AST-Pyridoxal-5'-phosphate Aspartate Aminotransferase

For In Vitro Diagnostic Use

Rx Only

ANNUAL REVIEW

<table>
<thead>
<tr>
<th>Reviewed by</th>
<th>Date</th>
<th>Reviewed by</th>
<th>Date</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>

PRINCIPLE

INTENDED USE

AST- reagent, when used in conjunction with UniCel DxC 600/800 System(s) and SYNCHRON Systems Enzyme Validator Set, is intended for quantitative determination of pyridoxal-5'-phosphate aspartate aminotransferase concentration in human serum or plasma. Use of this product, in conjunction with the SYNCHRON Systems Enzyme Validator Set, will result in assay values which are compatible with the methods recommended by the International Federation of Clinical Chemistry (IFCC). 1

CLINICAL SIGNIFICANCE

Aspartate aminotransferase measurements are used in the diagnosis and treatment of certain types of liver and heart disease.

METHODOLOGY

The AST- reagent is used to measure aspartate aminotransferase activity by an enzymatic rate method. 2,3 In the assay reaction, the AST catalyzes the reversible transamination of L-aspartate and α-ketoglutarate to oxaloacetate and L-glutamine. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase (MDH) with the concurrent oxidation of β-Nicotinamide Adenine Dinucleotide (reduced form) (NADH) to β-Nicotinamide Adenine Dinucleotide (NAD).

The AST- assay is based on the IFCC standard for enzyme determination. 4 Pyridoxal-5'-phosphate is a cofactor that is required for transaminase activity by binding to the enzyme using Schiff-base linkage. 5

The SYNCHRON System(s) automatically proportions the appropriate sample and reagent volumes into a cuvette. The ratio used is one part sample to 11 parts reagent. The system monitors the rate of change in absorbance at 340 nanometers over a fixed-time interval. This rate of change in absorbance is directly proportional to the activity of AST in the sample and is used by the SYNCHRON System(s) to calculate and express the AST activity.
One unit of enzyme activity is defined as the quantity of enzyme that catalyzes the reaction of 1 µmol of substrate per minute at +37°C.

CHEMICAL REACTION SCHEME

\[
\text{L-aspartate} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate} + \text{glutamate} \quad \text{(primary reaction)}
\]

\[
\text{Oxaloacetate} + \text{NADH} + H^+ \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}^+ \quad \text{(indicator reaction)}
\]

SPECIMEN

TYPE OF SPECIMEN

Biological fluid samples should be collected in the same manner routinely used for any laboratory test.\(^6\) Freshly drawn serum or plasma are the preferred specimens. Acceptable anticoagulants are listed in PROCEDURAL NOTES section of this chemistry information sheet. Whole blood, urine or cerebrospinal fluid are not recommended for use as a sample.

SPECIMEN STORAGE AND STABILITY

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.\(^7\)

2. Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.\(^7\)

Additional specimen storage and stability conditions as designated by this laboratory:

SAMPLE VOLUME

The optimum volume, when using a 0.5 mL sample cup, is 0.3 mL of sample. For optimum primary sample tube volumes and minimum volumes, refer to the Primary Tube Sample Template for your system.

CRITERIA FOR UNACCEPTABLE SPECIMENS

Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens.

Criteria for sample rejection as designated by this laboratory:
PATIENT PREPARATION

Special instructions for patient preparation as designated by this laboratory:

SPECIMEN HANDLING

Special instructions for specimen handling as designated by this laboratory:

REAGENTS

CONTENTS

Each kit contains the following items:
Two Aspartate Aminotransferase (AST-) Reagent Cartridges (2 x 300 tests) or (2 x 100 tests)

VOLUMES PER TEST

<table>
<thead>
<tr>
<th>Sample Volume</th>
<th>23 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORDAC Sample Volume</td>
<td>3 µL</td>
</tr>
<tr>
<td>Total Reagent Volume</td>
<td>258 µL</td>
</tr>
<tr>
<td>Cartridge Volumes</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>250 µL</td>
</tr>
<tr>
<td>B</td>
<td>8 µL</td>
</tr>
<tr>
<td>C</td>
<td>– –</td>
</tr>
</tbody>
</table>

REACTIVE INGREDIENTS
### REAGENT CONSTITUENTS

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Ketoglutarate</td>
<td>12.0 mmol/L</td>
</tr>
<tr>
<td>NADH</td>
<td>0.27 mmol/L</td>
</tr>
<tr>
<td>L-Aspartate</td>
<td>240.0 mmol/L</td>
</tr>
<tr>
<td>Lactate Dehydrogenase (porcine)</td>
<td>0.79 KU/L</td>
</tr>
<tr>
<td>Malate dehydrogenase (porcine)</td>
<td>0.53 KU/L</td>
</tr>
<tr>
<td>Pyridoxal-5’-phosphate</td>
<td>0.25 mmol/L</td>
</tr>
</tbody>
</table>

Also non-reactive chemicals necessary for optimal system performance.

Avoid skin contact with reagent. Use water to wash reagent from skin.

### GHS HAZARD CLASSIFICATION
Aspartate Aminotransferase Reagent (Pyridoxal-5'-phosphate) (Compartment A)

**WARNING**

H316 Causes mild skin irritation.
P332+P313 If skin irritation occurs: Get medical advice/attention.
Tris(hydroxymethyl)– aminomethane 1 - 5%

Aspartate Aminotransferase Reagent (Pyridoxal-5'-phosphate) (Compartment B)

**WARNING**

H302 Harmful if swallowed.
P264 Wash hands thoroughly after handling.
P301+P312 IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
Ethylene Glycol > 90%

Aspartate Aminotransferase Reagent (Pyridoxal-5'-phosphate) (Compartment C)

**WARNING**

H316 Causes mild skin irritation.
H319 Causes serious eye irritation.
P280 Wear protective gloves, protective clothing and eye/face protection.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P332+P313 If skin irritation occurs: Get medical advice/attention.
P337+P313 If eye irritation persists: Get medical advice/attention.
α-Ketoglutaric Acid < 3%

**SOS** Safety Data Sheet is available at techdocs.beckmancoulter.com

**MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT**

SYNCHRON Systems Enzyme Validator Set
At least two levels of control material
Saline

REAGENT PREPARATION

1. Transfer all of the contents of the smallest reagent compartment (C) into the largest reagent compartment (A).
2. Replace the cartridge caps and gently invert the cartridge several times to ensure adequate mixing.

ACCEPTABLE REAGENT PERFORMANCE

The acceptability of a reagent is determined by ensuring that quality control results are within your facility's acceptance criteria.

REAGENT STORAGE AND STABILITY

AST- reagent, when stored unopened at +2°C to +8°C, will obtain the shelf-life indicated on the cartridge label. Once opened and prepared, the reagent is stable for 10 days when stored at +2°C to +8°C unless the expiration date is exceeded.

Reagent storage location:

CALIBRATION

CALIBRATOR REQUIRED

SYNCHRON Enzyme Validator Set (Levels 1 and 2)

CALIBRATOR PREPARATION

No preparation is required.

CALIBRATOR STORAGE AND STABILITY

If unopened, the SYNCHRON Enzyme Validator should be stored at -15°C to -20°C until the expiration date printed on the calibrator bottle. Opened calibrators that are resealed and stored at -15°C to -20°C are stable for 60 days unless the expiration date is exceeded.
Because this product is of human origin, it should be handled as though capable of transmitting infectious diseases. Each serum or plasma donor unit used in the preparation of this material was tested by United States Food and Drug Administration (FDA) approved methods and found to be negative for antibodies to HIV and HCV and nonreactive for HbsAg. Because no test method can offer complete assurance that HIV, hepatitis B virus, and hepatitis C virus or other infectious agents are absent, this material should be handled as though capable of transmitting infectious diseases. This product may also contain other human source material for which there is no approved test. The FDA recommends such samples to be handled as specified in Centers for Disease Control's Biosafety Level 2 guidelines.

Calibrator storage location:

CALIBRATION INFORMATION

1. The system must have a valid calibration in memory before controls or patient samples can be run.

2. Under typical operating conditions the AST- reagent cartridge must be calibrated every 5 days and also with certain parts replacements or maintenance procedures, as defined in the UniCel DxC 600/800 Systems Instructions For Use (IFU) manual. This assay has within-lot calibration available. Refer to the UniCel DxC 600/800 Systems Instructions For Use (IFU) manual for information on this feature.

3. For detailed calibration instructions, refer to the UniCel DxC 600/800 System Instructions For Use (IFU) manual.

4. The system will automatically perform checks on the calibration and produce data at the end of calibration. In the event of a failed calibration, the data will be printed with error codes and the system will alert the operator of the failure. For information on error codes, refer to the UniCel DxC 600/800 System Instructions For Use (IFU) manual.

TRACEABILITY

For Traceability information refer to the Calibrator instructions for use.

QUALITY CONTROL

At least two levels of control material should be analyzed daily. In addition, these controls should be run with each new calibration, with each new reagent cartridge, and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws.

The following controls should be prepared and used in accordance with the package inserts. Discrepant quality control results should be evaluated by your facility.
Table 1.0 Quality Control Material

<table>
<thead>
<tr>
<th>CONTROL NAME</th>
<th>SAMPLE TYPE</th>
<th>STORAGE</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
</tr>
</tbody>
</table>

TESTING PROCEDURE(S)

1. If necessary, load the reagent onto the system.
2. After reagent load is completed, calibration may be required.
3. Program samples and controls for analysis.
4. After loading samples and controls onto the system, follow the protocols for system operations.

For detailed testing procedures, refer to the UniCel DxC 600/800 System Instructions For Use (IFU) manual.

CALCULATIONS

The SYNCHRON System(s) performs all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

REPORTING RESULTS

Equivalency between the SYNCHRON LX and UniCel DxC 600/800 Systems has been established. Chemistry results between these systems are in agreement and data from representative systems may be shown.

REFERENCE INTERVALS

Each laboratory should establish its own reference intervals based upon its patient population. The preliminary upper reference limits listed below were taken from literature.⁴

Table 2.0 Reference intervals

<table>
<thead>
<tr>
<th>INTERVALS</th>
<th>SAMPLE TYPE</th>
<th>CONVENTIONAL UNITS</th>
<th>S. I. UNITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Literature</td>
<td>Serum (Male)</td>
<td>35 IU/L</td>
<td>0.58 µkat/L</td>
</tr>
<tr>
<td></td>
<td>Serum (Female)</td>
<td>31 IU/L</td>
<td>0.52 µkat/L</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>INTERVALS</th>
<th>SAMPLE TYPE</th>
<th>CONVENTIONAL UNITS</th>
<th>S. I. UNITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Refer to References (9,10,11) for guidelines on establishing laboratory-specific reference intervals.

Additional reporting information as designated by this laboratory:

PROCEDURAL NOTES

ANTICOAGULANT TEST RESULTS

If plasma is the sample of choice, the following anticoagulants were found to be compatible with this method based on a study of 20 healthy volunteers:

Table 3.0 Acceptable Anticoagulants

<table>
<thead>
<tr>
<th>ANTICOAGULANT</th>
<th>LEVEL TESTED FOR IN VITRO INTERFERENCE</th>
<th>AVERAGE PLASMA-SERUM BIAS (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium Heparin</td>
<td>14 Units/mL</td>
<td>NSIb</td>
</tr>
<tr>
<td>Lithium Heparin</td>
<td>14 Units/mL</td>
<td>NSI</td>
</tr>
<tr>
<td>Sodium Heparin</td>
<td>14 Units/mL</td>
<td>NSI</td>
</tr>
</tbody>
</table>

a Data shown was collected using SYNCHRON CX Systems. Equivalency between SYNCHRON LX Systems has been established by Deming regression analysis to SYNCHRON CX Systems.
b NSI = No Significant Interference (within ± 6 IU/L or 7%).

LIMITATIONS

Samples with extremely high enzyme activity (>12,000 IU/L or >200.04 µkat/L) may consume all of the NADH substrate before the first absorbance measurement is taken after sample addition. These samples can report either very low enzyme activities or suppress the result as "OIR LO". These samples should be diluted 1:20 with saline and rerun.

INTERFERENCES

1. The following substances were tested for interference with this methodology:

Table 4.0 Interferences

<table>
<thead>
<tr>
<th>SUBSTANCE</th>
<th>SOURCE</th>
<th>LEVEL TESTED</th>
<th>OBSERVED EFFECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin (unconjugated)</td>
<td>Bovine</td>
<td>30 mg/dL</td>
<td>NSIb</td>
</tr>
<tr>
<td>Lipemia</td>
<td>IntralipidC</td>
<td>400 mg/dL</td>
<td>NSI</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>Pyruvic acid</td>
<td>6 mg/dL</td>
<td>NSI</td>
</tr>
</tbody>
</table>

a Data shown was collected using SYNCHRON CX Systems. Equivalency between SYNCHRON LX Systems has been established by Deming regression analysis to SYNCHRON CX Systems.
b NSI = No Significant Interference (within ± 6 IU/L or 7%).
c Intralipid is a registered trademark of KabiVitrum, Inc., Clayton, NC 27250.

2. Samples showing evidence of hemolysis should not be used. Hemolysis may cause falsely elevated results.

3. Refer to References (12,13,14) for other interferences caused by drugs, disease and preanalytical variables.
PERFORMANCE CHARACTERISTICS

ANALYTIC RANGE

The SYNCHRON System(s) method for the determination of this analyte provides the following analytical range:

Table 5.0 Analytical Range

<table>
<thead>
<tr>
<th>SAMPLE TYPE</th>
<th>CONVENTIONAL UNITS</th>
<th>S.I. UNITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum or Plasma</td>
<td>10 – 400 IU/L</td>
<td>0.2 – 6.7 µkat/L</td>
</tr>
<tr>
<td>Serum or Plasma (ORDAC)</td>
<td>350 – 2600 IU/L</td>
<td>5.8 – 43.3 µkat/L</td>
</tr>
</tbody>
</table>

a Overrange Detection and Correction. Refer to the UniCel DxC 600/800 System Instructions For Use (IFU) manual for more details on this function. Samples with activities exceeding the high end of the analytical range should be rerun with ORDAC enabled or diluted with saline and reanalyzed.

REPORTABLE RANGE (AS DETERMINED ON SITE):

Table 6.0 Reportable Range

<table>
<thead>
<tr>
<th>SAMPLE TYPE</th>
<th>CONVENTIONAL UNITS</th>
<th>S.I. UNITS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

SENSITIVITY

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for AST- determination is 10 IU/L (0.2 µkat/L).

EQUIVALENCY

Equivalency was assessed by Deming regression analysis of patient samples to accepted clinical methods.

Serum:

\[ Y \text{ (SYNCHRON LX Systems)} = 1.002X - 4.2 \]
\[ N = 113 \]
\[ \text{MEAN (SYNCHRON LX Systems)} = 60.9 \]
\[ \text{MEAN (IFCC Formulation)} = 65.0 \]
\[ \text{CORRELATION COEFFICIENT (r)} = 0.9968 \]

Refer to References (15) for guidelines on performing equivalency testing.

PRECISION

A properly operating SYNCHRON System(s) should exhibit imprecision values less than or equal to the following:
Table 7.0 Precision Values

<table>
<thead>
<tr>
<th>TYPE OF PRECISION</th>
<th>SAMPLE TYPE</th>
<th>1 SD IU/L</th>
<th>1 SD µkat/L</th>
<th>CHANGEOVER VALUEa IU/L</th>
<th>CHANGEOVER VALUEa µkat/L</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-run</td>
<td>Serum/Plasma</td>
<td>3.0</td>
<td>0.05</td>
<td>85.7</td>
<td>1.43</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Serum/Plasma (ORDAC)</td>
<td>NAb</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>10.0</td>
</tr>
<tr>
<td>Total</td>
<td>Serum/Plasma</td>
<td>4.5</td>
<td>0.08</td>
<td>85.7</td>
<td>1.43</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>Serum/Plasma (ORDAC)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>10.0</td>
</tr>
</tbody>
</table>

a When the mean of the test precision data is less than or equal to the changeover value, compare the test SD to the SD guideline given above to determine the acceptability of the precision testing. When the mean of the test precision data is greater than the changeover value, compare the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.
b NA = Not applicable.

Comparative performance data for a SYNCHRON LX System evaluated using the NCCLS Proposed Guideline EP5-T2 appears in the table below. Each laboratory should characterize their own instrument performance for comparison purposes.

Table 8.0 NCCLS EP5-T2 Precision Estimate Method

<table>
<thead>
<tr>
<th>TYPE OF IMPRECISION</th>
<th>SAMPLE TYPE</th>
<th>No. Systems</th>
<th>No. Data Pointsa</th>
<th>Test Mean Value (IU/L)</th>
<th>EP5-T2 Calculated Point Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SD</td>
</tr>
<tr>
<td>Within-run</td>
<td>Serum Control 1</td>
<td>1</td>
<td>80</td>
<td>22.7</td>
<td>1.49</td>
</tr>
<tr>
<td></td>
<td>Serum Control 2</td>
<td>1</td>
<td>80</td>
<td>175.4</td>
<td>1.37</td>
</tr>
<tr>
<td></td>
<td>Serum Control 3</td>
<td>1</td>
<td>80</td>
<td>327.0</td>
<td>1.96</td>
</tr>
<tr>
<td>Total</td>
<td>Serum Control 1</td>
<td>1</td>
<td>80</td>
<td>22.7</td>
<td>2.41</td>
</tr>
<tr>
<td></td>
<td>Serum Control 2</td>
<td>1</td>
<td>80</td>
<td>175.4</td>
<td>2.47</td>
</tr>
<tr>
<td></td>
<td>Serum Control 3</td>
<td>1</td>
<td>80</td>
<td>327.0</td>
<td>3.50</td>
</tr>
</tbody>
</table>

a The point estimate is based on the data from one system, run for twenty days, two runs per day, two observations per run on an instrument operated and maintained according to the manufacturer’s instructions.

NOTICE
These degrees of precision and equivalency were obtained in typical testing procedures on a SYNCHRON LX System and are not intended to represent the performance specifications for this reagent.

ADDITIONAL INFORMATION

For more detailed information on UniCel DxC Systems, refer to the appropriate system manual.

Beckman Coulter, the stylized logo, and the Beckman Coulter product and service marks mentioned herein are trademarks or registered trademarks of Beckman Coulter, Inc. in the United States and other countries.

May be covered by one or more pat. -see www.beckmancoulter.com/patents.

SHIPPING DAMAGE

If damaged product is received, notify your Beckman Coulter Clinical Support Center.
REVISION HISTORY

Revision AE
Revised Quality Control section

Revision AF
Updated corporate address; updated European Hazard Classification and OSHA precaution, and removed EDTA as an Acceptable Anticoagulant claim.

Revision AG
Added Reagent Preparation visual aid to the Reagent Preparation section.

Revision AH
Added Revision History

Revision AJ
Added new language requirement: Czech, and Korean.

Revision AK
Removed references to CX and LX systems as they are discontinued effective 12/2013.
Added Beckman Coulter trademark statement and disclaimer.

Revision AL
Added GHS Classification information

Revision AM
Added GHS Classification information

Revision AN
Added new language requirement: Romanian

Revision AP
Updates to comply with requirements per Beckman Coulter Global Labeling Policy.

Revision AR
Additional changes to comply with requirements per Beckman Coulter Global Labeling Policy.

Revision AT
Added new language requirement: Bulgarian, Serbian, and Vietnamese. Additional changes to comply with requirements per Beckman Coulter Global Labeling Policy.
# SYMBOLS KEY

## Table 9.0

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>REP</td>
<td>Catalogue Number</td>
</tr>
<tr>
<td>CONTENTS</td>
<td>Contents</td>
</tr>
<tr>
<td>🚚</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>LOT</td>
<td>Batch code</td>
</tr>
<tr>
<td>CE</td>
<td>CE Mark</td>
</tr>
<tr>
<td>Authorized Representative in the European Community</td>
<td>Date of Manufacture</td>
</tr>
<tr>
<td>Biological risks</td>
<td>WARNING</td>
</tr>
<tr>
<td>Do not reuse</td>
<td>WARNING</td>
</tr>
</tbody>
</table>

Made In USA of US and Foreign Components

Made in USA of US and Foreign Components
REFERENCES


Beckman Coulter Eurocenter S.A., 22, rue Juste-Olivier. Case Postale 1044, CH - 1260 Nyon 1, Switzerland
Tel: +41 (0)22 365 36 11

Beckman Coulter, Inc., 250 S. Kraemer Blvd., Brea, CA 92821 U.S.A.