VALP2

cobas®

ONLINE TDM Valproic Acid

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08058814190	08058814500	ONLINE TDM Valproic Acid (500 tests)	System-ID 2120 001	**cobas c** 303, **cobas c** 503
08445575190	08058814500	ONLINE TDM Valproic Acid (200 tests)	System-ID 2120 002	**cobas c** 303, **cobas c** 503

Materials required (but not provided):

03375790190	Preciset TDM I CAL A-F (1 x 5 mL) Diluent (1 x 10 mL)	Codes 20691-20696	
04521536190	TDM Control Set Level I (2 x 5 mL) Level II (2 x 5 mL) Level III (2 x 5 mL)	 Code 20310 Code 20311 Code 20312	

English

System information

VALP2: ACN 21200

Intended use

In vitro test for the quantitative determination of valproic acid in serum and plasma on **cobas c** systems.

Summary

Valproic acid measurements performed with this assay in human serum and plasma are used for monitoring valproic acid treatment to ensure appropriate therapy.

Valproic acid belongs to the anti-epileptic drugs, also referred to as anticonvulsants or antiseizure medications. It also has mood-stabilizing activity. It is used in the mono- or adjunctive treatment of neurological conditions such as partial seizures, absence seizures and bipolar disorder. The mechanism of action of valproate has not yet been established. It has been suggested that its activity in epilepsy is related to increased GABAergic activity.[1,2,3,4]

In epileptic patients, the clinical endpoint (absence of seizures) can only be assessed prospectively, namely if the patient remains without seizures. Therefore, monitoring of antiseizure medications is used to reduce the risk of drug toxicity on one hand, and on the other hand to indicate likely therapeutic concentrations.[5] In addition, monitoring antiseizure medication levels can be useful in detection of non-adherence to the prescribed medication.[6] Valproic acid is extensively metabolized, mainly in the liver, and the primary route of elimination is through the kidneys (a small percentage unchanged and a large percentage of metabolites).[2,3,4] In the circulation, valproic acid is highly protein bound.[1] In certain clinical or physiological conditions (e.g. uremia, cirrhosis, or concurrent drug therapy), total concentrations may appear to be normal whereas free concentrations may be substantially elevated; therefore, total concentrations have to be interpreted with caution.[1,2,3,4] Especially when enzyme-inducing substances are co-administered, there is a poor correlation between the valproic acid dose and serum/plasma concentrations, resulting in high interindividual variability.[7] High valproic acid concentrations are associated with an increased risk for hepatic toxicity and acute toxic encephalopathy.[1] Therefore, monitoring of valproic acid serum/plasma concentrations and adjustment of the valproic acid dosage, if appropriate, is recommended by scientific societies and drug manufacturers.[3,4,6,7,8]

Test principle

The assay is based on a homogeneous enzyme immunoassay technique used for the quantitative analysis of valproic acid (free and protein-bound) in human serum or plasma. The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts oxidized nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6PDH does not interfere because the coenzyme functions only with the bacterial *(Leuconostoc mesenteroides)* enzyme employed in the assay.

Reagents - working solutions

R1 Anti-valproic acid antibody (mouse monoclonal), G6P, NAD and bovine serum albumin in buffer, preservatives

R3 Valproic acid labeled with bacterial G6PDH, and bovine serum albumin in buffer, preservatives

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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317	May cause an allergic skin reaction.
H412	Harmful to aquatic life with long lasting effects.

Prevention:

P261	Avoid breathing mist or vapours.
P273	Avoid release to the environment.
P280	Wear protective gloves.

Response:

P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Reagent handling

Ready for use

VALP2

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Mix reagents by gentle inversion numerous times before placing on-board the analyzer.

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:	18 weeks

Do not freeze.

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: Collect serum using standard sampling tubes.
Plasma: sodium or lithium heparin, K_2- or K_3-EDTA.

Stability:[9]	2 days capped at 20-25 °C
	7 days capped at 4-8 °C
	3 months capped at -20 °C (± 5 °C)

Freeze only once.

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.

Do not induce foaming of specimens.

Invert thawed specimens several times prior to testing.

Specimens for valproic acid analysis should be drawn just prior to dose, preferably in the fasting state. More frequent monitoring may be necessary when administering valproic acid in the presence or during the withdrawal of other anti-epileptic agents.[10]

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

Reporting time	10 min		
Wavelength (sub/main)	415/340 nm		
Reagent pipetting		Diluent (H_2O)	
R1	66 µL	–	
R3	32 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.5 µL	–	–
Decreased	1.5 µL	–	–
Increased	1.5 µL	–	–

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

Calibration

Calibrators (full calibration)	S1-6: Preciset TDM I calibrators
Calibrators (2-point calibration)	S2: Preciset TDM I-B S5: Preciset TDM I-E
Calibration mode	Non-linear
Calibration frequency **cobas c** 303 analyzer	2-point calibration - every 3 days on-board Full calibration - after reagent lot change - every 2 weeks during shelf life - as required following quality control procedures
Calibration frequency **cobas c** 503 analyzer	Full calibration - after reagent lot change - every 2 weeks during shelf life - 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 USP reference standards.[11] The calibrators are prepared to contain known quantities of valproic acid in normal human serum.

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 18 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 concentration of each sample in the unit µg/mL (µmol/L, mg/L).

Conversion factor:[12]	µg/mL x 6.93 = µmol/L
	µg/mL x 1.0 = mg/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at valproic acid concentrations of approximately 50 and 100 µg/mL (346.5 and 693 µmol/L).

Icterus:[13] No significant interference up to an I index of 30 for conjugated bilirubin and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 30 mg/dL or 513 µmol/L).

Hemolysis:[13] No significant interference up to an H index of 500 (approximate hemoglobin concentration: 500 mg/dL or 310 µmol/L).

Lipemia (Intralipid):[13] No significant interference up to an L index of 500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Criterion: Recovery within ± 10 % of initial value at a valproic acid level of approximately 50 µg/mL (346.5 µmol/L).

Triglycerides: No significant interference from triglycerides up to a concentration of 1000 mg/dL (11.3 mmol/L).

Rheumatoid factors: No significant interference from rheumatoid factors up to a concentration of 100 IU/mL.

Total protein: No significant interference from total protein in the concentration range of 2-12 g/dL.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[14]

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

2.8-150 µg/mL (19.4-1040 µmol/L)

Manually dilute samples above the measuring range 1 + 1 with the Preciset TDM I diluent (0 µg/mL) and reassay. Multiply the result by 2 to obtain the specimen value.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 2.8 µg/mL (19.4 µmol/L)
Limit of Detection	= 2.8 µg/mL (19.4 µmol/L)
Limit of Quantitation	= 6.0 µg/mL (41.6 µmol/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.

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 concentration 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 concentration samples.

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

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

Expected values

Investigator	Therapeutic		Toxic	
	µg/mL	µmol/L	µg/mL	µmol/L
Schobben et al.[15]	50-100	346.5-693.0	—	—
Cloyd and Leppik[16]	50-100	346.5-693.0	> 100	> 693.0
Klotz and Schweizer[17]	40-90	277.2-623.7	—	—
Turnbull et al.[18]	50-100	346.5-693.0	> 100	> 693.0

Several factors complicate interpretation of VPA levels,[19] including time interval between drug administration and blood sampling, the type of seizures treated, albumin concentration and factors affecting albumin binding of VPA, and the presence of other anti-epileptic drugs and pharmacologically-active metabolites of VPA.

Some overlap of toxic and non-toxic values has been reported.[16,18] The ranges, therefore, are provided only as a guide for interpretation along with other clinical symptoms, and are not to be taken as the sole indicator for adjustment of dosage.

Expected values reflect the data and information provided in the reference and do not necessarily represent therapeutic recommendations and/or dosage instructions. For therapeutic recommendations and dosage instructions refer to applicable guidelines and the full prescription information of the drug.

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*	*SD*	*CV*
	µg/mL	*µg/mL*	*%*
TDMC 1[a)]	34.0	0.703	2.1
TDMC 2[b)]	72.1	0.971	1.3
TDMC 3[c)]	109	1.82	1.7
Human serum 1	5.62	0.338	6.0
Human serum 2	52.9	0.849	1.6
Human serum 3	79.5	1.18	1.5
Human serum 4	102	1.84	1.8
Human serum 5	130	1.80	1.4
Intermediate precision	*Mean*	*SD*	*CV*
	µg/mL	*µg/mL*	*%*
TDMC 1[a)]	34.0	1.17	3.4
TDMC 2[b)]	72.1	1.80	2.5
TDMC 3[c)]	108	2.85	2.6
Human serum 1	5.85	0.569	9.7
Human serum 2	53.3	1.40	2.6
Human serum 3	79.5	1.97	2.5
Human serum 4	102	2.79	2.7
Human serum 5	130	3.97	3.1

a) TDM Control Set Level I

b) TDM Control Set Level II

c) TDM Control Set Level III

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

Method comparison

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

Sample size (n) = 70

Passing/Bablok[20]	Linear regression
y = 1.028x + 0.490 µg/mL	y = 1.015x + 1.24 µg/mL
τ = 0.957	r = 0.998

The sample concentrations were between 3.90 and 145 µg/mL.

Valproic acid 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** 501 analyzer (x).

Sample size (n) = 75

Passing/Bablok[20]	Linear regression
y = 1.059x - 1.24 µg/mL	y = 1.050x - 0.899 µg/mL
τ = 0.942	r = 0.996

The sample concentrations were between 3.70 and 150 µg/mL.

Analytical specificity

The following compounds were tested for cross-reactivity.

Compound	Concentration Tested (µg/mL)	% Cross-reactivity
2-Propyl glutaric acid	400	1.6
Carbamazepine	1000	ND
Clonazepam	100	ND
Diazepam	100	ND
Ethosuximide	1000	ND
Phenobarbital	750	ND
Phenytoin	1000	ND
Primidone	1000	ND
2-n-Propyl-3-hydroxy-pentanoic acid (*Rac-erythreo* -3-hydroxy valproic acid)	100	ND
2-n-Propyl-3-hydroxy-pentanoic acid (*Rac-threo* -3-hydroxy valproic acid)	100	4.1
2-n-Propyl-4-hydroxy-pentanoic acid	100	4.5
2-n-Propyl-5-hydroxy-pentanoic acid	50	ND
2-Propyl-2-pentenoic acid	20	ND
2-Propyl-4-pentenoic acid	10	35.5
2-n-Propyl-3-oxo-pentanoic acid	100	ND
2-Propyl succinic acid	500	ND

Cross-reactivity was designated as “not detectable” (ND) if the obtained value was less than the sensitivity of the assay.

Tests were performed on 16 drugs. No significant interference with the assay was found.

Acetaminophen	Doxycycline (Tetracycline)
Acetyl cysteine	Ibuprofen
Acetylsalicylic acid	Levodopa
Ampicillin-Na	Methyldopa + 1.5 H_2O
Ascorbic acid	Metronidazole
Ca-Dobesilate	Phenylbutazone
Cefoxitin	Rifampicin
Cyclosporine	Theophylline

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.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

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 for reconstitution
GTIN	Global Trade Item Number

VALP2

ONLINE TDM Valproic Acid

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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