BECKMAN COULTER CE

AU

Instructions For Use

AST
AST

REF OSR6009 4 x 6 mL R1, 4 x 6 mL R2
OSR6109 4 x 25 mL R1, 4 x 25 mL R2
OSR6209 4 x 50 mL R1, 4 x 50 mL R2
OSR6609 4 x 173 mL R1, 4 x 173 mL R2
OSR60180 4 x 17 mL Liquid P-5-P R1-2
60106 6 x 4 mL Liquid Pyridoxal Phosphate

For *in vitro* diagnostic use only.

PRINCIPLE

INTENDED USE

Kinetic UV test for the quantitative determination of aspartate aminotransferase, EC 2.6.1.1 (AST), in human serum and plasma on Beckman Coulter analysers.

OSR6609 for use on the AU5800, AU2700 and AU5400 systems only.

OSR60180 for use with 3-part-reagent enabled systems only.

SUMMARY AND EXPLANATION

References[1,2,3]

AST occurs in a wide variety of tissues including liver, cardiac muscle, skeletal muscle, brain, kidneys, lungs, pancreas, erythrocytes and leucocytes, with highest activities found in liver and skeletal muscle. Measurement of AST is indicated in the diagnosis, differentiation and monitoring of hepatobiliary disease, myocardial infarction and skeletal muscle damage. AST measurement may also be performed as part of medical screening examinations. In some cases, AST may be useful in monitoring the course of myocardial infarction. Where recent myocardial infarction is suspected, AST has a diagnostic sensitivity of 96%, with a diagnostic sensitivity of 86% at 12 hours after onset of chest pain. AST levels may be increased in viral hepatitis and liver disease associated with hepatic necrosis, with 20 to 50 fold elevations frequently encountered. The evaluation of AST activity in relation to ALT (De Ritis ratio; AST/ALT) is a useful indicator of liver damage. Ratios <1.0 are indicative of mild liver damage, and are particularly associated with diseases of an inflammatory nature. Ratios >1.0 are indicative of severe liver disease, usually involving necrosis. Increased AST levels may be detected in cirrhosis, extrahepatic cholestasis, progressive muscular dystrophy, dermatomyositis, acute pancreatitis, haemolytic disease, gangrene, crushed muscle injuries and pulmonary emboli. Slight or moderate increases in AST levels may also be observed after ingestion of alcohol, or administration of drugs including penicillin, salicylates or opiates.

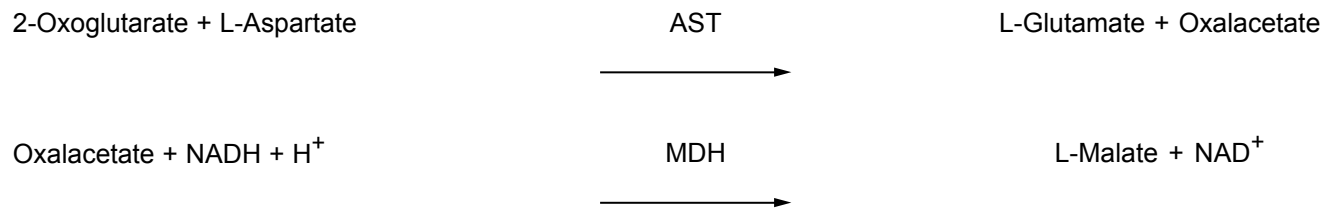
METHODOLOGY

Reference[4]

Method based on the recommendations of the “International Federation for Clinical Chemistry” (IFCC).

In this method, aspartate aminotransferase (AST) catalyses the transamination of aspartate and 2-oxoglutarate, forming L-glutamate and oxalacetate. The addition of pyridoxal phosphate to the reaction mixture ensures maximum catalytic activity of AST. The oxalacetate is reduced to L-malate by malate dehydrogenase (MDH), while NADH is simultaneously converted to NAD^+. The decrease in absorbance due to the consumption of NADH is measured at 340 nm and is proportional to the AST activity in the sample. Endogenous pyruvate is removed by the LDH-reaction during the incubation period.

CHEMICAL REACTION SCHEME



SPECIMEN

TYPE OF SPECIMEN

Serum and heparinised plasma: Stable in serum for 7 days when stored at 2…8°C and 4 days when stored at 15…25°C.[5]

Haemolysed samples should be avoided as the concentration of AST in erythrocytes is approximately 15 times higher than that of normal serum.

Specimen storage and stability information provides guidance to the laboratory. Based on specific needs, each laboratory may establish alternative storage and stability information according to good laboratory practice or from alternative reference documentation.

REAGENTS

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents.

Dispose of all waste material in accordance with local guidelines.

This product contains material of animal origin. The product should be considered as potentially capable of transmitting infectious diseases.

REACTIVE INGREDIENTS

Final concentration of reactive ingredients:

Tris buffer, pH 7.65 (37°C)	80 mmol/L
L-aspartate	240 mmol/L
2-Oxoglutarate	12 mmol/L
LDH	≥ 0.9 kU/L
MDH	≥ 0.6 kU/L
NADH	0.20 mmol/L
Pyridoxal phosphate (P-5-P)	0.1 mmol/L (when Cat. No. 60106 or OSR60180 is used)
Preservative	

The concentrations of the reactive components of the reagents shown on the kit label are the actual concentrations in the individual R1/R2 vials. The reagent composition which is shown in the Instructions For Use is the final concentration of these components in the reaction cuvette after addition of R1, Sample, and R2.

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

AST R1	WARNING	
	H316	Causes mild skin irritation.
	P332+P313	If skin irritation occurs: Get medical advice/attention.
		Tris(hydroxymethyl)– aminomethane 5 - 8%

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

REAGENT PREPARATION

With manual pyridoxal phosphate addition

Pyridoxal Phosphate Liquid (Cat. No. 60106) is supplied separately for pyridoxal phosphate activation. Pipette Pyridoxal Phosphate Liquid 60106 into the R1 bottle according to the table below, and mix by gentle inversion. R2 is ready for use and can be placed directly on board the instrument. This method can also be used for 3-part-reagent enabled systems.

Cat No.	Volume Pyridoxal Phosphate Liquid
OSR6009	0.25 mL
OSR6109	1 mL
OSR6209	2 mL
OSR6609	0.5mL inside the pipe and 6.2mL outside the pipe

Outside the pipe is the gap between the pipe and bottle neck.

Without pyridoxal phosphate activation

The reagents are ready for use and can be placed directly on board the instrument.

REAGENT STORAGE AND STABILITY

Reagents are stable, unopened, up to the stated expiry date when stored at 2…8°C.

Without pyridoxal phosphate activation

Once open, reagents stored on board the instrument are stable for 30 days.

With pyridoxal phosphate activation

After addition of pyridoxal phosphate, R1 stored on board the instrument is stable for 7 days.

R2 stored on board the instrument is stable for 30 days.

Pyridoxal Phosphate Liquid reagent (Cat. No 60106)

Once open, Pyridoxal Phosphate Liquid reagent is stable until the expiry date printed on the label, provided that contamination is avoided through adherence to GLP, the cap is replaced immediately after use and the reagent is stored at 2…8°C.

3-part-reagent enabled systems with P-5-P activation

Reagent Preparation

P-5-P Liquid (Cat. No. OSR60180) is specifically for use on board 3-part-reagent enabled systems. The reagent is ready for use and can be placed directly on board the instrument in the R1 carousel. R1 and R2 are also ready for use and can be placed directly on board the instrument.

STORAGE AND STABILITY

Once open, P-5-P (Cat no OSR60180) is stable on board the instrument for 60 days. R1 and R2 reagent stored on board the instrument are stable for 30 days.

CALIBRATION

CALIBRATION INFORMATION

The test is run in MB-mode. To provide a robust approach to generate the analyser specific MB factor it is recommended that 5 separate calibration events should be used. A fresh vial of calibrator, utilising System Calibrator Cat No. 66300 in the AB calibration mode, should be used for each of these runs. When calculating the mean factor from the separate runs the data should be examined for obvious outliers which should be repeated and replaced. For the AU2700/AU5400 this procedure needs to be performed for each ring. Quality control procedures should be undertaken immediately following calibration in accordance with good laboratory practice.

With pyridoxal phosphate activation the calibrator value is traceable to the IFCC reference method. Without pyridoxal phosphate activation,the calibrator value is traceable to a Beckman Coulter Master Calibrator.

Re-establishment of the analyser specific MB factor is recommended when a critical part of the analyser is replaced.

Reagent blank measurement is recommended when changing to a new lot of reagent.

QUALITY CONTROL

Controls Cat. No. ODC0003 and ODC0004 or other control materials with values determined by this Beckman Coulter system may be used.

Each laboratory should establish its own control frequency however good laboratory practice suggests that controls be tested each day patient samples are tested and each time calibration/blanking is performed.

The results obtained by any individual laboratory may vary from the given mean value. It is therefore recommended that each laboratory generates analyte specific control target values and intervals based on multiple runs according to their requirements. These target values should fall within the corresponding acceptable ranges given in the relevant product literature.

If any trends or sudden shifts in values are detected, review all operating parameters.

Each laboratory should establish guidelines for corrective action to be taken if controls do not recover within the specified limits.

TESTING PROCEDURE(S)

Refer to the appropriate Beckman Coulter AU analyser User Guide/Instructions For Use (IFU) for analyser-specific assay instructions for the sample type as listed in the Intended Use statement. The paediatric application is suitable for use with small volume serum/plasma samples.

CALCULATIONS

The Beckman Coulter analyzers automatically compute the AST activity of each sample.

REPORTING RESULTS

REFERENCE INTERVALS

Reference[6,7]

Male (Adult)	< 50 U/L (0.85 µkat/L)
Female (Adult)	< 35 U/L (0.60 µkat/L)
Newborn	25 − 75 U/L (0.42 − 1.25 µkat/L)
Infant	15 − 60 U/L (0.25 − 1 µkat/L)

Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population, and if necessary determine its own reference interval according to good laboratory practice. For diagnostic purposes, results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

PROCEDURAL NOTES

LIMITATIONS

Highly lipemic samples may exceed the reaction absorbance and will be flagged with a "@". Such samples should be diluted and re-run.

INTERFERENCES

Results of studies conducted to evaluate the susceptibility of the method to interference were as follows:

Icterus:	Interference less than 10% up to 40 mg/dL or 684 µmol/L bilirubin
Lipemia:	Interference less than 5% up to 300 mg/dL Intralipid
Pyruvate:	Interference less than 10% up to 1 mmol/L pyruvate

Refer to Young[8] for further information on interfering substances.

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

Data contained within this section is representative of performance on Beckman Coulter systems. Data obtained in your laboratory may differ from these values.

LINEARITY

The test is linear within an enzyme activity range of 3 – 1,000 U/L (0.05 – 16.7 µkat/L).

SENSITIVITY

The lowest detectable level in serum on an AU5800 analyzer was estimated at 1 U/L.

The lowest detectable level represents the lowest measurable level of AST that can be distinguished from zero. It is calculated as the absolute mean plus three standard deviations of 20 replicates of an analyte free sample.

METHODS COMPARISON

Method Comparison with pyridoxal phosphate activation

Patient serum samples were used to compare this AST OSR6109 assay on the AU640 against the IFCC reference method. Results of linear regression analysis were as follows:

y = 1.032x - 0.2	r = 0.997	n = 112	Sample range = 9 – 250 U/L

PRECISION

The following data was obtained on an AU5800 using 3 serum pools analysed over 20 days.

n = 80	**Within-run**		**Total**	
Mean U/L	**SD**	**CV %**	**SD**	**CV %**
19	0.36	1.9	0.54	2.9
58	0.65	1.1	0.91	1.6
378	2.97	0.8	5.29	1.4

ADDITIONAL INFORMATION

DxC 700 AU requires that each reagent application has a standard format of abbreviated Closed Test Name. This Closed Test Name is required to allow automated loading of the calibrator information for each application as part of the DxC 700 AU Closed System. Refer to the table below for the Closed Test Name assigned to each application for this assay.

Test Name	**Description**
AST1N	AST (Without P5P)
AST1NP	AST (Paediatric without P5P)
AST2N	AST (with P5P 60106)
AST2NP	AST (Paediatric with P5P 60106)
AST3N	AST (with P5P OSR60180)
AST3NP	AST (Paediatric with P5P OSR60180)

Setting Sheet Footnotes
User defined
* Values set for working in U/L. To work in SI units (µkat/L) divide by 60.
§ For use in AB mode only, refer to leaflet for further instruction.

REVISION HISTORY

Added new languages

Preceding version revision history

IFU updated to add Vietnamese language.

REFERENCES

1. Thomas L. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST). In:Thomas L, ed. Clinical laboratory diagnostics. Use and assessment of clinical laboratory results. Frankfurt/Main: TH-Books Verlagsgesellschaft, 1998:55-65.

2. Moss DW, Henderson RA, Kachmar JF. Enzymes. In: Tietz NW, ed. Fundamentals of clinical chemistry. Philadelphia:WB Saunders Company, 1987:369-373.

3. Schmidt E, Schmidt FW. Diagnosis of icteric diseases. Dtsch Med Wschr 1984;109:139-146.

4. 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 Concentration of Aspartate Aminotransferase. Clin Chem Lab Med 2002;40:725-733.

5. Ehret W, Heil W, Schmitt Y, Töpfer G, Wisser H, Zawta B, et al. Use of anticoagulants in diagnostic laboratory investigations and stability of blood, plasma and serum samples. WHO/DIL/LAB/99.1 Rev.2:23pp.

6. 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;29:301-08.

7. Painter PC, Cope JY, Smith JL. Reference information for the clinical laboratory. In: Burtis CA, Ashwood ER, eds. Tietz textbook of clinical chemistry. Philadelphia:WB Saunders Company, 1999;1802pp.

8. Young DS. Effects of Drugs on Clinical Laboratory Tests, AACC, 5th ed. AACC Press, 2000.

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