AST

cobas®

Aspartate Aminotransferase acc. to IFCC without pyridoxal phosphate activation

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
05850819 190	Aspartate Aminotransferase acc. to IFCC (1100 tests)	System-ID 05 7500 2	Roche/Hitachi **cobas c** 701/702

Materials required (but not provided):

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	
10171735122	Precinorm U (4 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	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05947626 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
05947774 160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Code 392	
05172152 190	Diluent NaCl 9 % (119 mL)	System-ID 08 6869 3	

English

System information

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 two 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.

Two 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^+.

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

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

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
R3 (STAT R2)	NADH: ≥ 1.7 mmol/L; 2-oxoglutarate: 94 mmol/L; preservative

R1 is in position B and R3 (STAT R2) 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

AST

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	4 weeks
On-board on the Reagent Manager:	24 hours

Diluent NaCl 9 %

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On-board in use and refrigerated on the analyzer:	4 weeks
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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.

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 701/702 **test definition** for ASTL (ACN 8687):

Assay type	Rate A		
Reaction time / Assay points	10 / 24-38		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Decrease		
Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	44 µL	57 µL	
R3	19 µL	22 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	10 µL	–	–
Decreased	10 µL	15 µL	135 µL
Increased	20 µL	–	–

cobas c 701/702 **test definition** for SASTL (ACN 8587):

Assay type	Rate A		
Reaction time / Assay points	7 / 12-26		
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

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

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 AST activity of 30 U/L (0.50 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 40 (approximate hemoglobin concentration: 25.6 µmol/L or 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):[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. Choose diluted sample treatment for automatic rerun.

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

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

Cyanokit (Hydroxocobalamin) may cause interference with 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 Roche/Hitachi **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is required in certain cases. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SmpCln1+2/SCCS Method Sheet and for further instructions refer to the operator's manual.

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).

Values below the lower detection limit (< 5 U/L) will not be flagged by the instrument.

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.

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:

ASTL:

Repeatability	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L µkat/L)*	*%*
Precinorm U	44.7 (0.746)	0.8 (0.013)	1.9
Precipath U	156 (2.61)	1 (0.02)	0.6
Human serum A	21.3 (0.356)	0.7 (0.012)	3.2
Human serum B	82.4 (1.38)	0.7 (0.012)	0.8
Human serum C	564 (9.42)	3 (0.05)	0.5

SASTL:

Repeatability	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	45.8 (0.765)	0.7 (0.012)	1.5
Precipath U	156 (2.61)	1 (0.02)	0.4
Human serum A	14.8 (0.247)	0.8 (0.013)	5.4
Human serum B	130 (2.17)	1 (0.02)	0.9
Human serum C	642 (10.7)	3 (0.1)	0.5

ASTL / SASTL:

Intermediate precision	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	36.7 (0.61)	0.5 (0.01)	1.3
Precipath U	130 (2.17)	1 (0.02)	0.8
Human serum 3	30.0 (0.50)	0.7 (0.01)	2.3
Human serum 4	121 (2.02)	2 (0.03)	1.9

Results for intermediate precision were obtained on the master system **cobas c** 501 analyzer.

Method comparison

AST values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 701 analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi **cobas c** 501 analyzer (x).

ASTL:

Sample size (n) = 73

Passing/Bablok[14]	Linear regression
y = 1.000x + 0.408 U/L	y = 0.993x + 1.45 U/L
τ = 0.989	r = 1.000

The sample activities were between 13.1 and 686 U/L (0.219 and 11.5 µkat/L).

SASTL:

Sample size (n) = 305

Passing/Bablok[14]	Linear regression
y = 1.007x + 0.769 U/L	y = 0.999x + 1.17 U/L
τ = 0.928	r = 1.000

The sample activities were between 5.20 and 659 U/L (0.087 and 11.0 µkat/L).

References

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

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14 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 after reconstitution or mixing
GTIN	Global Trade Item Number

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.

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