VALP2

cobas®

Valproic Acid

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04491041 190	ONLINE TDM Valproic Acid 100 tests	System-ID 07 6913 4	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
05108438 190	ONLINE TDM Valproic Acid 200 tests	System-ID 07 6913 4	
03375790 190	Preciset TDM I calibrators CAL A-F (1 x 5 mL) Diluent (1 x 10 mL)	 Codes 691-696	
04521536 190	TDM Control Set Level I (2 x 5 mL) Level II (2 x 5 mL) Level III (2 x 5 mL)	 Code 310 Code 311 Code 312	

English

System information

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

VALP2: ACN 207

For **cobas c** 502 analyzer:

VALP2: ACN 8207

Intended use

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

Summary

Valproic acid (VPA; 2-propylpentanoic acid; Depakene) is a relatively new anticonvulsant medication which is used chiefly for the treatment of primary and secondary generalized seizures, but is also effective against absence seizures.[1,2,3,4,5] It is particularly effective in myoclonus,[6] and is the drug of choice in photosensitive epilepsy.[2] Although VPA is used in conjunction with other anti-epileptic medications, more recent studies have shown benefits of converting treatment to monotherapy with VPA.[7,8] Also, a growing body of evidence suggests that VPA is useful in treatment of affective disorders; in particular, lithium-insensitive bipolar disorders.[9,10]

At therapeutic concentrations, over 90 % of VPA in the circulation is bound to plasma proteins, primarily albumin.[11] Binding is saturable, and at high VPA concentrations, the free fraction increases.[12] Other compounds can compete for VPA binding to albumin; these include salicylic acid[13] and free fatty acids.[14] The concentration of VPA in cerebrospinal fluid is correlated to both the total and unbound concentrations of the drug in plasma.[15]

VPA is converted to a complex mixture of metabolites via β and ω-oxidation and conjugation.[16,17] Some metabolites show significant anti-convulsant activity,[16,17,18] while others may be responsible for some of the drug's toxic side effects.[19]

VPA has the fewest adverse effects of all the widely-used anti-epileptic agents.[20,21] The most common side effects are gastrointestinal disturbances such as nausea and vomiting. Some incidences of tremor, coma or stupor have been noted; these often occur in conjunction with co-administration of other anti-epileptic drugs. Rare occurrences of hepatic failure, Reye-like syndrome, pancreatitis or thrombocytopenia are thought to be individualized reactions unrelated to drug levels.[20] Pharmacokinetics of VPA are highly variable, depending on the form of the drug and route of administration, as well as individual variations in volume of distribution, metabolism and clearance.[13,14] Moreover, co-administration of other anti-epileptic drugs can significantly affect VPA metabolism.[22] Therefore, monitoring VPA concentrations during therapy is essential in order to provide the physician with an indicator for adjusting dosage.

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

R2 Valproic acid labeled with bacterial G6PDH, and bovine serum albumin in buffer

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

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: For prescription use only.

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

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P272 Contaminated work clothing should not be allowed out of the workplace.

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:

Product safety labeling primarily follows EU GHS guidance.

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

Contact phone: all countries: +49-621-7590, USA: 1-800-428-2336

Reagent handling

Ready for use

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

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On-board in use and refrigerated on the analyzer:	12 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:[23]	2 days capped at 20-25 °C
	7 days capped at 4-8 °C
	3 months capped at -20 °C

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.

Do not induce foaming of specimens. Specimens should not be repeatedly frozen and thawed.

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.[2]

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

Deselect Automatic Rerun for these applications in the Utility menu, Application screen, Range tab.

cobas c 311 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 10-15		
Wavelength (sub/main)	415 /340 nm		
Reaction direction	Increase		
Unit	µg/mL		
Reagent pipetting		Diluent (H_2O)	
R1	88 µL	–	
R2	43 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.0 µL	–	–
Decreased	2.0 µL	–	–
Increased	2.0 µL	–	–

cobas c 501/502 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 16-22		
Wavelength (sub/main)	415 /340 nm		
Reaction direction	Increase		
Unit	µg/mL		
Reagent pipetting		Diluent (H_2O)	
R1	88 µL	–	
R2	43 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.0 µL	–	–
Decreased	2.0 µL	–	–
Increased	2.0 µL	–	–

Calibration

Calibrators	S1-6: Preciset TDM I calibrators
Calibration mode	RCM
Calibration frequency	6-point calibration • after reagent lot change • every 2 weeks • as required following quality control procedures

Traceability: This method has been standardized against USP reference standards. 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. 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

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factor:[24] µg/mL x 6.93 = µmol/L

Limitations - interference

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

Serum/Plasma

Icterus:[25] 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:[25] No significant interference up to an H index of 500 (approximate hemoglobin concentration: 500 mg/dL or 310 µmol/L).

Lipemia (Intralipid):[25] 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).

No significant interference from triglycerides up to 1000 mg/dL (11.3 mmol/L).

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

Total protein: No significant interference from protein from 2-12 g/dL.

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In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[26]

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.

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

Lower detection limit of the test

2.8 µg/mL (19.4 µmol/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 2 standard deviations above that of the 0 µg/mL calibrator (standard 1 + 2 SD, repeatability, n = 21).

Expected values

Investigator	Therapeutic		Toxic	
	µg/mL	µmol/L	µg/mL	µmol/L
Schobben et al.[27]	50-100	346.5-693.0	—	—
Cloyd and Leppik[28]	50-100	346.5-693.0	> 100	> 693.0
Klotz and Schweizer[29]	40-90	277.2-623.7	—	—
Turnbull et al.[30]	50-100	346.5-693.0	> 100	> 693.0

Several factors complicate interpretation of VPA levels,[3] 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.[28,30] 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.

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

Specific performane data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in a modified NCCLS EP5-T2 protocol (repeatability n = 63, intermediate precision n = 63). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Serum/Plasma

Repeatability	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	37.9	262.6	1.1	7.8	3.0
Control 2	80.5	557.9	1.7	11.8	2.1
Control 3	117.4	813.6	3.0	20.9	2.6
HS 1	51.6	357.6	1.2	8.0	2.2
HS 2	101.5	703.4	2.5	17.0	2.4

Intermediate precision	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	37.9	262.6	1.7	11.5	4.4
Control 2	80.5	557.9	2.6	18.2	3.3
Control 3	117.4	813.6	4.9	34.1	4.2
HS 1	51.6	357.6	1.7	12.0	3.4
HS 2	101.5	703.4	4.0	27.7	3.9

Method comparison

Serum/plasma

Valproic acid values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined with the corresponding reagent on a Roche/Hitachi 917 analyzer (x) and on a COBAS INTEGRA 800 analyzer (x).

Roche/Hitachi 917 analyzer	Sample size (n) = 65
Passing/Bablok[31]	Linear regression
y = 1.020x + 0.859 µg/mL	y = 1.019x + 0.638 µg/mL
τ = 0.925	r = 0.989

The sample concentrations were between 12.9 and 122.6 µg/mL (89.4 and 849.6 µmol/L).

COBAS INTEGRA 800 analyzer	Sample size (n) = 65
Passing/Bablok[31]	Linear regression
y = 1.012x – 0.031 µg/mL	y = 1.012x – 0.032 µg/mL
τ = 0.941	r = 0.993

The sample concentrations were between 13.5 and 120.1 µg/mL (93.6 and 832.3 µmol/L).

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

1 Chadwick D. Comparison of monotherapy with valproate and other antiepileptic drugs in the treatment of seizure disorders. Am J Med 1988;84(suppl 1A):3-6.

2 Wallace SJ. Use of ethosuximide and valproate in the treatment of epilepsy. Neurol Clin 1986;4:601-616.

3 Rimmer EM, Richens A. An update on sodium valproate Pharmacotherapy 1985;5:171-184.

4 Gram L, Bentsen KD. Valproate: an updated review. Acta Neurol Scand 1985;72:129-139.

5 Clancy RR. New anticonvulsants in pediatrics: Carbamazepine and valproate. Curr Probl Pediatr 1987;17:133-209.

6 Dreifuss FE. Juvenile myoclonic epilepsy: characteristics of a primary generalized epilepsy. Epilepsia 1989;30(4):1-7

7 Wilder BJ, Rangel RJ. Review of valproate monotherapy in the treatment of generalized tonic-clonic seizures. Am J Med 1988;84(suppl 1A):7-13.

8 Chadwick DW. Valproate monotherapy in the management of generalized and partial seizures. Epilepsia 1987;28(suppl 2):12-17.

9 Post RM. Emerging perspectives on valproate in affective disorders. J Clin Psychiatry 1989;50(suppl):3-9.

10 McElroy SL, Keck PE Jr, Pope HG Jr, Hudson JI. Valproate in psychiatric disorders: Literature review and clinical guidelines. J Clin Psychiatry 1989;50(suppl):23-29.

11 Gugler R, Mueller G. Plasma protein binding of valproic acid in healthy subjects and in patients with renal disease. Br J Clin Pharmacol 1978;5:441-446.

12 Gugler R, von Unruh GE. Clinical pharmacokinetics of valproic acid. Clin Pharmocokinet 1980;5:67-83.

13 Miners JO. Drug interactions involving aspirin (acetylsalicylic acid) and salicylic acid. Clin Pharmacokinet 1989;17:327-344.

14 Zaccara G, Messori A, Moroni F. Clinical pharmacokinetics of valproic acid. Clin Pharmacokinet 1988;15:367-389.

15 Löscher W, Nau H, Siemes H. Penetration of valproate and its active metabolites into cerebrospinal fluid of children with epilepsy. Epilepsia 1988;29:311-316.

16 Nau H, Löscher W. Valproic acid and metabolites: Pharmacological and toxicological studies. Epilepsia 1984;25(suppl 1):14-22.

17 Löscher W. Anticonvulsant activity of metabolites of valproic acid. Arch Int Phamacodyn 1981;249:158-163.

18 Löscher W, Hönack D, Nolting B, et al. Trans-2-en- valproate: reevaluation of its anticonvulsant efficacy in standardized seizure models in mice, rats and dogs. Epilepsy Res 1991;9:195-210.

19 Kesterson JW, Granneman GR, Machinist JM. The hepatotoxicity of valproic acid and its metabolites in rats. I. Toxicologic, biochemical and histopathologic studies. Hepatology 1984;4:1143-1152.

20 Dreifuss FE, Langer DH. Side effects of valproate. Am J Med 1988;84(Suppl 1A):34-41.

21 Schmidt D. Adverse effects of valproate. Epilepsia 1984;25(suppl 1):44-49.

22 Bourgeois BFD. Pharmacologic interactions between valproate and other drugs. Am J Med 1988;84(suppl 1A):29-33.

23 Guder W, Fonseca-Wollheim W, Heil O, et al. Maximum permissible transport and storage times for analysis of blood (serum, plasma), urine and cerebrospinal fluid. DG Klinische Chemische Mitteilungen 1995;26:205-224.

24 Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: WB Saunders Company 1995;884.

25 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

26 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

27 Schobben F, van der Kleijn E, Gabreëls FJM. Pharmacokinetics of di-N-propylacetate in epileptic patients. Eur J Clin Pharmacol 1975;8:97-105.

28 Cloyd JC, Leppik IE. Valproic acid: therapeutic use and serum concentration monitoring. "In: Taylor WJ, Finn AL, eds. Individualizing Drug Therapy: Practical Applications of Drug Monitoring. NewYork, NY: Gross, Townsend, Frank, Inc 1981:87-108.

29 Klotz U, Schweizer C. Valproic acid in childhood epilepsy: Anticonvulsive efficacy in relation to its plasma levels. Int J Clin Pharmacol Ther Toxicol 1981;18:461-465.

30 Turnbull DM, Rawlins MD, Weightman E, et al. Plasma concentrations of sodium valproate: Their clinical value. Ann Neurol 1983;14:38-42.

31 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.

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
GTIN	Global Trade Item Number

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

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