ASTLP

cobas®

Aspartate Aminotransferase acc. to IFCC with pyridoxal phosphate activation

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04467493190	Aspartate Aminotransferase acc. to IFCC (425 tests)	System-ID 07 6856 1	**cobas c** 311, **cobas c** 501/502

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 401	
10759350360	Calibrator f.a.s. (12 x 3 mL, for USA)	Code 401	
12149435122	Precinorm U plus (10 x 3 mL)	Code 300	
12149435160	Precinorm U plus (10 x 3 mL, for USA)	Code 300	
12149443122	Precipath U plus (10 x 3 mL)	Code 301	
12149443160	Precipath U plus (10 x 3 mL, for USA)	Code 301	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05947626160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Code 391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
05947774160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Code 392	
04489357190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

English

System information

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

ASTLP: ACN 686

For **cobas c** 502 analyzer:

ASTPM: ACN 8680

Intended use

In vitro test for the quantitative determination of aspartate aminotransferase (AST) with pyridoxal phosphate activation in human serum and plasma on **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.

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.

The addition of pyridoxal phosphate to the assay causes an increase in aminotransferase activity. The activation is higher for AST than for ALT. Pyridoxal phosphate activation prevents falsely low aminotransferase activity in patient samples with insufficient endogenous pyridoxal phosphate (vitamin B_6 deficiency).

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^+. Pyridoxal phosphate serves as a coenzyme in the amino transfer reaction. It ensures full enzyme activation.

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

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

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 Pyridoxal phosphate: 730 µmol/L; preservative

R3 NADH (yeast): ≥ 1.7 mmol/L; 2-oxoglutarate: 94 mmol/L; preservative

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

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

ASTLP

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.

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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.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Stability:[5]	4 days at 20-25 °C
	7 days at 4-8 °C
	3 months at -20 °C

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 / 40-57		
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	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 / 54-70		
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	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 / 54-70		
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	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.[6]

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

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 AST activity of 35 U/L (0.58 µkat/L).

Icterus:[7] 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:[7] No significant interference up to an H index of 20 (approximate hemoglobin concentration: 12.4 µmol/L or 20 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):[7] 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.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[8,9]

Cyanokit (Hydroxocobalamin) may cause interference with results.

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

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[10]

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 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

Acc. to IFCC/Standard Method 94 with pyridoxal phosphate activation measured at 37 °C:[11]

Males	10-50 U/L	(0.17-0.85 µkat/L)
Females	10-35 U/L	(0.17-0.60 µkat/L)

Consensus values with pyridoxal phosphate activation:[12]

Males	up to 50 U/L	(up to 0.85 µkat/L)
Females	up to 35 U/L	(up to 0.60 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:

Repeatability	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	48.0 (0.802)	0.6 (0.010)	1.3
Precipath U	147 (2.46)	1 (0.02)	0.6
Human serum 1	151 (2.52)	1 (0.02)	0.7
Human serum 2	17.0 (0.284)	1.0 (0.017)	6.0

Intermediate precision	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	49.0 (0.818)	0.8 (0.013)	1.6
Precipath U	149 (2.49)	1 (0.02)	0.8
Human serum 3	40.7 (0.680)	1.3 (0.022)	3.1
Human serum 4	190 (3.17)	3 (0.05)	1.3

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

Method comparison

AST 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) = 177

Passing/Bablok[13]	Linear regression
y = 1.000x - 2.45 U/L	y = 0.998x - 2.87 U/L
τ = 0.960	r = 0.998

The sample activities were between 45.0 and 675 U/L (0.752 and 11.3 µkat/L).

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

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, 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 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 WHO Publication: Use of anticoagulants in diagnostic laboratory investigations, WHO/DIL/LAB/99.1 Rev.2:Jan 2002.

6 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 Activity Concentrations of Aspartate Aminotransferase. Clin Chem Lab Med 2002;40(7):725-733.

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

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

9 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.

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

11 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;31:907-909.

12 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.

13 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

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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.

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