ALDOLASE

Kinetics UV Aldolase determination in serum and plasma

TEST SUMMARY

Aldolase convert fructose-1,6-difosphate (F-1,6-DP) to glyceraldehyde-3-phosfate (GAP) and dihydroxyacetone phosphate (DAP). The addition of triosephosphate isomerase (TPI), glycerolphosphate dehydrogenase (GDH) and NADH converts the dihydroxyacetone 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 in NAD^+.

F-1,6-DP —(Aldolase)→ GAP + DAP

GAP —(TPI)→ DAP

$2DAP + 2NADH + 2H^+$ —(GDH)→ 2 glycerol-1-phosphate + $2\ NAD^+$

SAMPLE

Serum, plasma (heparinate or EDTA).

REAGENTS

Reagent A:	Collidine buffer pH 7.4,mono-iodoacetate, F-1,6-DP. Liophile
Reagent B:	NADH.
Reagent C:	GDH, TPI, LDH.

MATERIAL REQUIRED BUT NOT SUPPLIED

Normal laboratory equipment. Spectrophotometer UV/VIS with thermostatation. Automatic Micropipette. Cuvette in optical glass or monouse in optical polystyrene. Physiologic solution.

PRECAUTION

Reagent may contain not reactive and conservative components. It is opportune to avoid contacts with the skin and do not swallow.
Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

REAGENTS PREPARATION

Reconstitute one vial of Reagent A with 20 ml of redistilled water. Melt carefully until complete dissolving.
Reagent B and Reagent C are ready to use.
Reagents are stable until expiration date on label, stored at 2-8°C.
Reconstituted Reagent A is stable 15 days at 2-8°C.
Bring the reagents at work temperature before use.

Concentration of each component in the final volume of reaction:
collidine 51 mmol/l; mono-iodoacetate 0.27 mmol/l; F-1,6-DP 2.7 mmol/l; NADH 0.22 mmol/l; GDH ≥ 326 U/l; TPI ≥ 4350 U/l; LDH ≥ 616 U/l.

PROCEDURE

Method:	kinetics in decrease
Wavelength:	340 nm (334 – 365)
Cuvette:	1 cm
Temperature:	37°C
Zero:	sample blank

Reagents	Sample Blank	Sample
Sample	200 µl	200 µl
Reagent A	--	2.5 ml
Physiologic Solution	2.5 ml	--
Reagent B	--	50 µl
Reagent C	--	10 µl

Mix carefully and incubate for 5 min at 37°C.
Read absorbance A1 against sample blank.
Allow to stand at 37°C for exactly 20 min after first reading and then measure absorbance A2 against blank.

CALCULATION

$\Delta A = A1 - A2$

Aldolase Activity (U/l) a 37°C

340 nm = ΔA x 54,8

334 nm = ΔA x 55,8

365 nm = ΔA x 101,5

Aldolase Activity (U/l) a 25°C

Obtained value a 37°C x 0,41

Aldolase Activity (U/l) a 37°C

Obtained value a 25°C x 2,44

(If A1 < 0.95 dilute the sample, perform again the test and multiply the result by 2)

EXPECTED VALUES

SERUM/PLASMA

25°C	≤ 3.1 U/
37°C	≤ 7.6 U/l

Every laboratory should establish his own intervals reference according to his own population.

NOTES

- If the results are incompatible with clinical presentation, they have to be evaluated within a total clinical study.
- Only for IVD use.

CALIBRATION/ QUALITY CONTROL

It is suggested to perform an internal quality control. For this purpose the following control sera on animal base are available on request:

CC03330 **2 x 1 ml**
ALDOLASI Control set (2 levels)

CC03340 **2 x 1 ml**
ALDOLASI Calibration Sera

TEST PERFORMANCE

Precision

Intra-assay (n = 10)	Mean (U/l)	SD (U/l)	CV%
Sample 1	3.03	0.094	3.13
Sample 2	8.08	0.168	2.09
Sample 3	31.56	0.783	2.48

Inter-assay (n = 20)	Mean (U/l)	SD (U/l)	CV%
Sample 1	6.19	0.185	2.99
Sample 2	43.82	1.911	4.38

Sensitivity/cut-off
Cut-off as from 1.1 U/l.

Linearity
For ΔA exceeds 0.500 at 334/340nm or 0.250 a 365nm, repeat the determination on a dilute sample 1: 10 with physiological solution and multiplying results by 10.

Methods comparison
A comparison with an available commercial method gave following results on 102 samples compared:

Aldolase LTA = y
Aldolase competitors = x
n = 102

y = 0,15177 + 0,96281 x r = 0,99879

Interferences
Haemolysis interferes with the test.

WASTE DISPOSAL

Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

PACKAGING

CODE CC02700	(40 TESTS)	
Reagent A	5 x 20 ml	(liophile)
Reagent B	1 x 2 ml	(liquid)
Reagent C	1 x 0,5 ml	(liquid)

REFERENCES

Feissil, S., et All., (1966). Klin. Wschr. 44: 390.

MANUFACTURER

LTA s.r.l.
Via Milano 15/F
20060 Bussero (Milan) ITALY
Tel: ++39 02 95409034
Fax: ++39 02 95334185
e-mail: info@ltaonline.it
Website: http://www.ltaonline.it

SYMBOLS

IVD	Only for IVD use
LOT	Lot of manufacturing
REF	Code number
	Storage temperature interval
	Expiration date (year, month)
	Warning, read enclosed documents
	Read the directions
	Biologic risk

Mod. 01.06 (ver. 1.6 - 04/03/2006)

CE