GOT - GPT

Reitman-Frankel. Colorimetric

Quantitative determination of transaminases GOT and GPT

CE IVD

PACKAGING

Ref.: 101-0025	Cont.: 200 tests
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Store at 2-8º C

CLINICAL SIGNIFICANCE

Transaminases GOT and GPT are cellular enzymes, found in highest concentration in heart muscle, the cells of the liver, the cells of the skeletal muscle and in smaller amounts in other weaves. Although an elevated level of GOT and GPT in the serum is not specific of the hepatic disease, is used mainly to diagnostic and to verify the course of this disease. When GOT is used in conjunction with GPT aid in the diagnosis of infarcts in the myocardium, since the value of the GPT stays within the normal limits in the presence of elevated levels of GOT[1,2,5,6].

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD

The glutamic transaminase enzymes, serum glutamic oxalacetic (GOT) and serum glutamic pyruvic (GPT), catalyse the transfers of the amino group of glutamic acid to oxalacetic acid and pyruvic acid in reversible reactions. The transaminase activity is proportional to the amount of oxalate or pyruvate formed over a definite period of time and is measured by a reaction with 2,4- Dinitrophenylhydrazine (DNPH) in alkaline sol.[1,2].

REAGENTS

Ref: 101-0025 **GOT**			
R 1 a Substrate GOT	DL-Aspartate α-Ketoglutarate	pH 7.4	100 mmol/L 2 mmol/L

Ref: 101-0026 **GPT**			
R 1 b Substrate GPT	DL-Alanine α -Ketoglutarate	pH 7.4	200 mmol/L 2 mmol/L

R 2 Developer	2,4-Dinitrophenylhydrazine (DNPH)	1 mmol/L
GOT / GPT –R&F CAL	Primary calibrator of pyruvic acid 1.2 mmol/L	

Adicional reagent: Sodium hydroxide (NaOH) 0.4 N

Optional (not included in the kit)

Contro-N	Ref.: 101-0252	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0083	20 x 5 mL	
Contro-P	Ref.: 101-0253	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0084	20 x 5 mL	

PRECAUTIONS

R2: Corrosive (C). R35: Causes severe burns.

PREPARATION

All the reagents are ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8º C, protected from light and contaminations prevented during their use.

Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 505 nm.
- Thermostatic bath at 37º C (± 1º C)
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Serum[1,2]: Stability 7 days at 2 - 8º C.

PROCEDURE

Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

1. Assay conditions:
   Wavelength: . . . . . 505 nm
   Cuvette: . . . . . 1 cm light path
   Constant temperature . . . . . 15 - 25º C
2. Adjust the instrument to zero with distilled.
3. Pipette into a tubes:

	GOT	GPT
R 1 a Substrate GOT	0.5 mL	--
R 1 b Substrate GPT	--	0.5 mL

4. Mix, incubate for 5 min. at 37º C, add.

Sample	100 µL	100 µL

5. Mix. Return to the bath for: 30 min. 30 min.

R 2 Developer	0.5 mL	0.5 mL

6. Mix. Allow to stand for 20 min at room temperature.

NaOH 0.4 N	5.0 mL	5.0 mL

7. Mix. Let stand for 5 min. at room temperature.
8. Read the initial absorbance (A) against a water blank. The color is stable at least 1 hour.

CALCULATIONS

From absorbances, read units of GOT or GPT from the corresponding calibration curves.

Calibration curve

1. Set up six tubes and pipette (mL):

Tube	1	2	3	4	5	6
Water	0.2	0.2	0.2	0.2	0.2	0.2
GOT	1.0	0.9	0.8	0.7	0.6	0.5
Substrate	0.0	0.1	0.2	0.3	0.4	0.5
Calibrator	1.0	1.0	1.0	1.0	1.0	1.0
DNFH						

Mix. Allow to stand for 20 minutes at room temperature.

NaOH 0.4 N	10.0	10.0	10.0	10.0	10.0	10.0

Mix. Allow to stand for at least 5 minutes.

2. Read against a water blank at 505 nm.

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3. Plot a calibration curve of the absorbances found vs. The corresponding units, on a graph paper, according to the following:

GOT	WU/mL	0	22	55	95	150	215
	U/L	0	11	27	46	72	104
GPT	WU/mL	0	25	50	83	126	--
	U/L	0	12	24	40	62	--

Units
- One Wróblewski unit (WU) of GOT or GPT is defined as the amount of enzyme that will form 4.82 x 10^{-4} µmol of Glutamate/min (25º C).
- To convert WU into international units (U/I), multiply results by 0.482.

QUALITY CONTROL
Control sera are recommended to monitor the performance of assay procedures.
If control values are found outside the defined range, check the instrument, reagents and technique for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

GOT: 8-40 UW / ml (3-18 U/L) **GPT**: 5-30 UW / ml (2-16 U/L)

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS
Measuring range: Up to linearity limit of GOT 180 WU (85 U/L) and GPT 126 WU (62 U/L).
If the results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl (9 g/L) and multiply the result by 10.

Accuracy: Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES
Haemolysis interferes with the assay[1,2]. A list of drugs and other interfering substances with GOT - GOT determination has been reported by Young et. al[3,4].

BIBLIOGRAPHY
1. Murray R. Aspartate aminotransferase. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1112-116.
2. Reitman S, Frankel S.J. Clin Path 1957; 28-56.
3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
4. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
5. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
6. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

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