

GOT-GPT

Colorimetric test. Reitman-Frankel.

Store at 2-8°C

For *in vitro* diagnostic use only.

PRINCIPLE

The glutamic transaminase enzymes, serum glutamic oxalacetic (GOT) and serum glutamic pyruvic (GPT), catalyze the transfer 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 oxaloacetate pyruvate formed over a definite period of time and is measured by a reaction with 2,4-dinitrophenylhydrazine (DNPH) in alkaline solution.

REAGENTS

Reagent -1a	Sustrate GOT (AST)	
	L-aspartate	100 mmol/L
	Ketoglutarate	2 mmol/L
Reagent -1b	Sustrate GPT (ALT)	
	DL-Alanine	200 mmol/L
	Ketoglutarate	2 mmol/L
Reagent -2	Colour reagent	
	2,4-dinitrophenylhydrazine	1 mmol/L
Pyruvic standard.		1.2 mmol/L
Auxiliar reagent. 250ml NaOH 4N (disol. 1:10).		

SAMPLES

Serum, free of hemolysis.

PROCEDURE

	GOT	GPT
Sustrate GOT (R.1a)	0.5 ml	--
Sustrate GPT (R.1b)	--	0.5 ml
Preincubate for 5 min at 37°C.		
Serum	100 µL	100 µL
Mix. Return to bath for	60 min	30 min
DNPH R.2	0.5 ml	0.5 ml
Mix. Allow to stand for 20 min at room temperature.		
NaOH 0.4 N	5.0 ml	5.0 ml
MIX. Let stand for 15 min at room temperature. Read at 505 nm against a water blank. The colour is stable at least 60 minutes.		

CALCULATIONS

From absorbencies, read units of GOT and GPT from corresponding curves.

CALIBRATION (mL)

	1	2	3	4	5	6
Water	0.2	0.2	0.2	0.2	0.2	0.2
GOT R.1a	1.0	0.9	0.8	0.7	0.6	0.5
Pyruvic stand.	--	0.1	0.2	0.3	0.4	0.5
DNPH R.2	1.0	1.0	1.0	1.0	1.0	1.0
Mix. Allow to stand for 20 min at room temperature.						
NaOH 0.4 N	10	10	10	10	10	10
MIX. Allow to stand for at least 15 min. Read against water blank at 505 nm. Plot a calibrator curve of the absorbances found vs. the corresponding units, on a graph paper, according to the following concentrations:						
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	--

LINEARITY

When GOT value exceeds 180 WU/MI (87 U/L) or GPT exceeds 126 WU/MI (61 u/l) repeat test using a 1:10 dilution of serum with saline sol.(9g/L). Multiply the result by 10.

UNITS

$$1 \text{ WU/mL} = 1 \text{ U/L} \times 2.07$$

$$1 \text{ U/L} = 1 \text{ WU/mL} \times 0.483$$

NORMAL VALUES

$$\text{GOT/AST} = 8-40 \text{ WU/ml (3.85-19.3 U/L)}$$

$$\text{GPT/ALT} = 5-30 \text{ WU/ml (2.4 -14.5 U/L)}$$

REFERENCES

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ATLAS Medical
William James House,
Cowley Road, Cambridge, CB4 0WX, UK

Tel: ++44 (0) 1223 858 910

Fax: ++44 (0) 1223 858 524

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