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

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 -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

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

Composition in the test: Tris buffer 100 mM pH 7.15, L-Alanine 500 mM, 2-Oxoglutarate 15 mM, NADH 0.18 mM, LDH ≥ 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)

Wavelenght:	340 nm
Ligthpath:	1 cm
Temperature:	37°C
dispense in cuvette working reagent:	1 ml
preincubate at 37°C for 5 minutes.	
add sample:	100 µl
Mix, execute a first reading of absorbance after 90 seconds, incubating at 37°C. Perform other 3 readings at 60 seconds intervals. Calculate the ΔA/min.	

TEST PROCEDURE (reagent as starter)

Wavelenght:	340 nm
Ligthpath:	1 cm
Temperature:	37°C
dispense in cuvette reagent R1:	1 ml
add sample	125 µl
incubate at 37°C for 5 minutes.	
dispense in cuvette reagent R2:	250 µl
Mix, execute a first reading of absorbance after 90 seconds, incubating at 37°C. Perform other 3 readings at 60 seconds intervals. Calculate the ΔA/min.	

RESULTS CALCULATION

Perform calculation in units per litre, multiplying the ΔA/min by the factor as it is indicated.

Calculation in U/l: ΔA/min x 1746

Activity in µkat/l: U/l x 0.0167 = µkat/l

EXPECTED VALUES

Men:	< 45 U/l	(< 0.74 µkat/l)
Women:	< 34 U/l	(< 0.56 µkat/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 ΔA/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:

GPT Chema = x
GPT competitor = y
n = 126

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

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.

MANUFACTURER

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SYMBOLS

IVD	*in vitro* diagnostic medical device
LOT	batch code
REF	catalogue number
	temperature limit
	use-by date
	caution
	consult instructions for use

CE