ALT (ALANINE AMINOTRANSFERASE)

Diagnostic reagent for determination of ALT concentration.
Liquid. Dual Reagents. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). **Do not freeze.**

Ref No	Pack
MH-192	100 mL
MH-193	75 mL

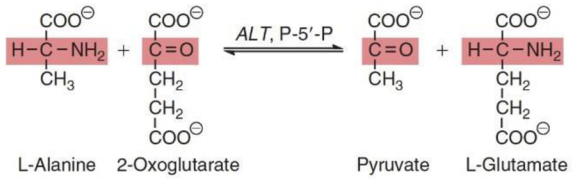
Changes made in the instructions for use are marked as grey.

INTENDED USE

The test is applied for the quantitative determination of ALT (SGPT: Serum Glutamic Pyruvic Transaminase) in serum and plasma.

GENERAL INFORMATION

Aminotransferases constitute a group of enzymes that catalyze the conversion of amino acids to 2-oxo-acids through the transfer of amino groups. The 2-oxoglutarate/l-glutamate couple serves as one amino group acceptor and donor pair in all amino-transfer reactions; the specificity of the individual enzymes derives from the particular amino acid that serves as the other donor of an amino group. Thus, ALT catalyzes the following reaction:



The reactions are reversible, but the equilibria of the ALT reactions favor formation of alanine. Pyridoxal-5′-phosphate (P-5′-P) and its amino analog pyridoxamine-5′-phosphate function as coenzymes in the in vivo amino transfer reactions. P-5′-P binds to the inactive apoenzyme and functions as a true prosthetic group. P-5′-P bound to the apoenzyme accepts the amino group from the first substrate, alanine, to form enzyme-bound pyridoxamine-5′-phosphate and the first reaction product, pyruvate. The coenzyme in its amino form then transfers its amino group to the second substrate, 2-oxoglutarate, to form the second product, glutamate. P-5′-P is thus reconstituted. Since both coenzyme-depleted apoenzymes and holoenzymes can be present in serum, the addition of P-5′-P under measurement conditions that allow recombination with enzymes usually results in an increase in aminotransferase activity.[1]

Unlike aspartate aminotransferase (AST), which is found in both the cytoplasm and mitochondria, ALT is found only in the cytoplasm. When considering ALT activity per gram of wet tissue in an adult human, liver, kidney, heart and skeletal muscle are the organs with the highest levels, respectively.[2] Liver disease, especially hepatocyte necrosis, is the most important cause of increased ALT activity. Because serum activities of ALT are abnormally sensitive to liver injury, increases in ALT readily occur after moderate to excessive alcohol use or exposure to various hepatotoxic agents. ALT is often used as part of a series of enzymes to detect the presence and extent of liver damage. The half-life of ALT is approximately 47 ± 10 hours.[2,3] ALT is usually higher than AST in most types of liver disease where the activity of both enzymes is predominantly from the hepatocyte cytosol. When liver necrosis is severe, as in individuals with alcoholic and viral hepatitis, mitochondrial AST is also released into the blood and AST activity is usually higher than ALT. The ratio of AST to ALT (AAO), sometimes called the De Ritis Ratio, is often used to assess the severity of liver disease in alcoholic liver disease[2,4] and viral hepatitis.[2,5,6]

In most types of liver disease, ALT activity is higher than that of AST, with exceptions in alcoholic hepatitis, hepatic cirrhosis and liver neoplasia. Depending on the severity of the cirrhosis, aminotransferase activities vary, with elevations in enzyme activity up to 4 times the "Upper Reference Limit" (URL) and AAO values that may be greater than 1. This indicates decreased ALT production in the damaged liver and decreased AST clearance due to advanced liver fibrosis. An AAO of 1 or greater has a positive predictive value of approximately 90% for diagnosing the presence of advanced fibrosis in patients with chronic liver disease. A two- to fivefold increase in aminotransferases may occur in patients with primary or metastatic liver carcinoma.

AST is usually higher than ALT, but activities are usually normal in the early stages of malignant infiltration in the liver. In viral hepatitis and other forms of liver disease associated with acute hepatic necrosis, serum AST and ALT activities are increased even before clinical signs and symptoms of the disease (e.g. jaundice) appear. The activities of both enzymes can reach values up to 100 times the URL, but often 10- to 40-fold increases are encountered. The most effective aminotransferase threshold for the diagnosis of acute liver injury is seven times the URL (sensitivity and specificity >95%).[1] Peak values of aminotransferase activity in acute viral hepatitis occur between days 7 and 12; activities then gradually decline and return to physiologic concentrations by weeks 3 to 5 if recovery is occurring. Peak values are not associated with prognosis.[7] The picture in toxic or ischemic hepatitis is different from that in infectious

hepatitis. In acetaminophen intoxication, the peak aminotransferase is more than 85 times the URL in 90% of cases, a value rarely seen in acute viral hepatitis. Furthermore, AST and ALT activities usually peak early and decline even more rapidly.[1,7]

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of elevated aminotransferases other than viral and alcoholic hepatitis. Due to the high prevalence and potential morbidity of NAFLD, there is a suggestion that aminotransferases, especially ALT, should be used as a screening test for early diagnosis. However, since the use of URL has a low diagnostic sensitivity (<50%), there are expert groups who argue that a lower predictive value should be used instead, as well as opinions stating that this will lead to misdiagnosis and unnecessary further investigations.[1,8]

Mild or moderate increases in AST and ALT activities have been observed after administration of various prescription drugs such as non-steroidal anti-inflammatory drugs, antibiotics, antiepileptic drugs, statins or opiates. Over-the-counter drugs and herbal preparations are also included.[1] In patients with elevated amino transferases, negative viral markers and a negative history of drug or alcohol intake, the diagnosis should suggest less common causes of chronic liver injury such as autoimmune hepatitis, primary biliary cholangitis, sclerosing cholangitis, celiac disease, hemochromatosis, Wilson's disease and α1-antitrypsin deficiency.[9]

In pregnancy-associated liver disorders (e.g. intrahepatic cholestasis of pregnancy and acute fatty liver of pregnancy) and other pregnancy-specific diseases (e.g. hyperemesis gravidarum and pre-eclampsia/eclampsia) with possible liver involvement, serum aminotransferase activities may increase mildly to 20-fold.[10] ALT elevation persisting for more than 6 months after an episode of acute hepatitis is used to diagnose chronic hepatitis. In most patients with chronic hepatitis, the maximum ALT is less than five times the URL. ALT has been reported to be persistently normal in 15 to 50% of patients with chronic hepatitis C, but the probability of consistently normal ALT decreases with increasing number of measurements. Therefore, in patients with acute hepatitis C, ALT should be measured periodically for 1 to 2 years to determine whether it has returned to normal.[1,7]

TEST PRINCIPLE

UV, Enzymatic method

ALT in the sample to be measured catalyzes the formation of pyruvate and L-glutamate by the transfer of an amino group between L-alanine and 2-oxoglutarate. Pyruvate is then reduced to L-lactate by reaction with $NADH+H^+$ in the presence of lactate dehydrogenase (LDH), while $NADH+H^+$ is oxidized to NAD^+.

$$\text{L-Alanine + 2-oxoglutarate} \xrightarrow{ALT} \text{pyruvate + L-glutamate}$$

$$\text{Pyruvate + NADH + H}^+ \xrightarrow{LDH} \text{L-Lactate + NAD}^+$$

The rate of NADH oxidation is directly proportional to the catalytic ALT activity. The amount of NADH decreased per unit time is monitored by measuring the decrease in absorbance at 340 nm. The method conforms to IFCC (International Federation of Clinical Chemistry) (2002) recommendations but has been optimized for performance and stability.[11-13]

REAGENT COMPONENTS

Tris buffer	: ≤ 120 mmol/L
L-Alanine	: ≤ 550 mmol/L
2-Oxoglutarate	: ≤ 18 mmol/L
NADH	: ≤ 0.18 mmol/L
LDH	: ≥ 1700 U/L

REAGENT PREPARATION

Reagents are ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8°C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 45 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using CLSI EP25-A protocol.[14]

SAMPLE REQUIREMENTS

Serum and plasma can be used and are collected according to the standard procedures. For plasma, sample collection tube with Li heparin should be preferred. Non-haemolysed and non- icteric samples must be used.Multiple sample freezing and thawing should be avoided.

ALT activity stability in serum and plasma:

3 days at +20/+25ºC [28]
7 days at +2/+8ºC [28]
30 days at -20°C [29]

Annotation:

- Oxalate, heparin and citrate do not inhibit enzymatic activity but may cause slight turbidity. Hemolyzed samples should be avoided as erythrocytes contain three to five times more ALT activity than that found in serum.[2]
- ALT has been found to be stable in whole blood for up to 24 hours.[15] Urine has little or no enzyme activity and is not recommended for analysis.[2]

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of Arcal Auto Calibrator.
Arcal Auto Calibrator
Ref.No: VT-003

Calibration stability is 30 days. Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

Traceability is provided by ERM-AD454k_IFCC Alanine Aminotransferase material.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Arcon N Level 1 Control- Lyophilized
Ref.No: VT-001

Arcon P Level 2 Control- Lyophilized
Ref.No: VT-002

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

Serum/Plasma[30]
Men : < 45 U/L
Women : < 34 U/L

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.

Reference interval has been verified by using CLSI EP28-A3c protocol.[16]

Unit Conversion:
U/L x 0.0167 = µkat/L

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[17]

The determined analytic measuring interval for ALT is 5 – 500 U/L.

Detection Capability

Limit of Detection (LoD): 3 U/L

Limit of Quantitation (LoQ): 5 U/L

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[18]

Linearity

This method shows measurement linearity in the activities up to 500 U/L. Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For the manual dilution procedure, dilute the sample 1:10 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[19]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatibility and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[20]
Repeatibility (Within Run) and Repeatibility (Day to Day) SD (standard deviation) and CV% values of ALT have been given in the table 1 and 2 respectively.

Table 1. ALT Repeatibility (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
14 U/L	0.17	1.25	80
119 U/L	0.59	0.50	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[21]

Table 2. ALT Repeatibility (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
14 U/L	0.24	1.70	80
119 U/L	3.43	2.88	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[21]

Method Comparison

As a result of the statistical evaluation of the method comparison data:

Passing-Bablok equation:[22]
y= 1.032x – 1.344 U/L
r=0.997

Interference

Endogenous interferant and analyte concentrations that have been used in the ALT scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[23,24]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from ALT interference scanning test is appropriate, is determined as 10%.[25]

In ALT test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Interferant and Concentration	ALT Target (U/L)	N*	%Observed Recovery
Lipemia 667 mg/dL	17	3	94

** Total acceptable error rate determined as interference limit and repeatability (within run) pre-detected for the related method were used for the calculations of how many times the control and test samples prepared as a serum pool are going to be run repetitively. In the calculations, the accepted error rate for type 1 (α error) was 5% and for type 2 (β error) was 10% (90% power).*[24]
Non-hemolysed and non-icteric samples must be used.

Annotation:

- Erythrocytes have significant ALT activity and significant hemolysis (>300 mg/dL hemoglobin) may falsely increase ALT activity.[2,26]
- Metronidazole (Flagyl) may interfere with ALT methods by its relatively high concentration and absorbance near 340 nm.[27]

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[24]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.
Contains sodium azide.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA (Occupational Safety and Health Administration) standards.

Danger

EUH032 :Releases a very toxic gas if contacts with acid.
H317 :May cause allergic skin reaction.

Precaution

P280 :Use protective gloves / clothes / glasses / mask.
P264 :Wash your hands properly after using.
P272 :Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352 :Wash with plenty of water and soap if it contacts with skin.
P333+P313 :Seek medical help if it irritates your skin or develops rash.
P362+P364 :Remove contaminated clothes and wash properly before using.

Disposal

P501 :Dispose the vials and contents according to the local regulations.

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Mahmutbey Mah. Halkalı Cad. No:124 Kat:4
Bağcılar/İstanbul/Turkey
Tel: + 90 212 444 08 92
Fax: +90 212 629 98 89
info@archem.com.tr **www.archem.com.tr**
info@validity.com.tr **www.validity.com.tr**

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
R2	Reagent 2
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests