08056773500V6.0

ALTP

cobas®

Alanine Aminotransferase acc. to IFCC with pyridoxal phosphate activation

Order information

REF	(i)	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08056773190	08056773500	Alanine Aminotransferase acc. to IFCC (450 tests)	System-ID 2013 001	**cobas c** 303, **cobas c** 503

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	
08062986190	Pyridoxal phosphate (950 tests)	System-ID 2012 001	

English

System information

ALTP: ACN 20130

Intended use

In vitro test for the quantitative determination of alanine aminotransferase (ALT) with pyridoxal phosphate activation in human serum and plasma on **cobas c** systems.

Summary

Alanine aminotransferase (ALT) measurements, performed with this device, in human serum and plasma are used as an aid in diagnosis of hepatocellular injury and in monitoring chronic liver injury.

The enzyme alanine aminotransferase (ALT) is present in highest concentrations in the liver, in the cytosol of the hepatocytes, although it is also found in the kidney, and, in much smaller quantities, in heart and skeletal muscle cells.[1] ALT catalyzes the transfer of amino groups from L-alanine to α-ketoglutarate, resulting in the converted products L-glutamate and pyruvate. This is a critical process of the tricarboxylic acid cycle, in which the coenzyme pyridoxal phosphate (also known as pyridoxal-5-phosphate or active vitamin B6) is required. When liver injury occurs, ALT is released from injured liver cells and causes a significant serum elevation.[1] Measurement of ALT activity is therefore used for the diagnosis of hepatic diseases such as acute and chronic viral hepatitis, nonalcoholic fatty liver disease (NAFLD), alcohol-related liver disease, ischemic hepatopathy, autoimmune hepatitis, biliary injury, suspected malignant infiltration, cholestasis.[1] Serum elevations of ALT activity are rarely observed in conditions other than parenchymal liver disease.[2] In addition, ALT testing is recommended for monitoring chronic hepatitis status and progression.[3]

Although both serum aspartate aminotransferase (AST) and ALT become elevated whenever disease processes affect liver cell integrity, evidence suggests that ALT is a more specific marker of hepatic injury than AST. Moreover, elevations of ALT activity persist longer than elevations of AST activity.[1,4]

In patients with vitamin B6 deficiency (insufficient endogenous pyridoxal phosphate), serum aminotransferase activity may be decreased. The addition of pyridoxal phosphate to this assay causes an increase in aminotransferase activity (activation higher for AST than for ALT). Pyridoxal phosphate activation prevents falsely low aminotransferase activity in patient samples with insufficient endogenous pyridoxal phosphate (vitamin B6 deficiency).[5]

Test principle

This assay follows the recommendations of the IFCC, but was optimized for performance and stability.[6,7]

ALT catalyzes the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD^+ . Pyridoxal phosphate serves as a coenzyme in the amino transfer reaction. It ensures full enzyme activation.

$$\text{L-Alanine + 2-oxoglutarate} \xrightarrow{\text{ALT}} \text{pyruvate + L-glutamate}$$

$$\text{Pyruvate + NADH + H}^+ \xrightarrow{\text{LDH}} \text{L-lactate + NAD}^+$$

The rate of the NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance.

Reagents - working solutions

Alanine Aminotransferase acc. to IFCC (ALTL)

R1 TRIS buffer: 224 mmol/L, pH 7.3 (37 °C); L-alanine: 1120 mmol/L; albumin (bovine): 0.25 %; LDH (microorganisms): ≥ 45 µkat/L; stabilizers; preservative

R3 2-Oxoglutarate: 94 mmol/L; NADH: ≥ 1.7 mmol/L; additives; preservative

R1 is in position B and R3 is in position C.

Pyridoxal phosphate (PYP, Cat. No 08062986190)

R2 Pyridoxal phosphate: 730 µmol/L; additives; preservative

R2 is in position B.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum
Plasma: Li-heparin and K_2-EDTA plasma

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

Stability:	3 days at 15-25 °C[8]
	7 days at 2-8 °C[8]
	> 7 days at (-60)-(-80) °C

Freeze only once.

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section

General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

Test definition

Reporting time	10 min		
Wavelength (sub/main)	700/340 nm		
Reagent pipetting		Diluent (H_2O)	
R1	44 µL	24 µL	
R2	15 µL	–	
R3	15 µL	15 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	6.8 µL	–	–
Decreased	6.8 µL	10 µL	90 µL
Increased	6.8 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against the original IFCC formulation using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.[5]

Quality control

For quality control, use control materials as listed in the “Order information” section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory’s individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 12 weeks. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

cobas c systems automatically calculate the analyte activity of each sample in the unit U/L (µkat/L).

Conversion factor: U/L × 0.0167 = µkat/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at an ALT activity of 35 U/L.

Icterus:[9] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[9] No significant interference up to an H index of 170 (approximate hemoglobin concentration: 106 µmol/L or 170 mg/dL). Contamination with erythrocytes will elevate results, because the analyte level in erythrocytes is higher than in normal sera. The level of interference may be variable depending on the content of analyte in the lysed erythrocytes.

Lipemia (Intralipid):[9] No significant interference up to an L index of 150. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.
Lipemic samples may cause > Abs flagging.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[10,11] Exception: Calcium dobesilate can cause artificially low ALT results at therapeutic concentrations.

Cyanokit (Hydroxocobalamin) may cause interference with results.

Physiological plasma concentrations of Sulfasalazine or Sulfapyridine may lead to false results.

In very rare cases, gammopathy, in particular type IgM (Waldenström’s macroglobulinemia), may cause unreliable results.[12]

For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

ACTION REQUIRED
Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator’s manual.

Limits and ranges

Measuring range

5-700 U/L (0.08-11.7 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 5 U/L (0.08 µkat/L)
Limit of Detection	= 5 U/L (0.08 µkat/L)
Limit of Quantitation	= 6 U/L (0.10 µkat/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the activity below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low activity samples.

The Limit of Detection corresponds to the lowest analyte activity which can be detected (value above the Limit of Blank with a probability of 95 %).

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The Limit of Quantitation is the lowest analyte activity that can be reproducibly measured with a total error of 20 %. It has been determined using low activity alanine aminotransferase samples.

Expected values

U/L*

Acc. to IFCC/Standard Method 94 with pyridoxal phosphate activation measured at 37 °C:[13]

Males	10-50 U/L	Females	10-35 U/L

Consensus values with pyridoxal phosphate activation:[14]

Males	up to 50 U/L	Females	up to 35 U/L

*calculated by unit conversion factor

µkat/L

Acc. to IFCC/Standard Method 94 with pyridoxal phosphate activation measured at 37 °C:[13]

Males	0.17-0.83 µkat/L	Females	0.17-0.58 µkat/L

Consensus values with pyridoxal phosphate activation:[14]

Males	up to 0.83 µkat/L	Females	up to 0.58 µkat/L

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Repeatability	*Mean U/L*	*SD U/L*	*CV %*
PCCC1[a)]	49.5	0.457	0.9
PCCC2[b)]	121	0.607	0.5
Human serum 1	12.0	0.265	2.2
Human serum 2	30.0	0.402	1.3
Human serum 3	49.6	0.440	0.9
Human serum 4	351	1.96	0.6
Human serum 5	620	2.96	0.5

Intermediate precision	*Mean U/L*	*SD U/L*	*CV %*
PCCC1[a)]	49.5	0.629	1.3
PCCC2[b)]	121	0.977	0.8
Human serum 1	12.0	0.341	2.9
Human serum 2	31.9	0.469	1.5
Human serum 3	49.6	1.20	2.4
Human serum 4	349	2.75	0.8
Human serum 5	634	3.67	0.6

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s).

Method comparison

ALT values for human serum and plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 91

Passing/Bablok[15]	Linear regression
y = 0.993x + 1.14 U/L	y = 0.992x + 1.22 U/L
τ = 0.987	r = 1.000

The sample activities were between 7.02 and 695 U/L.

ALT values for human serum and plasma samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 65

Passing/Bablok[15]	Linear regression
y = 1.000x + 1.83 U/L	y = 0.978x + 3.53 U/L
τ = 0.974	r = 1.000

The sample activities were between 9.63 and 684 U/L.

References

1 Kim WR, Flamm SL, Di Bisceglie AM, et al. Public Policy Committee of the American Association for the Study of Liver Disease. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology 2008 Apr;47(4):1363-1370. doi: 10.1002/hep.22109.

2 Panteghini M, Bais R. Amino Transferases. In: Burtis CA, Ashwood ER, Bruns DE. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 5th ed. 2012;573-576.

3 Guidelines for the Prevention, Care and Treatment of Persons with Chronic Hepatitis B Infection. Geneva: World Health Organization; 2015 Mar.

4 Kwo PY, Cohen SM, Lim JK. ACG Clinical Guideline: Evaluation of Abnormal Liver Chemistries. Am J Gastroenterol 2017 Jan;112(1):18-35. doi: 10.1038/ajg.2016.517.

5 Schumann G, Bonora R, Ceriotti F, et al. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 degrees C. International Federation of Clinical Chemistry and Laboratory Medicine. Part 4. Reference procedure for the measurement of catalytic concentration of alanine aminotransferase. Clin Chem Lab Med 2022 Jul;40(7):718-724. doi: 10.1515/CCLM.2002.124.

6 Bergmeyer HU, Hørder M, Rej R. Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase. J Clin Chem Clin Biochem 1986;24:481-495.

7 ECCLS. Determination of the catalytic activity concentration in serum of L-alanine aminotransferase (EC 2.6.1.2, ALAT). Klin Chem Mitt 1989;20:204-211.

8 Heins M, Heil W, Withold W. Storage of Serum or Whole Blood Samples? Effect of Time and Temperature on 22 Serum Analytes. Eur J Clin Chem Clin Biochem 1995;33:231-238.

9 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

10 Breuer J. Report on the Symposium “Drug effects in Clinical Chemistry Methods”. Eur J Clin Chem Clin Biochem 1996;34:385-386.

11 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

12 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

13 Klauke R, Schmidt E, Lorentz K. Recommendations for carrying out standard ECCLS procedures (1988) for the catalytic concentrations of creatine kinase, aspartate aminotransferase, alanine aminotransferase and γ-glutamyltransferase at 37 °C. Eur J Clin Chem Clin Biochem 1993;31:901-909.

14 Thomas L, Müller M, Schumann G, et al. Consensus of DGKL and VDGH for interim reference intervals on enzymes in serum. J Lab Med 2005;29(5):301-308.

15 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
⟶	Volume for reconstitution
GTIN	Global Trade Item Number

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

Roche