20764949322V9

ASTL

Aspartate Aminotransferase acc. to IFCC without pyridoxal phosphate activation

cobas®

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
Aspartate Aminotransferase acc. to IFCC			**cobas c** 311	**cobas c** 501/502
500 tests	Cat. No. **20764949** 322	System-ID 07 6494 9	•	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401		
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401		
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300		
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300		
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301		
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301		
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300		
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301		
PreciControl ClinChem Multi 1 (20 x 5 mL)	Cat. No. **05117003** 190	Code 391		
PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Cat. No. **05947626** 160	Code 391		
PreciControl ClinChem Multi 2 (20 x 5 mL)	Cat. No. **05117216** 190	Code 392		
PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Cat. No. **05947774** 160	Code 392		
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357**190	System-ID 07 6869 3		

English

System information

For **cobas c** 311/501 analyzers:
ASTL: ACN 687
SASTL: ACN 587 (STAT, reaction time: 7)
For **cobas c** 502 analyzer:
ASTL: ACN 8687
SASTL: ACN 8587 (STAT, reaction time: 7)

Intended use

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

Summary[1,2]

The enzyme aspartate aminotransferase (AST) is widely distributed in tissue, principally hepatic, cardiac, muscle, and kidney. Elevated serum levels are found in diseases involving these tissues. Hepatobiliary diseases, such as cirrhosis, metastatic carcinoma, and viral hepatitis also increase serum AST levels. Following myocardial infarction, serum AST is elevated and reaches a peak 2 days after onset.

In patients undergoing renal dialysis or those with vitamin B_6 deficiency, serum AST may be decreased. The apparent reduction in AST may be related to decreased pyridoxal phosphate, the prosthetic group for AST, resulting in an increase in the ratio of apoenzyme to holoenzyme.
2 isoenzymes of AST have been detected, cytoplasmic and mitochondrial. Only the cytoplasmic isoenzyme occurs in normal serum, while the mitochondrial, together with the cytoplasmic isoenzyme, has been detected in the serum of patients with coronary and hepatobiliary disease.

Test principle

This assay follows the recommendations of the IFCC, but was optimized for performance and stability.[3,4]
AST in the sample catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH), to form NAD^+.

L-Aspartate + 2-oxoglutarate $\xrightarrow{AST}$ oxaloacetate + L-glutamate

Oxaloacetate + NADH + H^+ $\xrightarrow{MDH}$ L-malate + NAD^+

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

Reagents - working solutions

R1 TRIS buffer: 264 mmol/L, pH 7.8 (37 °C); L-aspartate: 792 mmol/L; MDH (microorganism): ≥ 24 µkat/L; LDH (microorganisms): ≥ 48 µkat/L; albumin (bovine): 0.25 %; preservative

R2 NADH: ≥ 1.7 mmol/L; 2-oxoglutarate: 94 mmol/L; preservative

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Reagent handling

Ready for use.

Storage and stability

ASTL

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.
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.

Stability: 24 hours at 15-25 °C[5]
7 days at 2-8 °C[6]

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.

ASTL

Aspartate Aminotransferase acc. to IFCC without pyridoxal phosphate activation

cobas®

Application for serum and plasma

cobas c 311 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 12-31 (STAT 7 / 12-31)		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Decrease		
Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	40 µL	51 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	18 µL	–	–

cobas c 501/502 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 18-46 (STAT 7 / 18-46)		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Decrease		
Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	40 µL	51 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	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

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.[7]

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. 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

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

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

Limitations - interferences

Criterion: Recovery within ± 10 % of initial value at an AST activity of 30 U/L (0.50 µkat/L).

Icterus:[8] No significant interference up to an I index of 60 for conjugated bilirubin and 60 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 60 mg/dL or 1026 µmol/L; approximate unconjugated bilirubin concentration: 60 mg/dL or 1026 µmol/L).

Hemolysis:[8] No significant interference up to an H index of 40 (approximate hemoglobin concentration: 25.6 µmol/L (40 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 specimens 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: Isoniazid can cause artificially low and Furosemide artificially high AST 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.

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/Multiclean/SCCS or 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 0. 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[11]

Acc. to the optimized standard method (comparable to the IFCC method without pyridoxal phosphate activation[12]):

Males:	up to 40 U/L	(up to 0.67 µkat/L)
Females:	up to 32 U/L	(up to 0.53 µkat/L)

Calculated values: A factor of 2.13 is used for the conversion from 25 °C to 37 °C.[13]

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.

ASTL

Aspartate Aminotransferase acc. to IFCC without pyridoxal phosphate activation

cobas®

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:

*Repeatability**	*Mean U/L (µkat/L)*	*SD U/L (µkat/L)*	*CV %*
Precinorm U	36.6 (0.611)	0.3 (0.005)	0.8
Precipath U	128 (2.14)	1 (0.02)	0.4
Human serum 1	126 (2.10)	1 (0.02)	0.4
Human serum 2	12.0 (0.200)	0.4 (0.007)	3.1

*Intermediate precision***	*Mean U/L (µkat/L)*	*SD U/L (µkat/L)*	*CV %*
Precinorm U	36.7 (0.613)	0.5 (0.008)	1.3
Precipath U	130 (2.17)	1 (0.02)	0.8
Human serum 3	30.0 (0.501)	0.7 (0.012)	2.3
Human serum 4	121 (2.02)	2 (0.03)	1.9

* repeatability = within-run precision

** intermediate precision = total precision / between run precision / between day precision

Method comparison

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

Sample size (n) = 192

Passing/Bablok[14]	Linear regression
y = 1.000x - 0.149 U/L	y = 0.991x + 1.22 U/L
τ = 0.970	r = 0.999

The sample activities were between 30.4 and 674 U/L (0.508 and 11.3 µkat/L).

References

1. Nagy B. Muscle disease. In: Kaplan LA, Pesce AJ, eds. Clinical Chemistry, theory, analysis, and correlation. St. Louis: Mosby 1984:514.
2. Moss DW, Henderson AR, Kachmar JF. Enzymes. In: Tietz NW, ed. Fundamentals of Clinical Chemistry. 3rd ed. Philadelphia: 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 2. IFCC method for aspartate aminotransferase. J Clin Chem Clin Biochem 1986;24:497-510.
4. ECCLS. Determination of the catalytic activity concentration in serum of L-aspartate aminotransferase (EC 2.6.1.1, ASAT). Klin Chem Mitt 1989;20:198-204.
5. Tietz NW. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: WB Saunders,1995:76–77.
6. Use of Anticoagulants in Diagnostic Laboratory Investigations. WHO Publication WHO/DIL/LAB/99.1 Rev.2.
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 5. Reference Procedure for the Measurement of Catalytic Concentration of Aspartate Aminotransferase. Clin Chem Lab Med 2002;40(7):725–733.
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. 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;99:343-351.
12. 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.
13. Zawta B, Klein G, Bablok W. Temperaturumrechnung in der klinischen Enzymologie? Klin Lab 1994;40:23-32.
14. Passing H, Bablok W, Bender R, et al. A General Regression Procedure for Method Transformation. 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.

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

CE

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support 1-800-428-2336

Roche