

GPT/ALT FL IFCC

GP F080 CH	4 x 20 ml
GP F245 CH	12 x 20 ml
GP F400 CH	8 x 50 ml
GP F500 CH	5 x 100 ml
GP F600 CH	5 x 120 ml
GP 100F CH	5 x 200 ml

INTENDED USE

Reagent for quantitative *in vitro* determination of GPT in biological fluids.

SUMMARY OF TEST

The aminotransferases (transaminases) constitute a group of enzymes that catalyze the interconversion of amino acids and α -oxo-acids by transfer of amino group. 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.

PRINCIPLE OF THE METHOD

The enzyme alanine aminotransferase (EC 2.6.1.2; L-Alanine:2-Oxoglutarate Aminotransferase, ALT or A1aAT; Glutamate Pyruvate Transaminase, GPT) catalyzes the transaminase 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.

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.

GPT R1	F080: 4 x 16 ml (liquid) blue cap
	F245: 12 x 16 ml (liquid) blue cap
	F400: 8 x 40 ml (liquid) blue cap
	F500: 4 x 100 ml (liquid) blue cap
	F600: 4 x 120 ml (liquid) blue cap
100F: 4 x 200 ml (liquid) blue cap	

GPT R2	F080: 1 x 16 ml (liquid) red cap
	F245: 3 x 16 ml (liquid) red cap
	F400: 2 x 40 ml (liquid) red cap
	F500: 1 x 100 ml (liquid) red cap
	F600: 1 x 120 ml (liquid) red cap
100F: 1 x 200 ml (liquid) red cap	

Composition in the test: Tris buffer 100 mM pH 7.15, L-Alanine 500 mM, 2-Oxoglutarate 15 mM, NADH 0.18 mM, LDH \geq 1700 U/l.

Store all components at 2-8°C.

REAGENT PREPARATION

Serum as starter procedure:

Codes F080/F245: add 4 ml of reagent R2 to a bottle of reagent R1.

Code F400: add 10 ml of reagent R2 to a bottle of reagent R1.

Code F500/F600/100F: mix 1 part of reagent R2 with 4 parts of reagent R1.

Stability of working reagent: preferably within 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: preferably within 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 (preferred). Plasma is not recommended.

Collect blood with a minimum of venous stasis.

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

TEST PROCEDURE (sample as starter)

Wavelength:	340 nm
Lighthpath:	1 cm
Temperature:	37°C
dispense in cuvette working reagent:	1 ml
preincubate at 37°C for 5 minutes.	
add sample:	100 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
Lighthpath:	1 cm
Temperature:	37°C
dispense in cuvette reagent R1:	1 ml
add sample	125 μ l
incubate at 37°C for 5 minutes.	
dispense in cuvette reagent R2:	250 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: < 45 U/l (< 0.74 μ kat/l)

Women: < 34 U/l (< 0.56 μ 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:

QUANTINORM CHEMA

with normal or close to normal control values

QUANTIPATH CHEMA

with pathological control values.

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

AUTOCAL H

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 0.169 U/l.

Interferences

no interference was observed by the presence of:

hemoglobin	≤ 500 mg/dl
bilirubin	≤ 40 mg/dl
lipids	≤ 450 mg/dl

Precision

intra-assay (n=10)	mean (U/l)	SD (U/l)	CV%
sample 1	49.29	0.35	0.71
sample 2	132.15	0.57	0.43

inter-assay (n=20)	mean (U/l)	SD (U/l)	CV%
sample 1	49.31	1.66	3.37
sample 2	132.85	4.28	3.22

Methods comparison

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

$$\begin{aligned} \text{GPT Chema} &= x \\ \text{GPT competitor} &= y \\ n &= 126 \end{aligned}$$

$$y = 0.992x - 0.299 \quad r^2 = 0.999$$

WASTE DISPOSAL

This product is made to be used in professional laboratories.

P501: Dispose of contents according to national/international regulations.








REFERENCES

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.

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SYMBOLS

	<i>in vitro</i> diagnostic medical device
	batch code
	catalogue number
	temperature limit
	use-by date
	caution
	consult instructions for use