Application Sheet

Laboratory Name

Test Name: Alanine Aminotransferase acc. to IFCC without pyridoxal phosphate activation

System information

For **cobas c** 311/501 analyzers:
ALTL: ACN 685

Intended use

In vitro test for the quantitative determination of alanine aminotransferase (ALT) in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary[1,2]

The enzyme alanine aminotransferase (ALT) has been widely reported as present in a variety of tissues. The major source of ALT is the liver, which has led to the measurement of ALT activity for the diagnosis of hepatic diseases. Elevated serum ALT is found in hepatitis, cirrhosis, obstructive jaundice, carcinoma of the liver, and chronic alcohol abuse. ALT is only slightly elevated in patients who have an uncomplicated myocardial infarction.
Although both serum aspartate aminotransferase (AST) and ALT become elevated whenever disease processes affect liver cell integrity, ALT is the more liver-specific enzyme. Moreover, elevations of ALT activity persist longer than elevations of AST activity.
In patients with vitamin B_6 deficiency, serum aminotransferase activity may be decreased. The apparent reduction in aminotransferase activity may be related to decreased pyridoxal phosphate, the prosthetic group for aminotransferases, resulting in an increase in the ratio of apoenzyme to holoenzyme.

Test principle

This assay follows the recommendations of the IFCC, but was optimized for performance and stability.[3, 4]
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^+ .

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

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

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

R1 is in position B and R2 is in position C.

Precautions and warnings

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

Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

Reagent handling

Ready for use

Storage and stability

ALTL

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

Diluent NaCl 9 %

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 (free from hemolysis).
– Plasma (free from hemolysis): 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.
Separate the serum or plasma from the clot or cells promptly.
Centrifuge samples containing precipitates before performing the assay.

Stability:	3 days at 15-25 °C[5,6]
	7 days at 2-8 °C[5,6]
	> 7 days at (-60)-(-80) °C[6]

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

- See “Order information” section
- General laboratory equipment

In addition, other suitable control material can be used.

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.

Laboratory Name

Test Name: Alanine Aminotransferase acc. to IFCC without pyridoxal phosphate activation

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 501 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 18-46		
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	17 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	9 µL	–	–
Decreased	9 µL	15 µL	135 µL
Increased	9 µL	–	–

Calibration

Calibrator	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, but without Pyp, using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.[7]

Quality control

At least once daily run solutions at two levels of a quality control material with known concentrations.

Refer to Brown Clinic Quality Control Requirements, Rules and Reviews Policy

Refer to Brown Clinic Quality Control Specialty and Subspecialty Policy

Calculation

Roche/Hitachi **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 30 U/L (0.5 µkat/L).
Icterus:[8] 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:[8] No significant interference up to an H index of 90 (approximate hemoglobin concentration: 56 µmol/L or 90 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):[8] 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. Choose diluted sample treatment for automatic rerun.
Drugs: No interference was found at therapeutic concentrations using common drug panels.[9,10]
Exceptions: Physiological plasma concentrations of Sulfasalazine and Sulfapyridine may lead to false results.
Calcium dobesilate and Isoniazid can cause artificially low and Furosemide artificially high ALT results at therapeutic concentrations.
Cyanokit (Hydroxocobalamin) may cause interference with results.
In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[11]
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 Roche/Hitachi **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 not required.
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[12]

Acc. to the optimized standard method (comparable to the IFCC method without pyridoxal phosphate activation[13]):

Males	up to 41 U/L	(up to 0.68 µkat/L)
Females	up to 33 U/L	(up to 0.55 µkat/L)

Calculated values: A factor of 1.85 is used for the conversion from 25 °C to 37 °C.[14]
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

For Known Interfering Substances section refer to package insert.
For Known Non-Interfering Substance refer to package insert.
For Additional Technical Information refer to package insert.

References

1 Sherwin JE. Liver function. In: Kaplan LA, Pesce AJ, eds. Clinical Chemistry, theory, analysis, and correlation. St. Louis: Mosby 1984;420-438.
2 Moss DW, Henderson AR, Kachmar JF. Enzymes. In: Tietz NW, ed. Fundamentals of Clinical Chemistry, 3rd ed. Philadelphia, PA: WB Saunders 1987;346-421.
3 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.
4 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.
5 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.
6 Data on file at Roche Diagnostics.
7 Schumann G, Bonora R, Ceriotti F, et al. IFCC Primary Reference Procedures for the Measurement of Catalytic Activity Concentrations of Enzymes at 37 °C – Part 4. Reference Procedure for the Measurement of Catalytic Activity Concentration of Alanine Aminotransferase. Clin Chem Lab Med 2002;40(7):718-724.
8 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.
9 Breuer J. Report on the Symposium “Drug effects in Clinical Chemistry Methods”. Eur J Clin Chem Clin Biochem 1996;34:385-386.
10 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.
11 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.
12 Thefeld W, Hoffmeister H, Busch EW, et al. Referenzwerte für die Bestimmungen der Transaminasen GOT und GPT sowie der alkalischen Phosphatase im Serum mit optimierten Standardmethoden. Dtsch Med Wschr 1974;99(8):343-351.
13 Klein G, Lehmann P, Michel E, et al. Vergleich der IFCC-Methoden für ALAT, ASAT und GGT bei 37 °C mit den eingeführten Standardmethoden bei 25 °C und 37 °C. Lab Med 1994;18:403-404.
14 Zawta B, Klein G, Bablok W. Temperaturumrechnung in der klinischen Enzymologie? Klin Lab 1994;40:23-32.
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.

Alternative method

Laboratory Name

Test Name: Alanine Aminotransferase acc. to IFCC without pyridoxal phosphate activation

Refer to Brown Clinic Back-up Testing Policy

Source document

Reagent Name: ALTL
Method Sheet Version: V12.0 English

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
20764957 322	Alanine Aminotransferase acc. to IFCC 500 tests	System-ID 07 6495 7	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
10759350 190	Calibrator f.a.s. (12 x 3 mL)	Code 401	
10759350 360	Calibrator f.a.s. (12 x 3 mL, for USA)	Code 401	
12149435 122	Precinorm U plus (10 x 3 mL)	Code 300	
12149435 160	Precinorm U plus (10 x 3 mL, for USA)	Code 300	
12149443 122	Precipath U plus (10 x 3 mL)	Code 301	
12149443 160	Precipath U plus (10 x 3 mL, for USA)	Code 301	
10171743 122	Precinorm U (20 x 5 mL)	Code 300	
10171778 122	Precipath U (20 x 5 mL)	Code 301	
10171760 122	Precipath U (4 x 5 mL)	Code 301	
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

Effective date

Effective date for this procedure: _____

Author

Source documentation compiled by Roche Diagnostics

Revised by: Heather J Hall, MBA, MT(ASCP), CG(ASCP)^{cm} Date: 4/9/2018

Approved by: Aaron Shives MD (Signature on file Date: 4/11/2018

REVIEW – REVISION SUMMARY DOCUMENTATION

Date: By: Revision Summary: