MEDICA

IVD CE

REF 10205-4 4 X 31 mL / 7 mL

ALANINE AMINOTRANSFERASE (ALT)

Wedges each contain a usable volume of 31 mL of R1 reagent; The 10 mL bottle contains 7 mL of R2 reagent.

INTENDED USE

The EasyRA ALT reagent is intended for the quantitative determination of alanine aminotransferase activity in human serum and plasma (with lithium heparin as anticoagulant), using the MEDICA "EasyRA® Clinical Chemistry Analyzer."
For *in vitro* diagnostic use only. For professional use only.

SUMMARY AND EXPLANATION

Alanine Aminotransferase is an intracellular enzyme involved in amino acid and carbohydrate metabolism and is released with tissue damage. Alanine aminotransferase measurements are used in the diagnosis and treatment of certain liver diseases (e.g., viral hepatitis and cirrhosis). In hepatic necrosis, the elevation of ALT occurs prior to the appearance of jaundice.[1]

PRINCIPLE OF THE PROCEDURE

This method is based on the procedures of Wroblewski and Ladue[2], based on the oxidation of NADH by lactate dehydrogenase (LDH). The reagent is based on modifications of the IFCC[3] and Bergmeyer[4] methods.

$$\alpha\text{-Ketoglutarate} + \text{L-Alanine} \xrightarrow{\text{ALT}} \text{L-Glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{reduced cofactor} + H^+ \xrightarrow{\text{LDH}} \text{Lactate} + \text{cofactor}$$

The rate of decrease in absorbance of the reaction mixture at 340 nm, due to the oxidation of the reduced cofactor, is directly proportional to the ALT activity in the sample.

REAGENTS

ALT Buffer Reagent (R1):

L-Alanine	500 mmol/L
LDH (rabbit muscle)	1200 U/L
Tris buffer, pH 7.5	100 mmol/L

ALT Substrate Reagent (R2):

α-Ketoglutarate	15 mmol /L
NADH, disodium salt	0.18 mmol/L

Nonreactive ingredients:

Buffers, stabilizers and preservative.

PRECAUTIONS

1. Good laboratory safety practices should be followed when handling any laboratory reagent. (CLSI, GP17-A2).
2. The reagents contain less than 0.1% sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Refer to the Safety Data Sheet for risk, hazard and safety information.
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Do not use washed cuvettes.

INSTRUCTIONS FOR REAGENT HANDLING, STORAGE AND STABILITY

The R1 and R2 reagents must be combined in the wedge before use. Unopened reagents are stable until the expiration date listed on the label if stored at 2 – 8°C. The working reagent is stable on-board in the refrigerated reagent area of the EasyRA Analyzer for the number of days programmed on the RFID chip on the reagent wedge. Do not use the reagent if it is turbid or cloudy or if it fails to recover known serum control values.

SPECIMEN COLLECTION AND STORAGE / STABILITY

Lithium heparin coated tubes may be used for plasma collection. Clear unhemolyzed samples should be used. Serum ALT is stable for 24 hours at 18 – 25°C and 7 days at 2 – 8°C.[5]

Limitations
Hemolysis must be avoided, as the concentration of ALT in red blood cells is approximately 3-5 times that of the serum.[6]

PROCEDURE

Materials Provided
Medica ALT Reagent Wedge/R2 Bottle, REF 10205

Additional materials required
Medica EasyQC® Chemistry/Electrolytes – Level A, REF 10793
Medica EasyQC Chemistry/Electrolytes – Level B, REF 10794
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry & ISE, REF 10660 *or*
Medica Cleaner Wedge – Chemistry, REF 10661

Instructions for Use
The R1 and R2 reagents must be combined in the wedge before use. The R1 reagent is in the wedge. Add the entire contents of the small bottle containing the R2 reagent to the wedge and mix well by inversion before use. There will be a total of 38 mL of usable working reagent after mixing. Remove the cap on the working reagent and place the reagent in the EasyRA Analyzer reagent tray located in the reagent area. The on-board stability (44 days maximum) of the working reagent is programmed on the RFID chip on the reagent wedge.

Note: Check inside the neck of the wedge for foam after removing the cap and placing the wedge on the analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test.

Calibration
Not applicable.

Quality Control
It is recommended that two levels of human serum based controls (normal and abnormal) be run with the assay daily whenever patient testing is performed and with each reagent lot change. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction or procedural errors. The laboratory should follow local, state and federal quality control guidelines when using quality control materials.

Results
After completion of the assay, the EasyRA Analyzer calculates the ALT concentration from change in the absorbance per minute, sample volume, total reaction volume, pathlength(cm) of 0.6 and molar absorptivity of 6.22.

$$\text{ALT (U/L)} = (\Delta\text{A/Min}) \times \frac{(\text{Total Volume}(\mu l) \times 1000)}{(\text{Molar absorptivity} \times \text{Pathlength(cm)} \times \text{Sample Volume}(\mu l))}$$

Expected Values[5]
The reference range for ALT in serum is as follows:

Adult Male:	10-40 U/L
Adult Female:	7-35 U/L
Newborn:	13-45 U/L

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

Procedural Limitations (e.g., if sample is above assay range)
Only unhemolyzed serum samples should be used.

If the Absorbance Change per Minute (ΔA/Min) is greater than 0.032, which corresponds to 500 U/L, results will be flagged with "SD" (substrate depletion) by the analyzer. If the "Re-run" icon is selected by the operator, the sample may be re-tested using one half (1/2) the sample volume. The retest results are calculated to reflect the use of the smaller sample volume. This will extend the reportable range of the ALT test to 1000 U/L.

PERFORMANCE CHARACTERISTICS[7]

Reportable Range
The reportable range is 5.0 to 500 U/L. Extended range is 5.0 to 1000 U/L when half of the sample is used (1:1 dilution).

Inaccuracy / Correlation (CLSI, EP9-A2)
The following table lists the data obtained in a comparison of the new Medica Reagent for ALT (y) on the EasyRA Analyzer to the performance of the prior ALT reagent (x) on the EasyRA Analyzer. The data shown below represents single determinations with the new Medica reagent for ALT on the EasyRA Analyzer vs. the average of two replicate values obtained with the prior Medica reagent for ALT on the EasyRA Analyzer.

Number of samples	80	Range of Samples	11.5 to 455.1 U/L
Slope	1.0239	y Intercept	-0.8187
Correlation Coefficient	0.9996	Regression Equation	Y= 1.0239*X – 0.8187

The following table lists the data obtained in a comparison of matched serum (x) and plasma (y) samples using the Medica Reagent for ALT on the Medica EasyRA Chemistry Analyzer. The data below represents a single plasma determination vs. the average of two replicate serum values.

Number of Samples	60	Range of Samples	7.6 to 449.3 U/L
Slope	1.0027	y Intercept	-0.4762
Correlation Coefficient	0.9992	Regression Equation	Y = 1.0027*X – 0.4762

Imprecision (CLSI, EP5-A2)
Duplicate measurements of each of three levels of QC material were tested twice a day for 20 days. Both within-run precision and total precision were determined from these data.

Within run imprecision:

QC Level U/L	Within Run SD U/L	Within Run CV %
205.2	1.91	0.93
90.7	1.52	1.68
27.4	0.75	2.72

Total Imprecision:

QC Level U/L	Total Imprecision SD U/L	Total Imprecision CV %
205.2	3.02	1.47
90.7	1.95	2.16
27.4	1.00	3.65

Linearity (CLSI, EP6-A)
Linear from 5.0 to 500 U/L, based on the linear regression equation Y = 0.998*X + 1.2333.

Limit of Blank (LOB):	2.4 U/L	(NCCLS, EP17-A)
Limit of Detection (LOD):	4.2 U/L	(NCCLS, EP17-A)

Interfering Substances (NCCLS, EP7-A)
Less than 10% interference was classified as “no significant interference.”
There is significant interference from hemolysis. Hemolysis must be avoided, as the concentration of ALT in red blood cells is approximately 5 times that of the serum. Do not use hemolyzed samples.
No significant interference was found in levels up to 28 mg/dL of bilirubin.
No significant interference was found in levels up to 1500 mg/dL of triglycerides (using Intralipid*).

**Intralipid is a registered trademark of Pharmacia AB, Clayton, NC.*

Young provides a list of drugs and other substances that interfere with clinical chemistry tests.[8, 9]

REFERENCES

1 Burtis CA, Ashwood ER. *Tietz Textbook of Clinical Chemistry, 2nd ed.* Philadelphia, PA: WB Saunders Co.; 1994: 790-791.
2 Wroblewski, F. and LaDue, J.S., Proc. Soc. Exper. Biol. And Med. 91:569 (1956).
3 International Federation of Clinical Chemistry, Provisional Concentrations of Enzymes, Clin Chem 23: 887, 1977.
4 Bergmeyer HU, Scheibe P, Wahlefeld, AW: Optimization of methods for aspartate aminotransferase and alanine aminotrasferase. Clin Chem 24: 58, 1978.
5 Burtis, C.A., Ashwood, E.R. (Ed.) *Tietz Textbook of Clinical Chemistry,* W.B. Saunders Co. Toronto, p 1800 (1999).
6 Henry R.J. *Clinical Chemistry-Principles and Technics.* New York, NY: Harper & Row; 1974: 881, 888.
7 Data on file at Medica Corporation.

8 Young DS. *Effects of Drugs on Clinical Laboratory Tests* 4th ed. Washington, DC: AACC Press, 1995.
9 Young DS. *Effects of Preanalytical Variables on Clinical Laboratory Tests*. 2nd ed. Washington, DC. AACC Press,1997.

EasyRA Assay Parameters (ALT)

Wavelength (nm)	340/405 nm
Reaction Type	Enzyme (0)
Reagent Direction	Decrease
Reagent Blank	No
Sample Blank	No
Max. first interval Abs. change	0.032
Reaction Time	5.6 min
Calibration interval (maximum)	N/A
Reagent on-board stability	44 days

Serum/Plasma

Sample volume (µl)	8.0
Diluent Volume (µl)	32
Reagent Volume (µl)	152
Decimal Places (default)	1
Units (default values)	U/L
Dilution Factor	1:1 (to extend measuring range)
Linearity	5.0 to 500 U/L
Molar Absorptivity	6.22

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