PRINCIPLE:

AST catalyses the transfer of amino group from L-aspartate (ASP) to alpha ketoglutarate resulting in the formation of oxaloacetate and L-glutamate. The oxaloacetate (OA) is converted to L-malate with the help of malate dehydrogenase (MDH) using NADH. The Oxidation of NADH is measured at 340nm.

L-Aspartate + 2-Oxoglutarate $\xrightarrow{AST}$ OA+ L-Glutamate

OA + NADH + H+ $\xrightarrow{MDH}$ L-Lactate +NAD^+

REAGENT COMPOSITION:

1. Reagent 1 (Enzyme)
2. Reagent 2 (Substrate)

REAGENT PREPARATION:

Mix 4 parts of R1 with 1 part of R2. The combined Reagent is stable for 4 weeks at 2°-8° C.

STORAGE & STABILITY:

Store at 2-8° C, and keep away from light. Unopened reagent is stable until expiry date stated on the label.

SAMPLE:

Unhemolysed serum or heparinised plasma.
ALT is stable in serum or plasma for:

- 24 hours at room temperature.
- 7 days at 2-8° C.

AUTOMATED PARAMETERS

Parameter	Kinetic test
Reaction type	Kinetic
Wavelength	340 nm
Reaction temperature	37° C
Blank	Against distilled water
Reaction	Decreasing
Sample volume	100 µl
Reagent Volume (R1): (R2)	1000 µl (800 µl + 200 µl)
Sample / Reagent ratio	1: 10
Delay/ Lag time	60 sec
Delta absorbance (ΔA)	60sec
Measurement/ test time	180 sec
Blank absorbance limit	> 1. 0 O.D
Factor	1764
Linearity	300 U/L

PROCEDURE:

Let stand reagents and specimens at room temperature.

Sample	100µl
Reagent volume R1	800µl
Reagent volume R2	200 µl

Mix 4 parts of R1 with 1 part of R2 ONLY NEEDED.

CALCULATIONS:

Calculate the result as follows:

ALT activity (U/L) = (Abs/min) x 1746

Expected Value:

New born: 39-117 U/L
Infant : 23-94 U/L
Adult : 13-31 U/L

Each lab should optimize its own normal range.

REFERENCE:

1. Bergmeyer H.U. and Brwers G.N., Clin Chem Acta 70, 1942 F (1976).
2. Bergmeyer, H.U. at al., Clin. Chem 24, 58-73, (1978).