

GOT/AST FL

GO F245 CH	12 x 20 ml
GO F400 CH	8 x 50 ml
GO F600 CH	5 x 120 ml

SUMMARY OF TEST

The aminotransferases (transaminases) constitute a group of enzymes that catalyze the interconversion of amino acids and α -keto-acids by transfer of amino groups. Transaminases are widely distributed in animal tissues. Both AST and ALT are normally present in human plasma, bile, cerebrospinal fluid, and saliva, but none is found in urine unless a kidney lesion is present. In viral hepatitis and other forms of liver disease associated with hepatic necrosis, serum AST and ALT levels are elevated even before the clinical signs and symptoms of disease appear. Levels for both enzymes may reach values as high as 100 times the upper reference limit, although 20- to 50-fold elevations are most frequently encountered. Alcoholic hepatitis has more modest elevations. In infectious hepatitis and other inflammatory conditions affecting the liver, ALT is characteristically as high as or higher than AST, and the ALT/AST ratio, which normally and in other conditions is less than 1, becomes greater than unity. The picture in toxic hepatitis is similar to that in infectious hepatitis, with very high ALT and AST activities being observed in severe cases. Elevations up to 20 times the upper reference limit may be encountered in infectious mononucleosis with liver involvement and somewhat lower values in intrahepatic cholestasis. Increased levels may also be observed in extrahepatic cholestasis, with levels tending to be higher the more chronic the obstruction. The aminotransferase levels observed in cirrhosis vary with the status of the cirrhotic process; they range from upper normal to some four to five times normal, with the level of AST activity higher than that of ALT activity. Five- to 10-fold elevations of both enzymes occur in patients with primary or metastatic carcinoma of the liver, with AST usually being higher than ALT, but levels are often normal in the early stages of malignant infiltration of the liver. Slight or moderate elevations of both AST and ALT activities may be observed after intake of alcohol, in delirium tremens, and after administration of various drugs, such as opiates, salicylates, or ampicillin. Although serum levels of both AST and ALT become elevated whenever disease processes affect liver cell integrity, ALT is the more liver-specific enzyme. Serum elevations of ALT activity are rarely observed in conditions other than parenchymal liver disease. Moreover, elevations of ALT activity persist longer than do those of AST activity. Measurement of both AST and ALT has some value in distinguishing hepatitis from other parenchymal lesions. After myocardial infarction, increased AST activity appears in serum, as might be expected from the relatively high AST concentration in heart muscle. Abnormal AST levels are observed in more than 97% of cases of myocardial infarction when correctly timed blood specimens are analyzed. Peak values of AST activity are reached after 18 to 24 h, and the activity values fall to within the normal range by the fourth or fifth day, provided no new infarct has occurred. The peak values of AST activity are roughly proportional to the extent of cardiac damage. Average increases are of the order of four to five times the upper limit of normal.

ALT levels are within normal limits or are only marginally increased in uncomplicated myocardial infarction, because the concentration of ALT activity in heart muscle is only a fraction of that of AST activity.

AST (and occasionally ALT) activity levels are increased in progressive muscular dystrophy and dermatomyositis; they are usually normal in other types of muscle diseases, especially in those of neurogenic origin. Pulmonary emboli can raise AST levels to two to three times normal, and slight to moderate elevations are noted in acute pancreatitis, crushed muscle injuries, gangrene, and hemolytic disease.

It is not possible to monitor transaminase reactions directly, but the advantages of continuous-monitoring assays can be obtained by coupling the transaminase reactions to specific dehydrogenase reactions. The α -keto-acids formed in the transaminase reaction are measured indirectly by enzymatic reduction to the corresponding hydroxyacids, the accompanying change in NADH concentration being monitored spectrophotometrically.

PRINCIPLE OF THE METHOD

The enzyme aspartate aminotransferase (EC 2.6.1.1; L-Aspartate:2-Oxoglutarate Aminotransferase, AST or AspAT; Glutamate Oxaloacetate Transaminase, GOT) catalyzes the transaminase reaction between L-Aspartate and 2-Oxoglutarate. The 2-Oxalacetate formed, is reduced to malate in the presence of MDH. 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. The present method has been made according to IFCC (2002).

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Saline solution.

KIT COMPONENTS

For in vitro diagnostic use only.

The components of the kit are stable until expiration date on the label.

Keep away from direct light sources.

Reagent A F245: 12 x 16 ml (liquid) blue cap
F400: 8 x 40 ml (liquid) blue cap
F600: 4 x 120 ml (liquid) blue cap

Reagent B F245: 3 x 16 ml (liquid) red cap
F400: 2 x 40 ml (liquid) red cap
F600: 1 x 120 ml (liquid) red cap

Composition in the test: Tris buffer 80 mM pH 7.65, L-aspartate 240 mM, 2-Oxoglutarate 12 mM, NADH 0.18 mM, MDH \geq 600 U/l, LDH \geq 900 U/l.

Store all components at 2-8°C.

REAGENT PREPARATION

Serum as starter procedure:

Code F245: add 4 ml of reagent B to a bottle of reagent A.
Code F400: add 10 ml of reagent B to a bottle of reagent A.
Code F600: mix 1 part of reagent B with 4 parts of reagent A.
Stability of working reagent: 30 days at 2-8°C, away from light sources.

Reagent as starter procedure:

use separate reagents ready to use.

Stability: up to expiration date on labels at 2-8°C;

Stability since first opening of vials: \geq 60 days at 2-8°C.

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.

Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

SPECIMEN

Serum, plasma.

Collect blood with a minimum of venous stasis.

GOT is stable up to 4 days at 2-8°C or 1 month at -20°C.

TEST PROCEDURE (sample as starter)

Wavelength:	340 nm
Lightpath:	1 cm
Temperature:	37°C
dispense in cuvette working reagent:	2 ml
preincubate at 37°C for 5 minutes.	
add sample:	200 μ l
Mix, execute a first reading of absorbance after 90 seconds, incubating at 37°C. Perform other 3 readings at 60 seconds intervals. Calculate the $\Delta A/min$.	

TEST PROCEDURE (reagent as starter)

Wavelength:	340 nm
Lightpath:	1 cm
Temperature:	37°C
dispense in cuvette reagent A:	2 ml
add sample	250 μ l
incubate at 37°C for 5 minutes.	
dispense in cuvette reagent B:	500 μ l
Mix, execute a first reading of absorbance after 90 seconds, incubating at 37°C. Perform other 3 readings at 60 seconds intervals. Calculate the $\Delta A/min$.	

RESULTS CALCULATION

Perform calculation in units per litre, multiplying the $\Delta A/min$ by the factor as it is indicated.

Calculation in U/l: $\Delta A/min \times 1746$

Activity in μ kat/l: $U/l \times 0.0167 = \mu$ kat/l

EXPECTED VALUES

Men: < 35 U/l ($< 0.58 \mu$ kat/l)
Women: < 31 U/l ($< 0.52 \mu$ kat/l)

Each laboratory should establish appropriate reference intervals related to its population.

QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control. For this purpose the following human based control sera are available:

QN 0050 CH QUANTINORM CHEMA 10 x 5 ml
with normal or close to normal control values

QP 0050 CH QUANTIPATH CHEMA 10 x 5 ml
with pathological control values.

If required, a multiparametric, human based calibrator is available:

AT 0030 CH AUTOCAL H 10 x 3 ml

Please contact Customer Care for further information.

TEST PERFORMANCE

Linearity

the method is linear up to 440 U/l.

If a $\Delta A/min$ of 0.200 is exceeded, it is suggested to dilute sample 1+9 with saline and to repeat the test, multiplying the result by 10.

Sensitivity/limit of detection (LOD)

the limit of detection is 0.47 U/l.

Interferences

no interference was observed by the presence of:

hemoglobin	\leq 400 mg/dl
bilirubin	\leq 25 mg/dl
lipids	\leq 500 mg/dl

Precision

intra-assay (n=10)	mean (U/l)	SD (U/l)	CV%
sample 1	38.88	0.56	1.40
sample 2	132.50	0.96	0.70

inter-assay (n=20)	mean (U/l)	SD (U/l)	CV%
sample 1	37.87	0.46	1.20
sample 2	134.48	1.48	1.10

Methods comparison

a comparison between Chema and a commercially available product gave the following results:

GOT Chema = x
GOT competitor = y
n = 112

y = 0.986x + 1.636 U/l r = 0.997

WASTE DISPOSAL

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. Refer to special instructions/safety data sheets.

REFERENCES

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
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ence procedure for aspartate aminotransferase.

MANUFACTURER

Chema Diagnostica
Via Padre Vincenzo Pellegrini 3
60035 Jesi (AN) - ITALY - EU
phone +39 0731 213360
fax +39 0731 213361
e-mail: mail@chema.com
website: http://www.chema.com

SYMBOLS

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