Manual Procedure

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Aldolase

Cat. No. 14041
For 40 tests

R1	5 x	Lyo. For 20 ml
R2	2 x	Lyo. For 1 ml
R3	1 x	0.5 ml

UV-Test, Improved Sensitivity

Lyo. Reagents

Test principle

$$\text{F-1,6-DP} \xrightarrow{\text{Aldolase}} \text{GAP + DAP}$$

$$\text{GAP} \xrightarrow{\text{TPI}} \text{DAP}$$

$$\text{2DAP + 2NADH + 2H}^{+} \xrightarrow{\text{GDH}} \text{2Glycerol-1-phosphate + 2NAD}^{+}$$

Aldolase converts fructose-1,6-diphosphate (F-1,6-DP) to glyceral-dehyde-3-phosphate (GAP) and dihydroxyacetone phosphate (DAP). The addition of triosephosphate isomerase (TPI), glycerol-phosphate dehydrogenase (GDH) and NADH converts the dihy-droxyacetone phosphate to glycerol-1-phosphate. The rate of the aldolase reaction is measured by the decrease in absorbance at 340 nm as a consequence of the conversion of NADH to NAD^{+}.

Concentrations in the test

Reagent R1:		
Collidine buffer pH=7.4	51.0	mmol/L
Mono-iodoacetate	0.27	mmol/L
F-1 ,6-DP	2.7	mmol/L
Reagent R2:		
NADH	12	mmol/L
Reagent R3:		
GDH	≥ 80	KU/L
TPI	≥ 10	KU/L
LDH	≥ 150	kU/L

Stability and preparation of working reagent

Reagent R1: *lyo.*
Reagent R2: *lyo.*
Reagent R3: *liquid*

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

Reagent R1:
Reconstitute the contents of each vial with 20 ml distilled water, let stand for 10 minutes and mix gently. Stability 15 days at 2 - 8 °C.

Reagent R2:
Reconstitute the contents of each vial with 1 ml distilled water, let stand for 10 minutes and mix gently. Stability 30 days at 2 - 8 °C.

Specimen collection and handling

1. Non-hemolyzed serum, heparinized or EDTA plasma is recommended.
2. Stability: 15 days at 2 - 8° C.

Procedure

Wavelength	Hg 340 nm (334 - 365 nm)
Spectrophotometer	340 nm
Cuvette	1 cm light path
Temperature	37°C
Measurement	against sample blank
Reaction	fixed time

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

	Sample Blank	Sample
Sample	200 µl	200 µl
Reagent R1	--	2500 µl
NaCl 0.9% Solution	2500 µl	--
Reagent R2	--	50 µl
Reagent R3	--	10 µl
Mix, incubate for 5 min. at 20-25 °C. Read absorbance A1. incubate for exactly 20 min. at 37 °C after the first reading and Read absorb-ance A2. against sample blank. Calculated **A= A1- A2**.		

Note: if A1 is more than 0.95 dilute the sample (1+1) with sodium chloride solution (0.9 %) and re-assay. multiply the result by 2.

Calculation

Aldolase activity in sample (U/L) **= A X Factor**

Factors

Wavelength	334 nm	340 nm	365 nm
Factors at 37°C	55.8	54.8	101.5

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

Up to 30 U/L.
If the result exceeds 30 U/L, repeat the test using diluted serum (1+9) with sodium chloride solution (0.9 %) and multiply the result by 10.

Interferences

A number of drugs and substances affect ALT activity.

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 dis-posal, flush with large amounts of water to prevent azide build up.

Reference range

Adults	< 7.6	U/L

References

1. Kaplan, L.A., Pesce, A.J.:"Clinical Chemistry", Mosbay Ed.(1996)
2. Tietz, N.W., Fundamentals of Clinical Chemistry, W.B. Saunders Co.,
3. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.