ALTLP

cobas®

Alanine Aminotransferase acc. to IFCC with pyridoxal phosphate activation

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04467388190	04467388500	Alanine Aminotransferase acc. to IFCC (275 tests)	System-ID 07 6858 8	**cobas c** 311, **cobas c** 501/502

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 401	
12149435122	Precinorm U plus (10 x 3 mL)	Code 300	
12149443122	Precipath U plus (10 x 3 mL)	Code 301	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
04489357190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

English

System information

For **cobas c** 311/501 analyzers:

ALTLP: ACN 684

For **cobas c** 502 analyzer:

ALTPM: ACN 8681

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

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

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

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

R1 is in position A, R2 is in position B, and R3 is in position C.

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.

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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

cobas c 311 **test definition**

Assay type	Rate A	
Reaction time / Assay points	10 / 29-57	
Wavelength (sub/main)	700/340 nm	
Reaction direction	Decrease	
Units	U/L (µkat/L)	
Reagent pipetting		Diluent (H_2O)
R1	59 µL	32 µL
R2	20 µL	–
R3	20 µL	20 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	9 µL	–	–
Decreased	9 µL	15 µL	135 µL
Increased	9 µL	–	–

cobas c 501 **test definition**

Assay type	Rate A	
Reaction time / Assay points	10 / 44-70	
Wavelength (sub/main)	700/340 nm	
Reaction direction	Decrease	
Units	U/L (µkat/L)	
Reagent pipetting		Diluent (H_2O)
R1	59 µL	32 µL
R2	20 µL	–
R3	20 µL	20 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	9 µL	–	–
Decreased	9 µL	15 µL	135 µL
Increased	9 µL	–	–

cobas c 502 **test definition**

Assay type	Rate A	
Reaction time / Assay points	10 / 44-70	
Wavelength (sub/main)	700/340 nm	
Reaction direction	Decrease	
Units	U/L (µkat/L)	
Reagent pipetting		Diluent (H_2O)
R1	59 µL	32 µL
R2	20 µL	–
R3	20 µL	20 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	9 µL	–	–
Decreased	9 µL	15 µL	135 µL
Increased	18 µL	–	–

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • 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.

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

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Calculation

cobas c systems automatically calculate the analyte activity of each sample.

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

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at an ALT activity of 35 U/L (0.58 µkat/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]

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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. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is required in certain cases.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

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

Lower detection limit of the test

5 U/L (0.08 µkat/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values

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

Males	10-50 U/L	(0.17-0.83 µkat/L)
Females	10-35 U/L	(0.17-0.58 µkat/L)

Consensus values with pyridoxal phosphate activation:[14]

Males	up to 50 U/L	(up to 0.83 µkat/L)
Females	up to 35 U/L	(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. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (3 aliquots per run, 1 run per day, 20 days). The following results were obtained on the **cobas c** 501 analyzer:

Repeatability	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	42.1 (0.70)	0.6 (0.01)	1.3
Precipath U	124 (2.07)	1 (0.01)	0.5
Human serum 1	122 (2.03)	1 (0.01)	0.6
Human serum 2	7.33 (0.12)	0.94 (0.02)	12.9

Intermediate precision	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	41.9 (0.70)	0.7 (0.01)	1.6
Precipath U	124 (2.07)	1 (0.02)	1.0
Human serum 3	26.3 (0.44)	0.8 (0.01)	2.9
Human serum 4	122 (2.03)	4 (0.07)	3.6

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

Method comparison

ALT values for human serum and plasma samples obtained on a **cobas c** 501 analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 198

Passing/Bablok[15]	Linear regression
y = 1.000x - 0.438 U/L	y = 0.994x - 1.72 U/L
τ = 0.931	r = 0.995

The sample activities were between 5.10 and 469 U/L (0.085 and 7.83 µkat/L).

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

References

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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.

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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

CE 0123

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