GOT (AST, SGOT)

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REF T2212 3498 Test
REF T2213 1795 Test

FOR USE WITH
ARCHITECT

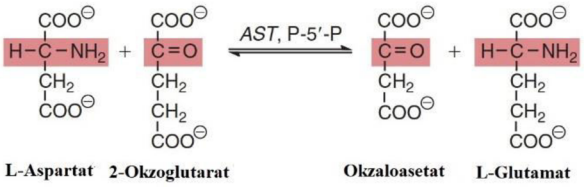
Diagnostic reagent for determination of GOT (Glutamate Oxaloacetate Transaminase) concentration.
Liquid. Dual Reagents. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). Do not freeze.
Products with T2212 / T2213 Ref Number are produced for Abbott Architect Biochemistry Autoanalyzer Series.
Changes made in the instructions for use are marked as grey.

INTENDED USE

The test is applied for the quantitative determination of AST (Aspartate Aminotransferase) / SGOT (Serum Glutamic – Oxaloacetic Transaminase) activity 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 pair acts as an amino group acceptor and donor pair in all amino transfer reactions; the specificity of the enzymes in the reactions is due to the specific amino acid acting as the other donor of one amino group. Thus AST catalyzes the following reaction:



The balance of their reactions is in favor of aspartate formation although the reaction is reversible, 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 serves as a true prosthetic group. P-5′-P bound to the apoenzyme accepts the amino group from the first substrate, aspartate, and forms enzyme-bound pyridoxamine-5′-phosphate and the first reaction product, oxaloacetate. 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]

Similar amounts of AST per gram of wet tissue are found in heart, liver, skeletal muscle and kidney. High enzyme activity indicates tissue damage although enzyme release is non-specific. The mitochondrial form represents 81% of the total AST present in human liver.[2] Alanine aminotransferase (ALT) activity is usually higher than AST in most types of liver disease where the activity of both enzymes is predominantly from the hepatocyte cytosol.[3] Both mitochondrial and cytoplasmic forms of AST are found in cells. These are genetically distinct AST isoenzymes with a dimeric structure consisting of two identical polypeptide subunits of approximately 400 amino acid residues. Approximately 5 to 10% of the total AST activity in the serum of healthy individuals is of mitochondrial origin.[1,4]

Liver disease is the most important cause of increased serum aminotransferase activity and is an indication for measurement. When disease processes affect liver cell integrity, AST serum activity increases, although it is not as liver-specific as ALT. Therefore, the indication for AST testing in addition to ALT is limited, especially in liver-related parenchymal diseases.[5,6]

In addition, when liver necrosis is significant, as in individuals with alcoholic and viral hepatitis, mitochondrial AST is also released into the blood and AST activity can often be higher than ALT. The ratio of AST to ALT, known as De Ritis Ratio, is often used to assess alcoholic liver disease[7] and the severity of liver disease in viral hepatitis.[4,8]
This ratio is only valid in isolated liver disease, when comorbidities that increase AST activity are not present.[3]

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 cirrhosis, aminotransferase activities vary, and elevations in enzyme activity can be up to 4 times the upper reference limit (URL). And AAR values may be higher than 1. This indicates decreased ALT production in the damaged liver and decreased AST clearance due to advanced liver fibrosis. An AAR 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 other forms of liver disease associated with viral hepatitis and acute hepatic necrosis, serum AST and ALT activities are increased even before clinical signs and symptoms of the disease (e.g. jaundice) develop. The activities of both enzymes can reach values up to 100 times the URL, although 10- to 40-fold increases are often encountered. The most effective aminotransferase threshold for diagnosing 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.[9]

The picture in toxic or ischemic hepatitis is different from that of infectious hepatitis. In acetaminophen intoxication, in 90% of cases the aminotransferase peak is greater than 85 times the URL, a value rarely seen in acute viral hepatitis. Furthermore, AST and ALT activities usually peak early and decline more rapidly.[1,9]

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 view 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, and there are also opinions stating that this will lead to misdiagnosis and unnecessary further investigations.[1,10]

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 a negative history of increased amino transferases, negative viral markers and negative drug or alcohol intake, the increased amino transferases should diagnostically 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.[11] In pregnancy-associated liver disorders (e.g. intrahepatic cholestasis of pregnancy and acute fatty liver of pregnancy) and other pregnancy-specific diseases with possible liver involvement (e.g. hyperemesis gravidarum and pre-eclampsia/eclampsia), serum aminotransferase activities may increase mildly to 20-fold.[12]

Due to the high concentration of AST in muscles, AST activity in serum is increased after acute MI, muscular dystrophy and myositis, with the exception of muscle diseases of neurogenic origin. Pulmonary embolism can increase AST up to two to three times the URL. Mild to moderate increases are also noted in acute pancreatitis, crush syndrome and hemolytic diseases [including HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome in pregnancy, where an increase in AST activity greater than twice the URL is seen]. Elevated AST may be associated with immunoglobulins or macro-AST. In these cases, there are no persistent increases in serum AST activity ranging from 2 to 50 times the URL, normal ALT concentrations in an asymptomatic person, and no demonstrable pathology in the organs where AST is found in high amounts.[1]

TEST PRINCIPLE

Enzymatic, UV colorimetric method

AST in the sample to be measured catalyzes the formation of oxaloacetate and L-glutamate by the transfer of an amino group between L-aspartate and 2-oxoglutarate. Oxaloacetate is then reduced to malate by reaction with $NADH+H^+$ in the presence of malate dehydrogenase (MDH), while $NADH+H^+$ is oxidized to NAD^+.

L-Aspartate + 2-Oxoglutarate $\xrightarrow{AST}$ Oxaloacetate + L-Glutamate

Oxaloacetate + 2-Oxoglutarate $\xrightarrow{MDH}$ L-malate + NAD^+

The rate of NADH oxidation is directly proportional to the catalytic AST 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 and has been optimized for performance and stability.[13-15]

REAGENT COMPONENTS

Tris buffer	≤ 90 mM pH 7.65,
L-aspartate	≤ 250 mM,
2-Oxoglutarate	≤ 14 mM,
NADH	≤ 0.18 mM,
MDH	≥ 600 U/L,
LDH	≥ 900 U/L.

REAGENT PREPARATION

Reagents are ready for use.

Annotation:

- Most reference methods, including the current IFCC procedure,[15] include pyridoxal phosphate. The necessity of adding this cofactor has been debated on a wide scale. The essential nature of the cofactor has long been recognized, but because it is usually present in sufficient quantities in human serum, most researchers do not add this component to the reaction mixture.[3]
- The presence of aminotransferases in reagents is possible if lactate dehydrogenase (LDH) is not carefully prepared. Good quality enzymes are not a

problem; in any case, blind reads will reveal the problem.[3]

- The presence of pyruvate is a potential source of error because in the presence of endogenous LD, pyruvate will be converted to lactate by concomitant NADH consumption. This problem is overcome by the addition of a large amount of LDH so that endogenous pyruvate is converted during the pre-incubation period, eliminating interference during measurement.[3]
- Activation of apoenzyme is more efficient in Tris buffer than in phosphate buffer. Although Tris buffer is more convenient to use, NADH is slightly less stable in Tris buffer than in phosphate buffer. Therefore, the Tris concentration is kept relatively low at approximately 80 mmol/L.[3]

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 Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[16]

SAMPLE REQUIREMENTS

Serum and plasma can be used and are collected according to the standard procedures. Heparin, oxalate, EDTA and citrate do not cause enzyme inhibition.[17] Anticoagulants containing ammonium as a cation should be avoided to reduce the possibility of error.[3] Multiple sample freezing and thawing should be avoided. The sample should be homogenized before testing.

AST activity stability in serum and plasma:[18]

4 days at +20/+25°C
7 days at +2/+8°C
3 months at -20°C

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

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of an Multiconstituent Calibrator.

Multiconstituent Calibrator.
Ref.No: 06R56-02

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

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

Men	: < 35 U/L (< 0.58 µkat/L)
Women	: < 31 U/L (< 0.52 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.

Reference interval has been verified by using CLSI EP28-A3c protocol.[19]

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).[20]
The determined analytic measuring interval for AST is 5 – 440 U/L.

Detection Capability

Limit of Detection (LoD): 0,47 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.[21]

Linearity

This method shows measurement linearity in the activities up to 440 U/L.

Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For manual dilution procedure, dilute the sample 10-fold using 0.90% isotonic. After the dilution, multiply the result of rerun 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.[22]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability 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.[23]

Repeatability (Within Run) and Repeatability (Day to Day) SD (standard deviation) and CV% values of Zinc have been given in the table 1 and 2 respectively.

Table 1. AST Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
16 U/L	0,27	1,66	80
248 U/L	1,01	0,40	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[24]

Table 2. AST Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
16 U/L	0,26	1,63	80
248 U/L	6,62	2,68	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[24]

Method Comparison

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

Passing-Bablock equation:[25]
y= 1.363x + 0.986 U/L
r=0.997

Interference

Endogenous interferant and analyte concentrations that have been used in the AST scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[26,27]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from AST interference scanning test is appropriate, is determined as ±25%.[28]

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

Interferant and Concentration	AST Target (U/L)	N*	%Observed Recovery
Bilirubin 4,11 mg/dL	16,4	3	96
Lipemia 412 mg/dL	18	3	102

** 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).*[27]

Annotation:

- Due to the high activity of AST in red blood cells, hemolyzed samples are considered unsuitable.[3]
- Potential sources of error in the enzymatic method include endogenous serum glutamate dehydrogenase, aminotransferases (in reagent preparation), too high serum pyruvate concentration or use of phosphate buffer.[3]
- Glutamate dehydrogenase is present in some pathologic sera, especially in the serum of patients with liver disease. This enzyme can cause interference by causing the reduction of NADH to NAD+.
- Since this source of error is only possible when ammonium ions are present, the IFCC method eliminates it (older methods sometimes included ammonium sulfate in the formulation).[3]

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.[27]
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 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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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 Practice
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

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