Manual Procedure

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 17121	R1	1	X	40	ml
For 50 tests	R2	1	X	10	ml
Cat. No. 17122	R1	4	X	40	ml
For 200 tests	R2	1	X	40	ml

SGOT/AST

Aspartate-aminotransferase

Kinetic UV method based on IFCC recommendations

Liquid Reagents

Test principle

L-aspartate + α-ketoglutatrate $\xrightleftharpoons{AST}$ L-glutamate + oxaloacetate

Oxaloacetate + NADH + H^+ $\xrightleftharpoons{MDH}$ L-malate + NAD^+

Aspartate aminotransferase (AST) catalyzes the transfer of the amino group from L-aspartate to α-ketoglutarate to yield oxaloacetate and L-glutamate. Malate dehydrogenase (MDH) catalyzes the reduction of oxaloacetate with simultaneous oxidation of $NADH^+$ to NAD. The resulting rate of decrease in absorbance at 340 nm is directly proportional to the AST activity. Lactate dehydrogenase (LDH) is added to prevent interference from endogenous pyruvate which is normally present in serum.

Concentrations in the test

Reagent R1		
Buffer Tris, pH = 7.5	125	mmol/L
L-aspartate	290	mmol/L
LDH	≥ 0.9	U/L
MDH	≥ 0.6	U/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 the 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.
Stability: 3 days at 2 - 8 °C.

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

Specimen collection and handling

1. Non-hemolyzed serum, or EDTA plasma.
2. Stability: 7 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

SGOT 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 sample (1+4) with sodium chloride solution (0.9 %) and multiply the result by 5.

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

Interferences

1. Hemolysis interferes with the test (red cells contain AST which can give falsely 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 AST activity.
4. Bilirubin up to 20 mg/dl, and hemoglobin up to 400 mg/dl have a negligible effect on this procedure.
5. Patient with severe vitamin B6 deficiency could have a decrease in the recovery of AST presumably due to lack of pyridoxal phosphate.

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.

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Reference range

1 d.		< 122	U/L
2 – 5 d.		< 110	U/L
6 d. – 6 mth.		< 84	U/L
7 – 12 mth.		< 89	U/L
1 – 3 yr.		< 56	U/L
4 – 6 yr.		< 39	U/L
7 – 12 yr.		< 50	U/L
13 – 17 yr.	women	< 27	U/L
	men	< 35	U/L
Adults	women	< 31	U/L
	men	< 37	U/L

References

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4. Kaplan, L.A., Pesce, A.J., Clinical Chemistry, St. Louis, C.V. Mosby, p.911 - 912 (1989).
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8. German Society for Clinical Chemistry. Recommendation of carrying out standard 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.