Manual Procedure

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 17081	R1	1	X	40	ml
For 50 tests	R2	1	X	10	ml
Cat. No. 17082	R1	4	X	40	ml
For 200 tests	R2	1	X	40	ml

SGPT/ALT

Alanine-aminotransferase

Kinetic UV method based on IFCC recommendations

Liquid Reagents

Test principle

L-alanine + α-ketoglutarate $\xrightleftharpoons{ALT}$ L-glutamate + pyruvate

pyruvate + NADH + H^+ $\xrightleftharpoons{LDH}$ Lactate + NAD^+

Alanine aminotransferase (ALT) catalyzes the transfer of the amino group from L-alanine to α-ketoglutarate resulting in the formation of pyruvate and L-glutamate. Lactate dehydrogenase (LDH) catalyzes the reduction of pyruvate and the simultaneous oxidation of $NADH^+$ to NAD. The resulting rate of decrease in absorbance at 340 nm is directly proportional to ALT activity.

Concentrations in the test

Reagent R1		
Buffer Tris, pH = 7.5	125	mmol/L
L-alanine	600	mmol/L
LDH	≥ 1.7	KU/L
Detergent, preservative.		
Reagent R2		
NADH	0.18	mmol/L
α-ketoglutarate	15	mmol/L
Detergent, preservative.		

Stability and preparation of working reagent

Reagent R1: *liquid.*

Reagent R2: *liquid.*

All reagents are stable up to expiry date given on the label when stored at +2 ➔ +8 °C.

Working Reagent: (4+1)

Mix 4 volumes of bottle R1 with 1 volume of bottle R2.
Avoid direct exposure to light.
Stability: 3 days at 2 - 8 °C.

Note: Don't use working reagent if its initial absorbance against water at 340 nm is below 0.800

Specimen collection and handling

1. Non-hemolyzed serum, heparinized or EDTA plasma is recommended.
2. Stability: 10 days at 2 - 8° C.

Calibrator

MediCal U Cat. No. 15011

Quality control

Meditrol N Cat. No. 15171
Meditrol P Cat. No. 15181

Procedure

Wavelength	Hg 340 nm (334 - 365 nm)
Spectrophotometer	340 nm
Cuvette	1 cm light path
Temperature	37°C
Measurement	against air or distilled water
Reaction	kinetic – decrease

Assay: Incubate Working Reagent at 37 °C before use:

Sample	50 µl
Working Reagent	1000 µl
Mix, incubate for 2 min. at 37 °C.Read change in absorbance per 1 min. for 3 min. Determine the mean absorbance change per 1 min. (ΔA/min).	

Calculation

SGPT activity in sample (U/L) = (ΔA/min.) X Factor

Factors

Wavelength	334 nm	340 nm	365 nm
Factors at 37°C	3400	3376	6176

Note: It is recommended that each laboratory (as per instrument performance) could make its own factor (F) by the use of a calibrator according to the following formula:

$$F = \frac{\text{Conc.}_{\text{Clibrator}}}{\Delta/\text{min}_{\text{Calibrator}}}$$

Linearity

Wavelength	334 nm	340 nm	365 nm
Linearity at 37°C	410 U/L	400 U/L	370 U/L

At higher concentrations dilute the sample (1+4) with sodium chloride solution (0.9 %) and multiply result by 5.

Very active sera "sometimes" present low initial ΔA since most of the NADH is already consumed prior to measurement. In this case dilute with sodium chloride (0.9 %) as specified and repeat the test as above.

Interferences

1. Hemolysis interferes with the test (Red cells contain ALT which can give false elevated results).
2. Triglycerides up to 400 mg/dl have been found to significantly influence this assay.
3. A number of drugs and substances affect ALT activity.
4. Bilirubin up to 20 mg/dl and hemoglobin up to 400 mg/dl have a negligible effect on this procedure.

SGPT/ALT Kinetic UV method based on IFCC recommendations

Precautions

Reagents contain sodium azide. Don't swallow. Avoid any contact with skin and mucous membranes. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Upon disposal, flush with large amounts of water to prevent azide build up.

Reference range

1 d.		< 31	U/L
2 – 5 d.		< 52	U/L
6 d. – 6 mth.		< 60	U/L
7 – 12 mth.		< 57	U/L
1 – 3 yr.		< 39	U/L
4 – 6 yr.		< 29	U/L
7 – 12 yr.		< 39	U/L
13 – 17 yr.	women	< 23	U/L
	men	< 26	U/L
Adults	women	< 31	U/L
	men	< 41	U/L

References

1. Tietz, N.W., Fundamentals of Clinical Chemistry, W.B. Saunders Co., p 674 (1982).
2. Henry, R.J., et al, Am. J. Clin. Path. 34 : 381 (1960).
3. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.
4. Henry , J,B. Clinical diagnosis and Management by laboratory methods Philadelphia .W. B. Saunders, (2001).
5. Clin. Chim. Acta 105 (1980), 155 - 166.
6. H. U. Bergmeyer, Tutzing (G.F.R.), G. N. Bowers, Conn. Clin. Chem. 23/5. (1977), 887 - 899.
7. International Federation of Clinical Chemistry (IFCC).
8. Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 2. IFCC method for alanine ami-notransferase. J Clin Chem Clin Biochem 1986, 24: 48 1 - 95. Part 3. IFCC method for aspartate aminotransferase. Clin Chem Clin Biochem 1986, 24: 497 - 510.
9. German Society for Clinical Chemistry. Recommendation of carrying out stan-dard ECCLS procedures (1988). for the catalytic concentrations of creatine kinase, aspartate aminotransferase, alanine aminotransferase and y-glutamyltransferase at 37 °C. Eur J Clin Chem Clin Biochem 1993, 31: 901 - 9.