

# GAMMA-GT FL

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

## SUMMARY OF TEST

Even though renal tissue has the highest level of GGT, the enzyme present in serum appears to originate primarily from the hepatobiliary system, and GGT activity is elevated in any and all forms of liver disease. It is highest in cases of intra- or posthepatic biliary obstruction, reaching levels some 5 to 30 times normal. Only moderate elevations occur in infectious hepatitis, and in this condition GGT determinations are less useful diagnostically than are measurements of the transaminases. High elevations of GGT are also observed in patients with either primary or secondary neoplasms. Small increases of GGT activity are observed in patients with fatty livers, and similar but transient increases are noted in cases of drug intoxication. In acute and chronic pancreatitis and in some pancreatic malignancies (especially if associated with hepatobiliary obstruction), enzyme activity may be 5 to 15 times the upper limit of normal. In summary, GGT is the most sensitive enzymatic indicator of hepatobiliary disease available at present. Normal values are rarely found in the presence of liver disease, although as mentioned later, elevations occur in response to certain stimuli when disease is absent. However, GGT is of little value in attempting to discriminate between different kinds of liver disease. Normal levels of the enzyme are found in cases of skeletal disease, in children older than 1 year and in healthy pregnant women, conditions in which ALP is elevated. Thus, measurement of GGT levels in serum can be used to ascertain whether observed elevations of ALP are due to skeletal disease or reflect the presence of hepatobiliary disease. Normal levels of GGT are observed in various muscle diseases and in renal failure, but mild elevations may be noted in untreated lipid nephrosis. In myocardial infarctions, GGT level is usually normal. Elevated levels of GGT are noted not only in the sera of patients with alcoholic cirrhosis but also in the majority of sera from persons who are heavy drinkers. The release of GGT into serum reflects the toxic effects of alcohol and other drugs on microsomal structures in liver cells. The enzyme level found correlates well with the duration of the drug action. Hepatic complications occurring in cystic fibrosis also lead to elevations of GGT. High levels of GGT are present in the prostate, and this may account for the fact that the activity of GGT in sera of males is approximately 50% higher than in sera from females. Prostatic malignancy may at times be the source of elevated GGT activity in serum. Irradiation of tumors in patients with cancer may be accompanied by a rise in GGT activity, although LDH activity in the course of such treatment remains unchanged. However, in malignant disease in general, increased serum GGT activity must first arouse suspicion that the disease is metastatic to the liver. GGT found in urine probably originates in the kidneys and genitourinary tract.

Although GGT was a subject of research interest for many years, its widespread and systematic use in diagnostic enzymology dates from the introduction in 1969 of a convenient method of assay by Szasz. This method and modifications of it use L- $\gamma$ -glutamyl-p-nitroanilide (GLUPA) as the substrate, with glycylglycine serving as the  $\gamma$ -glutamyl residue acceptor. Both two-point and continuous-monitoring methods have been described. Other substrates that have been investigated include the  $\gamma$ -glutamyl derivatives of aminopropionitrile,  $\alpha$ -naphthylamine, and aniline, but GLUPA has been found to be most convenient because it is most sensitive and the p-nitroaniline formed can be directly measured. However, the solubility of this substrate is limited. An alternative procedure has become available in recent years with the production of derivatives of GLUPA in which various groups have been introduced into the benzene ring to increase solubility in water. The most useful of these substrates is L- $\gamma$ -glutamyl-3-carboxy-4-nitroanilide (GLUPA-C). This compound is readily soluble in water and is split by GGT at a rate comparable to that observed with GLUPA.

## PRINCIPLE OF THE METHOD

The enzyme  $\gamma$ -GT (EC 2.3.2.2,  $\gamma$ -glutamyl-peptide:amino acid  $\gamma$ -glutamyltransferase; GGT) hydrolyzes the GLUPA-C to release p-nitroaniline. The p-nitroaniline formed is detected spectrophotometrically at 405 nm to give a measurement of GGT activity in the sample.

## 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 100 mM pH 8.25, glycylglycine 100 mM, L-Glutamyl-3-carboxy-4-nitroanilide 4 mM.

Store all components at 2-8°C.

## MATERIALS REQUIRED BUT NOT SUPPLIED

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

## 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:  $\geq$  60 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 EDTA. Avoid hemolysis.  
GGT is stable up to 7 days at both room temperature and 2-8°C. Store at -20°C for prolonged storage.

## TEST PROCEDURE (sample as starter)

Wavelength:	405 nm
Lighthpath:	1 cm
Temperature:	37°C
dispense in cuvette working reagent:	1 ml
preincubate at 37°C for 5 minutes.	
add sample:	100 $\mu$ l
Mix, execute a first reading of absorbance after 1 minute, incubating at 37°C. Perform other 3 readings at 60 seconds intervals. Calculate the $\Delta A/min$ .	

## TEST PROCEDURE (reagent as starter)

Wavelength:	405 nm
Lighthpath:	1 cm
Temperature:	37°C
dispense in cuvette reagent A:	1 ml
add sample:	100 $\mu$ l
incubate at 37°C for 5 minutes.	
dispense in cuvette reagent B:	250 $\mu$ l
Mix, execute a first reading of absorbance after 1 minute, 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:  $\Delta A/min \times 1280$  (sample starter)  
Calculation in U/l:  $\Delta A/min \times 1571$  (reagent starter)

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

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

## EXPECTED VALUES

Men: < 50 U/l ( $< 0.83 \mu$ kat/l)  
Women: < 30 U/l ( $< 0.50 \mu$ kat/l)

## 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 800 U/l.

If a  $\Delta A/min$  of 0.400 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 1 U/l.

### Interferences

no interference was observed by the presence of:  
hemoglobin  $\leq$  200 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	44.96	0.41	0.90
sample 2	187.72	1.15	0.60
inter-assay (n=20)	mean (U/l)	SD (U/l)	CV%
sample 1	44.37	0.51	1.10
sample 2	186.70	1.06	0.60

### Methods comparison

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

GGT Chema = x  
GGT competitor = y  
n = 112

$y = 1.10x - 1.11$  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

Szasz G. - Clin. Chem. 22, 2051 (1976)  
Tietz Textbook of Clinical Chemistry, Second Edition, Bur-tis-Ashwood (1994).  
HU Bergmeyer - Method of enzymatic analysis (1987)

## MANUFACTURER

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## 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

