ALT (SGPT)

IFCC without pyridoxal phosphate

Intended use:
In vitro test for the quantitative determination of alanine aminotransferase (ALT) in human serum and plasma.

Summary:
Alanine aminotransferase (glutamate-pyruvate-transaminase) belongs to the group of transaminases which catalyze the conversion of amino acids to the corresponding a-keto acids via the transfer of amino groups; they also catalyze the reverse process. Although higher activities exist in the liver, minor activity can also be detected in the kidneys, heart, skeletal muscle, pancreas, spleen, and lungs. Elevated levels of transaminases are indicative of myocardial infarction, hepatopathies, muscular dystrophy, and damage to internal organs. Increased ALT activity in the serum, however, is a rather specific indicator of damage to the liver parenchyma, while AST is not necessarily a liver-specific parameter. The International Federation of Clinical Chemistry (IFCC) recommended standardized methods for the determination of ALT with optimized substrate concentrations, use of TRIS buffer, simultaneous preincubation of serum with buffer (to avoid competing reactions with NADH), substrate start, and pyridoxal phos-phate activation.
The method described here is derived from the IFCC reference method.

Test principle:
UV test according to the IFCC method.

$$\text{L-Alanin + 2-Oxoglutarate} \xrightarrow{\text{ALT}} \text{L-Glutamate + Pyruvat}$$

$$\text{Pyruvat + NADH + H}^+ \xrightarrow{\text{LDH}} \text{D-Lactate+ NAD}^+$$

The enzyme alanine aminotransferase (EC 2.6.1.2; L-Alanine:2-Oxoglutarate Aminotransferase, ALT or A1aAT; Glutamate Pyruvate Transaminase, GPT) catalyzes the tran- saminase reaction between L-Alanine and 2-Oxoglutarate. The pyruvate formed, is reduced to lactate in the presence of LDH. As the reactions proceed, NADH is oxidized to NAD^+. The disappearance of NADH per unit time is followed by measuring the decrease in absorbance at 340 nm.

Reagent concentration:

R1:	
Tris buffer pH 7.8	100 mmol/l
L-Alanine	500 mmol/l
LDH	1200 U/l
R2:	
NADH	0.18 mmol/l
2-Oxoglutarate	15 mmol/l

Preparation and stability:
Serum start:
Mix 4 volumes of R1 with 1 volume of R2. This solution is stable
up to10 days at +2°C to +8°C or
up to 1 day at +20°C to +25°C.

Substrate start:
R1: Ready for use.
R2: Ready for use.
Unopened kit components: Up to the expiration date at +2°C to +8°C
Onboard stability: R1: 28 days
R2: 90 days

Specimen:
Collect serum using standard sampling tubes.
Heparin or EDTA plasma.
Stability: 24 hours at +20°C to +25°C
3 days at +2°C to +8°C

Separate serum/plasma from clot/cells within 8 hours at room temperature or 48 hours at +2°C to +8°C.
Centrifuge samples containing precipitate before performing the assay.

Limitations - interference:
Criterion: Recovery within ± 10% of initial value.
Hemolysis interferes due to ALT activity from erythrocytes.
Icterus: No significant interference up to an index I of 20 (approximate conjugated and unconjugated bilirubin: 20 mg/dl)
Hemolysis: No significant interference up to an index H of 1100 (approximate haemoglobin concentration: 1100 mg/dl).Lipemia (Intralipid): No significant interference up to a triglyceride concentration of
450 mg/dl). There is poor correlation between turbidity and triglycerides concentration.Lipemia may cause absorbance flagging as a result of an absorbance increase.

Testing procedure:
Applications for automated systems are available on request.
Materials provided
• Working solutions as described above
Additional materials required
• Calibrators and controls as indicated below
• 0.9% NaCl

Manual procedure for serum start:	
Wavelength:	Hg 334 nm, Hg 340 nm or Hg 365 nm
Temperature:	+25 / +30 / +37°C
Cuvette:	1 cm light path
Zero adjustment:	against water
R1	800 uL
Sample	100 uL
Mix, incubate 1-5 min. Then add;	
R2	200 uL
Mix, incubate for 1 min. and start stopwatch simultaneously. Read again after exactly 1, 2 and 3 minutes and calculate A/min.	
Calculation:	
Hg 365 nm	3235 x A/min
Hg 340 nm	1746 x A/min
Hg 334 nm	1780 x A/min

Measuring reportable range:
A/min 0.200 at 340 nm or A/min 0.100 at 365 nm
At higher activities dilute the sample with 0.9% NaCl (e.g. 1+6). Multiply the result
by the appropriate dilution factor (e.g. 7)

Determine samples with higher activities via the rerun function. On instruments without rerun function, manually dilute the samples with 0.9% NaCl (e.g. 1 + 6). Multiply the result by the appropriate dilution factor (e.g. factor 7)

Expected values:
Men < 45 U/L < 0,74 µkat/L
Women < 34 U/L < 0,56 µkat/L

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, the ALT results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Analytical sensitivity (lower detection limit):
Detection limit: 4 U/l or 0.07 µkat/l

Betwen day

Sample	Mean U/l	SD U/l	CV (%)
Sample 1	44,1	1,34	3,04
Sample 2	115	1,89	1,64
Sample 3	116	2,12	1,83

Within run

Sample	Mean U/l	SD U/l	CV (%)
Sample 1	33,5	1,22	3,63
Sample 2	88,3	1,5	1,69
Sample 3	129	1,81	1,41

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The lower detection limit represents the lowest measurable ALT concentration that can be distinguished from zero.

Imprecision:
Reproducibility was determined using human samples and controls in an internal protocol within day (n = 20). The following results were obtained:

Method comparison:
A comparison between BIOANALYTIC and a commercially available product gave the following results:

GPT BIOANALYTIC = x
GPT competitor = y
n = 126 y = 0.992x - 0.299
r^2= 0.999

Quality Control:
Control Serum:

BIOCON N	5 x 5 ml	#B10814
BIOCON P	5 x 5 ml	#B10817

The control intervals and limits must be adapted to the individual laboratory and country-specific requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Calibration:
S1: 0.9% NaCl
S2: BIOCAL H 5 x 3 ml #B11895

Calibration frequency:
A two-point-calibration is recommended in case of:
1-change of lot
2- quality control requirements

Literatur:
J. Clin.Chem.Clin.Biochem 8 (1970) 658; 10 (1972) 182
Tietz Textbook of Clinical Chemistry, Second Edition,
Burtis-Ashwood (1994).
HU Bergmeyer - Methods of enzymatic analysis, (1987).
CCLM 2002; 40(7):725-733, Schumann et al.
IFCC reference procedure for aspartate aminotransferase.

Order information (Cat No.) :

CC330	BGPT250	B25016	B31015	B36015	B80017
SH330	BGPT500	B27015	B32015	B36016	
CR330	B21015	B27016	B32016	B37015	
OL330	B21016	B28015	B33015	B37016	
KL330	B22015	B28016	B33016	B42015	
AB330	B24015	B30015	B34015	B80015	
BGPT125	B25015	B30016	B35015	B80016	

Manufacturer
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SYMBOLS

IVD	for in vitro diagnostic use only
LOT	lot of manufacturing
REF	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	Read the directions