physiological saline	**500 µl**
Mix and read the absorbance of the sample at 405 nm against the blank.	

2. The AT activity in the test plasma read directly from the calibration curve.

MEASURED VALUES

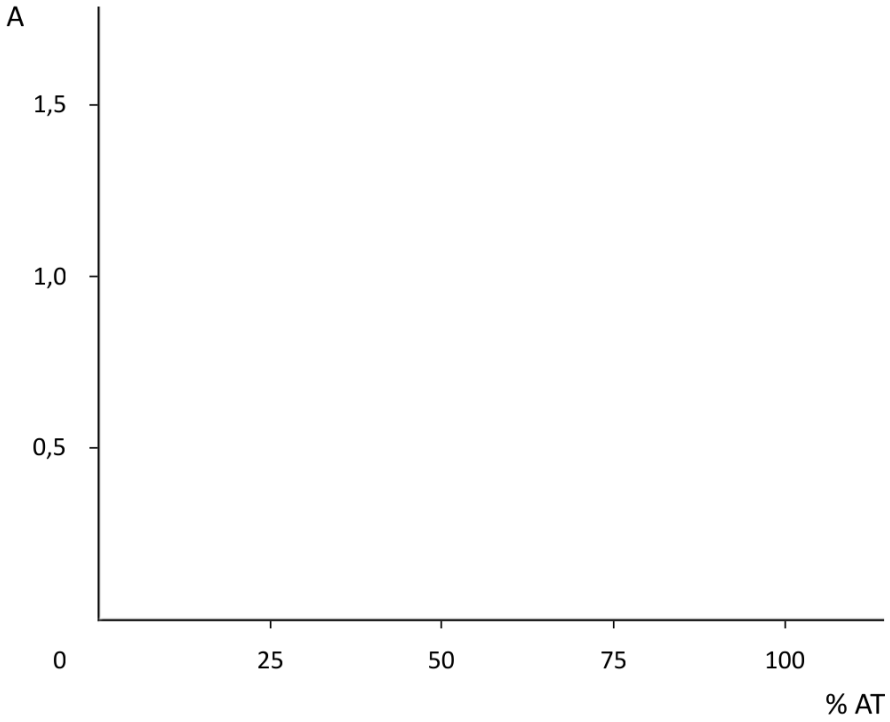
CALIBRATION CURVE

absorbance (A)	activity (% AT)
	0
	25
	50
	100

TEST SAMPLE

absorbance (A)	activity (% AT)

GRAPHICAL REPRESENTATION



CONCLUSION