

Valproic Acid

Order information

REF	CONTENT	Analyzer(s) on which cobas c pack(s) can be used
20737933 322	Valproic Acid (200 tests)	System-ID 07 3793 3 COBAS INTEGRA 400 plus COBAS INTEGRA 800
03375790 190	Preciset TDM I Calibrators A-F (6 × 1 × 5 mL) Diluent (1 × 10 mL)	System-ID 07 6830 8
04521536 190	TDM Control Set Level I (2 × 5 mL) Level II (2 × 5 mL) Level III (2 × 5 mL)	System-ID 07 6900 2 System-ID 07 6901 0 System-ID 07 6902 9
20720720 322	COBAS FP Sample Dilution Reagent II (1 × 200 mL)	System-ID 07 2072 0

English

System information

Test VALPM, test ID 0-893

Intended use

In vitro diagnostic test for the quantitative determination of valproic acid in serum or heparinized plasma on COBAS INTEGRA systems.

Summary

Valproic acid (dipropylacetic acid) is primarily used in the treatment of petit mal seizures and other generalized and partial complex seizures.¹ Although the mechanism of action is unclear, valproic acid has been shown to raise the brain concentration of gamma aminobutyric acid (GABA) while lowering the concentration of cyclic guanosine monophosphate (CGMP).² Due to the potential of severe adverse reactions, it is important that serum levels of valproic acid be monitored during therapy.

Test principle

Fluorescence polarization

COBAS INTEGRA therapeutic drug monitoring measurements are made on the COBAS INTEGRA systems using the principle of fluorescence polarization. When a fluorescent molecule, or fluorophore, is irradiated with light of the proper wavelength (the excitation wavelength) some of the light is absorbed. Within a few nanoseconds the absorbed light is emitted, although at a longer wavelength (the emission wavelength). Whether or not the emitted light is polarized depends on the freedom of the fluorophore to rotate in solution. A small molecule, such as fluorescein, can rotate rapidly before light emission occurs, resulting in depolarization of the emitted light. In contrast, a fluorescent macromolecule, such as a fluorescein-labeled protein, will rotate much more slowly. Thus, in the time frame between excitation and emission, the macromolecule will have rotated only very slightly and the emitted light will be polarized.³ Fluorescence polarization is a reproducible function of the drug concentration, and is suitable for the quantitative determination of drug concentrations in serum for the purpose of therapeutic drug monitoring.

Surface active agents are used to ensure dissociation of the drug from serum proteins and to prevent nonspecific binding of the tracer.

Reagents - working solutions

- R1** Antibody reagent
Anti-valproic acid monoclonal antibody (mouse) in buffer, pH 7.5, with stabilizer and preservative.
- SR** Tracer reagent
Fluorescein-labeled valproic acid derivative in buffer, pH 7.5, with stabilizer and preservative.

R1 is in position B and SR is in position C.

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C

See expiration date on **cobas c** pack label

COBAS INTEGRA 400 plus system

On-board in use at 10-15 °C

12 weeks

COBAS INTEGRA 800 system

On-board in use at 8 °C

26 weeks

The on-board in use stability period begins at the time of **cobas c** pack puncture.

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:

Unhemolyzed serum

Unhemolyzed heparinized 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.

Usual sampling time varies dependent upon route of delivery and desired measurement of peak or trough values.⁴ Specimens should be tested within 2 days of collection if kept capped at 15-25 °C. If specimens must be stored for later testing, they should be kept capped at 2-8 °C for up to 7 days or at -20 °C for 3 months.⁵ Avoid repeated freezing and thawing.

Invert thawed specimens several times prior to testing.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

COBAS FP Sample Dilution Reagent (SDR II), Cat. No. 20720720 322
The SDR II is placed as special diluent in its predefined rack position and is stable for 7 days on-board COBAS INTEGRA 400 plus/800 analyzers.

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.

Applications for serum and plasma

COBAS INTEGRA 400 plus test definition

Measuring mode	FP
Reaction mode	R1-SDR/S-SR
Wavelength	excitation 485 nm emission 515 nm
Reading cycle blank/test	29/45

Unit $\mu\text{g/mL}$ **Pipetting parameters**

		Diluent (H ₂ O)
R1	140 μL	10 μL
Sample	2 μL	5 μL
Special diluent SDR II	18 μL	
SR	15 μL	10 μL
Total volume	200 μL	

COBAS INTEGRA 800 test definition

Measuring mode	FP
Reaction mode	R1-SDR/S-SR
Wavelength	excitation 485 nm
	emission 515 nm
Reading cycle blank/test	40/60
Unit	$\mu\text{g/mL}$

Pipetting parameters

		Diluent (H ₂ O)
R1	140 μL	10 μL
Sample	2 μL	5 μL
Special diluent SDR II	18 μL	
SR	15 μL	10 μL
Total volume	200 μL	

Calibration

Calibrators	Preciset TDM I
	Calibrators A-F
Calibration mode	Exponential 5
Calibration replicate	Duplicate recommended
Deviation low/high	< 10 % at $\geq 12.5 \mu\text{g/mL}$ ($\geq 87 \mu\text{mol/L}$)
Calibration interval	
COBAS INTEGRA 400 plus analyzer	Each lot, every 16 weeks, and as required following quality control procedures
COBAS INTEGRA 800 analyzer	Each lot, every 20 weeks, and as required following quality control procedures

A calibration curve must be prepared using the Preciset TDM I calibrators. Calibrators must be placed from the highest concentration (F) first, to the lowest (A) last, on the CAL/QC rack. This curve is retained in memory by the COBAS INTEGRA systems and recalled for later use.

Traceability: The Preciset TDM I calibrators are prepared to contain known quantities of valproic acid in normal human serum and are traceable to USP reference standards.

Note

Calibrators should be assayed within 2 hours after placing on-board the instrument.

Quality control

Quality control	TDM Control Set
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

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.

Note

Controls should be assayed within 2 hours after placing on-board the instrument.

Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

Conversion factor: $\mu\text{g/mL} \times 6.93 = \mu\text{mol/L}$

Limitations - interference

See the Analytical specificity section of this method sheet for information on substances tested for cross-reactivity in this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors).

Specimens with assay values greater than the highest calibrator will be flagged by the system and must be repeated after appropriate dilution of the original sample with the Preciset TDM I Diluent (0 $\mu\text{g/mL}$). Specimens with high fluorescent backgrounds or those giving polarization values greater than the zero calibrator will also be flagged by the system.

Serum/plasma

Criterion: Recovery within $\pm 10 \%$ of initial value at a valproic acid concentration of 63 $\mu\text{g/mL}$ (437 $\mu\text{mol/L}$).

Icterus:⁶ No significant interference up to a bilirubin concentration of 657 $\mu\text{mol/L}$ or 38.4 mg/dL.

Hemolysis:⁶ No significant interference up to a hemoglobin concentration of 621 $\mu\text{mol/L}$ or 1000 mg/dL.

Lipemia:⁶ No significant interference up to a triglycerides concentration of 1894 mg/dL.

Total protein: No significant interference up to a total protein concentration of 2-12 g/dL.

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 INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges**Measuring range**

COBAS INTEGRA 400 plus analyzer:
2.4-150 $\mu\text{g/mL}$ (17-1040 $\mu\text{mol/L}$)

COBAS INTEGRA 800 analyzer:
3.15-150 $\mu\text{g/mL}$ (22-1040 $\mu\text{mol/L}$)

Lower limits of measurement

Lower detection limit of the test:

COBAS INTEGRA 400 plus analyzer:
2.4 $\mu\text{g/mL}$ (16.8 $\mu\text{mol/L}$)

COBAS INTEGRA 800 analyzer:
3.15 $\mu\text{g/mL}$ (22 $\mu\text{mol/L}$)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from the zero calibrator at a 95 % confidence level.

Expected values

Serum therapeutic concentrations for valproic acid are 50-100 $\mu\text{g/mL}$ (347-693 $\mu\text{mol/L}$), while toxic levels are > 150 $\mu\text{g/mL}$ (> 1040 $\mu\text{mol/L}$).^{1,7,8}

Adverse reactions to valproic acid therapy include liver dysfunction, nausea, vomiting, indigestion, sedation, stupor, and in some cases patients have developed psychiatric symptoms.^{2,9}

Valproic Acid

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 COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using controls in accordance with the NCCLS EP5-T2¹⁰ requirements with repeatability (n = 80) and intermediate precision (2 aliquots per run, 2 runs per day, 20 days). The following results were obtained:

COBAS INTEGRA 400 plus analyzer

Repeatability	Mean µg/mL (µmol/L)	SD µg/mL (µmol/L)	CV %
Level 1	35.2 (246)	1.0 (6.93)	2.8
Level 2	78.7 (550)	2.6 (18.02)	3.3
Level 3	126 (879)	3.7 (25.64)	3.0

Intermediate precision	Mean µg/mL (µmol/L)	SD µg/mL (µmol/L)	CV %
Level 1	35.2 (246)	1.5 (10.40)	4.1
Level 2	78.7 (550)	3.3 (22.87)	4.2
Level 3	126 (879)	5.6 (38.81)	4.4

COBAS INTEGRA 800 analyzer

Repeatability	Mean µg/mL (µmol/L)	SD µg/mL (µmol/L)	CV %
Level 1	26.2 (182)	0.46 (3.19)	1.7
Level 2	60.1 (416)	1.05 (7.28)	1.7
Level 3	102.0 (707)	2.46 (17.05)	2.4

Intermediate precision	Mean µg/mL (µmol/L)	SD µg/mL (µmol/L)	CV %
Level 1	26.2 (182)	0.61 (4.23)	2.3
Level 2	60.1 (416)	1.26 (8.73)	2.1
Level 3	102.0 (707)	2.46 (17.05)	2.4

Method comparison

Valproic acid values for human serum samples obtained on a COBAS INTEGRA 700 analyzer using the COBAS INTEGRA Valproic Acid reagent (y) were compared with those determined using a commercially available FPIA method (x).

	FPIA
Number of samples	207
Range of values	min. 3.2 µg/mL max. ≥ 150 µg/mL
Slope	0.956
Intercept	0.243 µg/mL
Correlation coefficient	0.997

Analytical specificity

The following cross-reactive substances were evaluated on the COBAS INTEGRA systems in normal human serum spiked with valproic acid at 112 µg/mL (776 µmol/L). Each substance was tested at 10 times the highest concentration for its therapeutic or normal range, as per the protocol described by NCCLS.¹¹ The imprecision of the assay was taken into account when determining cross-reactivity. Cross-reactivity was designated as "not detectable" (ND) if the obtained value was less than the sensitivity of the assay.

$$\text{Cross-reactivity (\%)} = \frac{100 \times (\text{analytical result} - \text{analyte concentration})}{\text{concentration of interferent}}$$

Drug	Level tested µg/mL	Cross-reactivity %
Carbamazepine	140	ND
Ethosuximide	1000	ND
2-Phenyl-2-ethyl-malonamide (PEMA)	100	ND
2-Propyl-glutaric acid	100	9.5
2-Propyl-4-pentenoic acid	100	27.2
Phenobarbital	400	ND
Phenytoin	200	ND

ND = Not Detectable

In a similar study, the following structurally related or potentially co-administered compounds were tested on the COBAS FARA II using normal human serum spiked with valproic acid at 75 µg/mL (520 µmol/L).

Drug	Level tested µg/mL	Cross-reactivity %
Carbamazepine-10,11-epoxide	140	ND
Clonazepam	1.2	ND
Diazepam	25	ND
Primidone	120	ND
Salicylate	100	ND

ND = Not Detectable

Any modification of the instrument as set forth in this labeling requires validation by the laboratory.

References

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- Guder WG, Fonseca-Wollheim F, Heil W, et al. Maximum permissible transport and storage times for analysis of blood (serum, plasma), urine and cerebrospinal fluid. DG Klinische Chemie Mitteilungen 1995;26:205-224.
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- National Committee for Clinical Laboratory Standards. User Evaluation of Precision Performance of Clinical Chemistry Devices; Tentative Guideline. Villanova, PA.: NCCLS;1992;4(12). NCCLS Publication EP5-T2.
- National Committee for Clinical Laboratory Standards. Interference Testing in Clinical Chemistry; Proposed Guideline. Villanova, PA.: NCCLS; 1986;6(13). NCCLS Publication EP7-P.

VALP

Valproic Acid

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.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT

Contents of kit



Volume after reconstitution or mixing

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