TECO DIAGNOSTICS
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ALT (SGPT) KINETIC METHOD
TC MATRIX-160/240

INTENDED USE
For the quantitative determination of alanine aminotransferase in serum used in routine examination and monitoring of therapy and relapses.

INTRODUCTION
The enzyme alanine aminotransferase is widely reported in a variety of tissue sources. The major source of ALT is of hepatic origin and has led to the application of ALT determinations to the study of hepatic diseases. Elevated serum levels are found in hepatitis, cirrhosis, and obstructive jaundice. Levels of ALT are only slightly elevated in patients following a myocardial infarction.

UV methods for ALT determination were first developed by Wroblewski and LaDue in 1956. The method was based on the oxidation of NADH by lactate dehydrogenase (LDH). In 1980, the International Federation of Clinical Chemistry recommended a reference procedure for the measurements of ALT based on the Wroblewski and LaDue procedure. The ALT reagent conforms to the formulation recommended by the IFCC.

PRINCIPLE
The enzymatic reaction sequence employed in the assay of ALT is as follows:

$$\text{L-Alanine} + \text{a-ketoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate} + \text{L-Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^+ + \text{H}_2\text{O}$$

The pyruvate formed in the first reaction is reduced to lactate in the presence of lactate dehydrogenase and NADH. The activity of ALT is determined by measuring the rate of oxidation of NADH at 340 nm. Endogenous sample pyruvate is converted to lactate by LDH during the lag phase prior to measurement.

REAGENT PREPARATION
No preparation is required.

REAGENT COMPOSITION
a-Ketoglutarate: 16 mmol/L
Lactate dehydrogenase (LD):>2300 IU/L
L-Alanine: 500 mmol/L
Tris Buffer: 97 mmol/L
NADH: 0.18 mmol/L
Also non-reactive chemicals for optimal system performance

REAGENT STORAGE AND STABILITY
Alanine Aminotransferase Reagent stored unopened at 2°C to 8°C is stable until the expiration date shown on the bottle label. Once opened, Alanine Aminotransferase Reagent is stable for 14 days, or until the expiration date on the bottle label whichever occurs first.
DO NOT FREEZE.

REAGENT STORAGE
Reagents are stable until the expiration date on their respective labels, when properly stored at 2 - 8°C and protected from light. Reagents should appear clear and colorless.

REAGENT DETERIORATION
1. Discard if either appears cloudy or contains particulate matter.
2. The working reagent is stable for 2 weeks at 2-8°C. The working reagent should be discarded if the initial absorbance, read against distilled water at 340 nm, is below 1.000.

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT
At least two levels of control material.

SPECIMEN COLLECTION AND STORAGE
Non-hemolyzed serum is the specimen of choice. Whenever possible specimens should be separated and analyzed on the day of collection. Store serum in stoppered tubes. About 10% ALT is lost in 3 days at 4°C and in 1 day at 25°C.

INTERFERING SUBSTANCES
Hemolysis must be avoided as the concentration of ALT in red cells is roughly 5 times that of serum.[2] Bilirubin levels up to 40 mg/dL and triglyceride levels up to 2000 mg/dL show no interference in this test. Certain drugs and other substances are also known to affect ALT values.

PROCEDURES
Settings for TC-Matrix 160

Test Name:	ALT	R1:	120
Full Name: Alanine Aminotransferase		R2:	25
Pri. Wave:	340 nm	Sample volume:	10.0
Sec. Wave:	700nm	Calibration Type:	2 point linear
Assay/ Point:	Kinetic	K Value:	/
Start - End:	14 - 21	Point:	2
Decimal place:	1	Blank Type:	Water
Unit:	U/L	Point 0 (Blank) Con.:	0.0
Linearity Range:	2.0000 - 750.0000	Point 1 (STD) Con.:	Standard/
Correlation Factor:	1.0000 - 0.0000		Calibrator

Settings for TC-Matrix 240

Test Name:	ALT	R1:	120
Full Name: Alanine Aminotransferase		R2:	25
Pri. Wave:	340 nm	Sample volume:	7.0
Sec. Wave:	700nm	Calibration Type:	2 point linear
Assay/ Point:	Kinetic	K Value:	/
Start - End:	20 - 27	Point:	2
Decimal place:	1	Blank Type:	Water
Unit:	U/L	Point 0 (Blank) Con.:	0.0
Linearity Range:	2.0000 - 750.0000	Point 1 (STD) Con.:	Standard/
Correlation Factor:	1.0000 - 0.0000		Calibrator

QUALITY CONTROL
It is recommended that controls be included in each set of assays. Commercially available control material with established ALT values may be routinely used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to

obtain the proper range of values in the assay of control material may indicate either reagent deterioration, instrument malfunction, or procedure errors.

CALIBRATION

1. Calibrator required: TECO MULTI Calibrator is needed but is not provided in the kit.
2. The system must have a valid calibration in memory before controls or patient samples can be run.
3. The TC Matrix system will automatically perform checks on the calibration and produce data at the end of calibration.

EXPECTED VALUES

Normal Range: Male: 10 – 40 U/L (37°C)
Female: 7 – 35 U/L (37°C)

It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories, and local populations.

PERFORMANCE CHARACTERISTICS

Analytical Range: 2 – 750 IU/L

For Alanine Aminotransferase analyte by Alanine Aminotransferase Reagent on TC Matrix System, this method has been demonstrated to be linear from **:** 2 – 750 IU/L

Accuracy: Comparison study was performed on TC Matrix system from 40 samples. Beckman Coulter Alanine Aminotransferase Reagent was used to compare with Alanine Aminotransferase Reagent. The results of this study in yield a correlation coefficient of 0.98 with a regression equation of y=0.98x – 1.2.

Precision: Within Run precision for Alanine Aminotransferase Reagent Set was determined following a modification of NCCLS EP5-A. Two commercial human sera were assayed on TC Matrix System for 25 times.

Sample	Sample 1	Sample 2
N	25	25
Mean (mg/dl)	39	97
Standard Deviation (mg/dl)	1.7	2.8
Coefficient of Variation (%)	3.7	3.3

Run-Day precision for Alanine Aminotransferase Reagent was determined following a modification of NCCLS EP5-A. Two commercial human sera were assayed on TC Matrix Systems five times per day for five days for the total of 25 values.

Sample	Sample 1	Sample 2
N	25	25
Mean (mg/dl)	38	97
Standard Deviation (mg/dl)	1.5	2.6
Coefficient of Variation (%)	3.7	2.7

WARNINGS AND PRECAUTIONS

Normal precautions exercised in handling laboratory reagents should be followed. The reagents contain sodium azide, which may be toxic if ingested. Sodium azide may also react with lead and copper plumbing to form highly explosive metal azides. Refer to Material Safety Data Sheet for any updated risk, hazard, or safety information.

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Manufactured by:

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