08104719500V8.0

ASTP2

cobas®

Aspartate Aminotransferase acc. to IFCC II

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08104719190*	08104719500	Aspartate Aminotransferase acc. to IFCC II (800 tests)	System-ID 2023 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08104719214*	08104719500	Aspartate Aminotransferase acc. to IFCC II (800 tests)	System-ID 2023 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

* Some kits shown may not be available in all countries.

English

System information

ASTP2: ACN 20230

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

Aspartate aminotransferase (AST) 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 aspartate aminotransferase (AST) is widely distributed in tissue, primarily in the liver, cardiac muscle, skeletal muscle, kidney, brain and erythrocytes.[1] AST catalyzes the transfer of amino groups from L-aspartate to α-ketoglutarate, resulting in L-glutamate and oxaloacetate. 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. In particular, AST is vital for aerobic glycolysis. AST exists in human tissues as 2 distinct isoenzymes, 1 located in the cytoplasm (c-AST), and the other in mitochondria (m-AST), which differ in amino acid composition and immunochemical and kinetic properties. In healthy individuals, the circulating AST levels consist mainly of cytoplasmic AST, originating from cytoplasmic leakage, on the other side, mitochondrial AST activity in serum shows a marked increase in patients with extensive liver cell degeneration and necrosis. Although AST activity is important in all cells with high metabolic activity, it is more relevant for liver and muscle cells.[2]

Primarily, AST is a marker of hepatocellular injury. Measurement of AST 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, suspected malignant infiltration, cholestasis.[3] Although alanine aminotransferase (ALT) is considered a more specific indicator of liver disease, the concentration of AST may be a more sensitive indicator of liver injury in conditions such as alcohol-related liver disease and in some cases of autoimmune hepatitis.[4] Several international guidelines recommend AST testing for monitoring chronic hepatitis status and progression.[4,5]

Non-liver causes for increases in AST include damage to cardiac or skeletal muscle cells and haemolysis. Serum elevation of AST without elevation in ALT is suggestive of cardiac or muscle disease.[3] In patients undergoing renal dialysis or those with vitamin B6 deficiency, serum AST may be decreased.[6] AST serum levels can be affected by age, gender, alcohol consumption, body mass index, dietary and living habits, nutrition, metabolic status, and drug treatment, among other factors.[7]

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) and prevents falsely low aminotransferase test results in these samples.[1]

Test principle

This assay follows the recommendations of the IFCC, but was optimized for performance and stability.[8]

AST 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 L-malate and NAD^+. Pyridoxal phosphate serves as a coenzyme in the amino transfer reaction. It ensures full enzyme activation.

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

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

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: 180 mmol/L, pH 7.65 (37 °C); L-aspartate: 550 mmol/L; MDH (microorganisms): ≥ 11 µkat/L; LDH (microorganisms): ≥ 80 µkat/L; pyridoxamine phosphate: 0.23 mmol/L; albumin (bovine): 0.25 %; stabilizers; preservative

R3 NADH: ≥ 0.71 mmol/L; 2-oxoglutarate: 96 mmol/L; preservative

R1 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.

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Only the specimens listed below were tested and found acceptable.
Serum
Plasma: Li-heparin and K_2- and K_3-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.

Stability:	4 days at 15-25 °C
	7 days at 2-8 °C
	3 months at -20 °C (± 5 °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

Test definition

Reaction time	10 min		
Wavelength (sub/main)	700/340 nm		
Reagent pipetting		Diluent (H_2O)	
R1	52 µL	48 µL	
R3	15 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	4.5 µL	–	–
Decreased	4.5 µL	10 µL	90 µL
Increased	4.5 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - 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.[8]

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. It is recommended to perform quality control always after lot calibration and subsequently at least every 12 weeks. 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 in the unit U/L (µkat/L).

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

Limitations - interference

Criterion: Recovery within ± 4.0 U/L of initial values of samples ≤ 40 U/L and ± 10 % for samples > 40 U/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 25 (approximate hemoglobin concentration: 15.6 µmol/L or 25 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 500. 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.[10,11]

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

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. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

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

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 5 U/L (0.08 µkat/L)
Limit of Detection	= 5 U/L (0.08 µkat/L)
Limit of Quantitation	= 5 U/L (0.08 µkat/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

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The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the activity below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low activity samples.

The Limit of Detection corresponds to the lowest analyte activity which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte activity that can be reproducibly measured with a total error of 20 %. It has been determined using low activity aspartate aminotransferase samples.

Expected values

U/L

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

Males:	10-50 U/L
Females:	10-35 U/L

Consensus values with pyridoxal phosphate activation:[14]

Males:	up to 50 U/L
Females:	up to 35 U/L

µkat/L*

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

Males:	0.17-0.84 µkat/L
Females:	0.17-0.58 µkat/L

Consensus values with pyridoxal phosphate activation:[14]

Males:	up to 0.84 µkat/L
Females:	up to 0.58 µkat/L

*calculated by unit conversion factor

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. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Repeatability	*Mean* U/L	*SD* U/L	*CV* %
PCCC1[a)]	46.4	0.551	1.2
PCCC2[b)]	149	1.49	1.0
Human serum 1	11.3	0.242	2.1
Human serum 2	33.0	0.497	1.5
Human serum 3	50.4	0.314	0.6
Human serum 4	345	1.31	0.4
Human serum 5	651	2.19	0.3

Intermediate precision	*Mean* U/L	*SD* U/L	*CV* %
PCCC1[a)]	46.5	1.33	2.9
PCCC2[b)]	148	3.30	2.2
Human serum 1	11.4	0.281	2.5
Human serum 2	33.0	0.552	1.7
Human serum 3	50.4	0.371	0.7
Human serum 4	345	1.79	0.5
Human serum 5	651	3.63	0.6

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

Method comparison

AST values for human serum and plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the test ASTLP on a **cobas c** 501 analyzer (x).

Sample size (n) = 111

Passing/Bablok[15]	Linear regression
y = 0.960x + 2.35 U/L	y = 0.931x + 6.22 U/L
τ = 0.978	r = 0.998

The sample activities were between 7.50 and 694 U/L.

AST values for human serum and plasma samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Sample size (n) = 50

Passing/Bablok[15]	Linear regression
y = 0.984x + 0.903 U/L	y = 0.980x + 1.37 U/L
τ = 0.989	r = 1.000

The sample activities were between 7.79 and 667 U/L.

AST values for human serum and plasma samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Sample size (n) = 66

Passing/Bablok[15]	Linear regression
y = 0.992x + 1.16 U/L	y = 0.990x + 1.36 U/L
τ = 0.981	r = 1.000

The sample concentrations were between 5.49 and 667 U/L.

References

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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 navifyportal.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

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