

# Application Sheet



Laboratory Name

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

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### System information

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

**ASTL:** ACN 687

**SASTL:** ACN 587 (STAT, reaction time: 7)

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### Intended use

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

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### Summary<sup>1,2</sup>

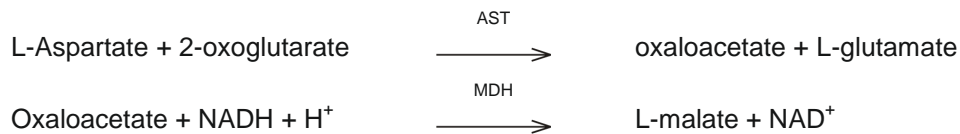
The enzyme aspartate aminotransferase (AST) is widely distributed in tissue, principally hepatic, cardiac, muscle, and kidney. Elevated serum levels are found in diseases involving these tissues. Hepatobiliary diseases, such as cirrhosis, metastatic carcinoma, and viral hepatitis also increase serum 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<sub>6</sub> 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.

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### Test principle

This assay follows the recommendations of the IFCC, but was optimized for performance and stability.<sup>3,4</sup> 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<sup>+</sup>.



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

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### 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

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

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**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.

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**Reagent handling**

Ready for use

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**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

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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<sub>2</sub>-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:<sup>5</sup> 4 days at 20-25 °C

7 days at 4-8 °C

3 months at -20 °C

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**Materials provided**

See "Reagents – working solutions" section for reagents.

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**Materials required (but not provided)**

- See "Order information" section
- General laboratory equipment

In addition, other suitable control material can be used.

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**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.

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**Application for serum and plasma**

**Cobas c 501 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 <sub>2</sub> O)	
R1	40 µL	51 µL	
R2	17 µL	20 µL	
<i>Sample volumes</i>	<i>Sample</i>	<i>Sample dilution</i>	
		<i>Sample</i>	<i>Diluent (NaCl)</i>
Normal	9 µL	–	–
Decreased	9 µL	15 µL	135 µL
Increased	9 µL	–	–

**Calibration**

Calibrators	S1: H <sub>2</sub> O 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,  $\epsilon$ .<sup>6</sup>

**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 Quatliy 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  $\pm 10\%$  of initial value at an AST activity of 30 U/L (0.50 µkat/L).  
Icterus:<sup>7</sup> 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:<sup>7</sup> 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

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than in normal sera. The level of interference may be variable depending on the content of analyte in the lysed erythrocytes.

Lipemia (Intralipid):<sup>7</sup> 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.<sup>8,9</sup>

Exceptions: Isoniazid can cause artificially low and Furosemide artificially high AST results at therapeutic concentrations.

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.<sup>10</sup>

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.**

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#### **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).

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#### **Expected values<sup>11</sup>**

Acc. to the optimized standard method (comparable to the IFCC method without pyridoxal phosphate activation<sup>12</sup>):

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.<sup>13</sup>

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

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#### **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.

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**References**

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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.

**Alternative method**

Refer to Brown Clinic Back-up Testing Policy

**Source document**

Reagent Name: ASTL  
 Method Sheet Version: V13.0 English

**Order information**

REF	CONTENT	Analyzer(s) on which <b>cobas c</b> pack(s) can be used
<b>20764949</b> 322	Aspartate Aminotransferase acc. to IFCC (500 tests)	System-ID 07 6494 9 <b>Roche/Hitachi cobas c 311,</b> <b>cobas c 501/502</b>
<b>10759350</b> 190	Calibrator f.a.s. (12 x 3 mL)	Code 401

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<b>10759350</b> 360	Calibrator f.a.s. (12 x 3 mL, for USA)	Code 401	
<b>12149435</b> 122	Precinorm U plus (10 x 3 mL)	Code 300	
<b>12149435</b> 160	Precinorm U plus (10 x 3 mL, for USA)	Code 300	
<b>12149443</b> 122	Precipath U plus (10 x 3 mL)	Code 301	
<b>12149443</b> 160	Precipath U plus (10 x 3 mL, for USA)	Code 301	
<b>10171743</b> 122	Precinorm U (20 x 5 mL)	Code 300	
<b>10171778</b> 122	Precipath U (20 x 5 mL)	Code 301	
<b>10171760</b> 122	Precipath U (4 x 5 mL)	Code 301	
<b>04489357</b> 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)<sup>cm</sup> \_\_\_\_\_ Date: 4/9/2018

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

**REVIEW – REVISION SUMMARY DOCUMENTATION**

Date: \_\_\_\_\_ By: \_\_\_\_\_ Revision Summary: \_\_\_\_\_