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Syva®

Emit® 2000 Valproic Acid Assay

See shaded sections:
Updated information from 2015-03 edition.

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Valproic Acid Assay

1 Intended Use

The Emit® 2000 Valproic Acid Assay is a homogeneous enzyme immunoassay intended for *in vitro* diagnostic use in the quantitative analysis of valproic acid in human serum or plasma.

2 Summary and Explanation of the Test

Monitoring valproic acid concentrations in serum helps to individualize drug therapy for safe and effective control of absence seizures, other generalized seizures, and partial seizures. Serum valproic acid monitoring is useful to assess patient compliance, or to explain changes in seizure control or drug toxicity.[1]

Achieving and maintaining therapeutic concentrations of serum valproic acid is difficult due to marked inter- and intra-patient variability in pharmacokinetics. The pharmacokinetics may be altered by age, pregnancy, renal failure, liver dysfunction, other drugs, low albumin and other factors.[1]

Valproic acid has pharmacokinetic parameters that make it susceptible to drug interactions. Valproic acid is extensively metabolized by the liver. Other coadministered drugs, including other antiepileptics, may induce or inhibit the drug metabolizing enzymes of the liver. When these drugs are added or removed from the therapeutic regimen of a patient, the clearance and concentration of valproic acid may be altered, requiring dosage adjustment.[1]

Adverse reactions associated with high concentrations of valproic acid are central nervous system depression, tremor, thrombocytopenia, and increases in liver function tests. These reactions may be minimized by dosage titration. Very high concentrations of valproic acid may also increase the risk of developing fatal hepatotoxicity, stupor, coma, or cerebral edema.[1]

The methods historically used to monitor valproic acid are nonisotopic immunoassay and gas chromatography.[2]

3 Principle

The Emit® 2000 Valproic Acid Assay is a homogeneous enzyme immunoassay technique used for the quantitative analysis of valproic acid (free and protein-bound) in human serum or plasma. In the performance of the Emit® 2000 Valproic Acid Assay, serum or plasma is mixed with Reagent 1, which contains antibodies to valproic acid and the coenzyme nicotinamide adenine dinucleotide (NAD). Subsequently, Reagent 2, containing valproic acid labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH), is added. Valproic acid in the sample and valproic acid labeled G6PDH compete for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the valproic acid concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts oxidized NAD to NADH, resulting in an absorbance change that can be measured spectrophotometrically. Endogenous G6PDH does not interfere because the coenzyme functions only with the bacterial *(Leuconostoc mesenteroides)* enzyme employed in the assay.

4 Reagents

REF	Product Description	Volume
4G019UL	**Emit® 2000 Valproic Acid Assay Antibody/Substrate Reagent 1** mouse monoclonal antibodies reactive to valproic acid (2 µg/mL),* G6P (22 mM), NAD (18 mM), bovine serum albumin, stabilizers, and preservatives	28 mL
	Enzyme Reagent 2 valproic acid labeled with bacterial G6PDH (0.39 U/mL),* Tris buffer, bovine serum albumin, stabilizers, and preservatives	14 mL

**** The antibody titer and enzyme conjugate activity may vary from lot to lot.***

Note: Reagents 1 and 2 are provided as a matched set. They should not be interchanged with components of kits with different lot numbers.

For *in vitro* diagnostic use.

Precautions

- Reagent 2 contains sodium azide (<0.1%) as a preservative. Sodium azide can react with copper or lead pipes in drain lines to form explosive compounds. Dispose of properly in accordance with local regulations.
- Reagents and calibrators contain materials that may cause sensitivity on contact with skin.
- Although the reagents contain a blocking agent for human anti-mouse antibody (HAMA), the HAMA in some patient samples may interfere with the method.

Preparation and Storage of Assay Components

Reagents:

The Emit® 2000 Valproic Acid Assay reagents are provided ready to use and may be used directly from the refrigerator. Close the reagent bottles when not in use.

Note: Caps must always be replaced on the original containers.

When not in use, store reagents at 2–8°C (36–46°F), upright and with screw caps tightly closed. If stored as directed, reagents are stable until the expiration date printed on the label. Refer to the analyzer-specific protocols for on-instrument stability information. Do not freeze reagents. Avoid prolonged exposure to temperatures above 32°C. **Improper storage of reagents can affect assay performance**.

5 Specimen Collection and Preparation

- Each assay requires serum or plasma. Whole blood cannot be used. The anticoagulants EDTA, heparin, citrate and oxalate/fluoride have been tested and may be used with this assay.
- Sample volume is instrument-dependent. Refer to the appropriate analyzer-specific protocol.
- To obtain a serum valproic acid concentration that best represents the peak tissue level, draw the sample 1 to 3 hours after an oral dose is given. Collect a trough sample just before the next scheduled dose.[1]
- Pharmacokinetic factors influence the correct time of sample collection after the last drug dose. These factors include dosage form, mode of administration, and biological variations affecting drug disposition.[1]
- Serum or plasma samples may be stored refrigerated at 2–8°C. For transporting, maintain the sample temperature at 2–8°C. Samples may be frozen (-20°C) for 1 year.[2]
- Human serum or plasma samples should be handled and disposed of as if they were potentially infectious. It is recommended that human specimens be handled in accordance with the *OSHA Standard on Bloodborne Pathogens* or other appropriate local practices.[3,4]

6 Procedure

Materials Provided

Emit® 2000 Valproic Acid Assay
- Reagent 1
- Reagent 2

Materials Required But Not Provided

4G109UL Emit® 2000 Valproic Acid Calibrators (0, 10, 25, 50, 100, 150 µg/mL)
Multi-level commercial controls
General chemistry analyzer (see Section 6, Procedure, Instruments)

Instruments

Siemens Healthcare Diagnostics provides instructions for using this assay on a number of chemistry analyzers. Contact the Technical Assistance Center in the USA or your local Siemens representative for application sheets.

Analyzers must be capable of maintaining a constant reaction temperature, pipetting specimens/reagents and measuring enzyme rates precisely, timing the reaction accurately, and mixing reagents thoroughly.

Calibration

Recalibrate whenever a new lot of reagents is used or as indicated by control results (see Quality Control, below). If a new set of reagents with the same lot number is used, validate the system by assaying controls.

Quality Control

1. Validate the calibration by assaying multi-level controls. Commercial controls are available for this purpose. Ensure that control results fall within acceptable limits as defined by your own laboratory. Once the calibration is validated, run samples.
2. Follow government regulations or accreditation requirements for quality control frequency. At least once each day of use, analyze two levels of a Quality Control (QC) material with known valproic acid concentrations. Follow your laboratory internal QC procedures if the results obtained are outside acceptable limits.
3. Refer to the instrument operator's manual for appropriate instrument checks.

Diluting High-Concentration Samples

To estimate valproic acid concentrations above the assay range, patient samples containing more than 150 µg/mL (1040 µmol/L) valproic acid may be diluted with 1 or 2 parts of distilled or deionized water or Emit® 2000 Valproic Acid Calibrator 0. After diluting the sample, test and multiply the results by the dilution factor.

Daily Maintenance

Refer to the instrument operator's manual for maintenance instructions.

7 Results

- Results are calculated automatically by the analyzers. No additional manipulation of data is required unless samples have been manually diluted.
- Consult the appropriate instrument operating manual and analyzer-specific protocol for complete instructions.
- The concentration of valproic acid in serum or plasma depends on the time of the last drug dose; time of sample collection; disease states that affect drug clearance; age; concomitant drug therapy; and individual variations in absorption, distribution, and elimination. These parameters must be considered when interpreting results.[1]
- An increase of the biologically active free fraction of the drug, caused by saturation of the protein binding sites or disease states that alter protein binding, can influence the relationship between serum or plasma valproic acid concentration and clinical response. The patient may exhibit toxic symptoms although the total drug concentration is within the therapeutic range.[1]
- Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.
- Siemens has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. User defined modifications are not supported by Siemens as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in Siemens Application Sheets or these instructions for use.

8 Limitations

When diluting patient samples containing high valproic acid concentrations, the following factors can affect the accuracy of the result: diluting with the correct fluid (Emit® 2000 Valproic Acid Calibrator 0 or distilled or deionized water), the accuracy of the dilution, and assay's specificity to drug metabolites.

9 Expected Values

The Emit® 2000 Valproic Acid Assay accurately quantitates valproic acid concentrations in human serum or plasma up to 150 µg/mL (1040 µmol/L). In most patients, valproic acid serum concentrations of 50–100 µg/mL (347–693 µmol/L) effectively control generalized and partial seizures. Seizure control may improve at levels greater than 100 µg/mL (693 µmol/L), but toxicity may occur at levels of 100–150 µg/mL (693–1040 µmol/L).[5]

Note: To convert from µg/mL to µmol/L valproic acid, multiply by 6.93.

For effective treatment, some patients may require serum or plasma levels outside this range. Therefore, the expected range is provided only as a guide, and individual patient results should be interpreted in light of other clinical signs and symptoms.

10 Specific Performance Characteristics

The data appearing in this section were collected on the Syva®-30R Biochemical System.

The following performance characteristics represent total system performance and should not be interpreted to pertain only to reagents. Performance may vary depending on the instrumentation used.

Specificity

The Emit® 2000 Valproic Acid Assay measures the total valproic acid concentration (protein-bound plus unbound) in serum or plasma. Compounds, whose chemical structure would suggest possible cross-reactivity or other therapeutics concurrently used, have been tested. The compounds listed in Table 1 do not interfere with the Emit® 2000 Valproic Acid Assay when tested in the presence of 50 µg/mL valproic acid. Levels tested were at or above maximum physiological or pharmacological concentrations.

Table 1 — Specificity

Compound	Concentration Tested (µg/mL)
Carbamazepine	1000
Clonazepam	100
Diazepam	100
Ethosuximide	1000
2-n-Propyl-3-hydroxy-pentanoic acid	100
2-n-Propyl-4-hydroxy-pentanoic acid	100
2-n-Propyl-5-hydroxy-pentanoic acid	50
2-n-Propyl-3-oxo-pentanoic acid	100
Phenobarbital	750
Phenytoin	1000
Primidone	1000
2-Propyl glutaric acid	400
2-Propyl-2-pentenoic acid	20
2-Propyl-4-pentenoic acid	10
2-Propyl succinic acid	500

For additional information, contact Siemens.

Sensitivity

The sensitivity level of the Emit® 2000 Valproic Acid Assay is less than 1 µg/mL valproic acid. This level represents the lowest concentration of valproic acid that can be distinguished from 0 µg/mL with a confidence level of 95%.

Endogenous Substances

No interference has been found in samples to which 50 µg/mL valproic acid and either 800 mg/dL hemoglobin or 30 mg/dL free bilirubin were added to simulate hemolytic or icteric samples.

No interference has been found in patient samples containing elevated concentrations of endogenous triglyceride (690–1040 mg/dL) to which 60 µg/mL valproic acid was added.

Calibration Stability

Studies have shown calibration stability of at least 14 days. The *permanent* quality control limits used in these studies were established by following the instructions in Section 6, Procedure, Quality Control. Calibration stability may vary from laboratory to laboratory depending on handling of reagents, maintenance of instruments, adherence to operating procedures, establishment of control limits, and verification of calibration.

Precision

Precision values were obtained using the Syva®-30R Biochemical System. Precision was determined by measuring 2 replicates each of in-house tri-level controls on 20 days with 2 runs per day. Precision was calculated according to National Committee for Clinical Laboratory Standards (NCCLS) Guideline EP5-A (February 1999). Results of these studies are summarized in Table 2.

Table 2 — Precision

Control	Number of Replicates	Mean (µg/mL)	Standard Deviation (µg/mL)	Coefficient of Variation (%)
Within-Run				
1	80	19.7	0.92	4.7
2	80	75.5	1.62	2.1
3	80	117.6	3.47	3
Total				
1	80	19.7	1.08	5.5
2	80	75.5	2.25	3
3	80	117.6	4.30	3.7

Accuracy

Patient serum samples with valproic acid concentrations ranging from 18.3 µg/mL to 146.9 µg/mL were analyzed on the Syva®-30R Biochemical System by the Emit® 2000 Valproic Acid Assay and by the Emit® Valproic Acid Assay, and the results were compared. Data are summarized in Table 3.

Table 3 — Accuracy

	Emit® 2000 vs Emit®
Slope	1.00
Intercept (µg/mL)	2.5
Mean (µg/mL)	
Emit® 2000 Assay	80.8
Comparison Method	78
Standard Error of the Estimate (µg/mL)	2.26
Correlation Coefficient	0.996
Number of Samples	99

11 Risk and Safety

H317
P280, P272, P302 + P352, P333 + P313, P501

Warning!
May cause an allergic skin reaction.

Wear protective gloves/protective clothing/eye protection/face protection. Contaminated work clothing should not be allowed out of the workplace. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention. Dispose of contents and container in accordance with all local, regional, and national regulations.

Contains: 5-chloro-2-methyl-3(2h)-isothiazolone mixture with 2-methyl-3(2h)-isothiazolone.

Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics

12 Bibliography

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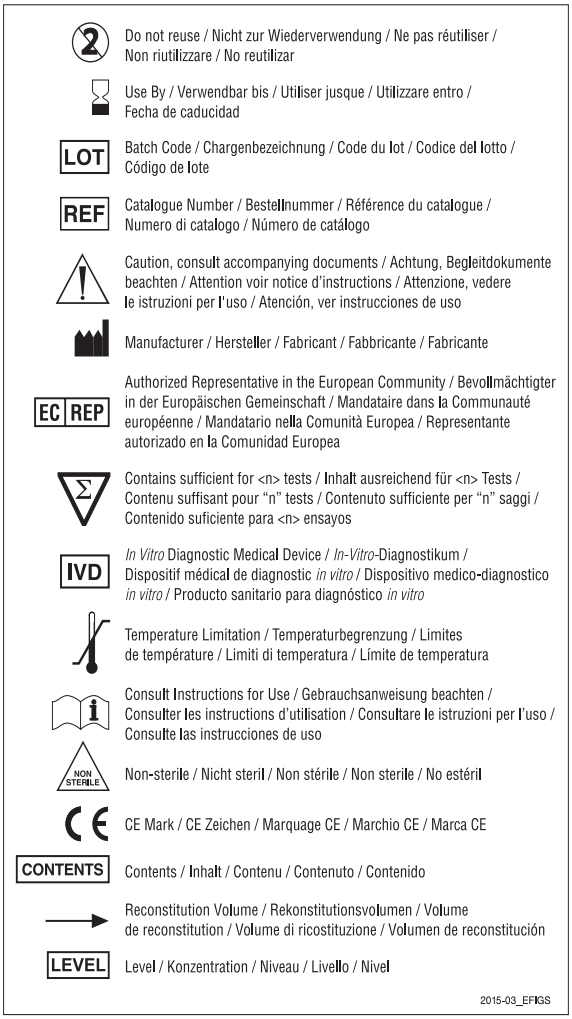
2. Tietz, NW. *Clinical Guide to Laboratory Tests*. 3rd ed. Philadephia, Pennsylvania: Saunders Co; 1995:884–885.
3. Occupational exposure to bloodborne pathogens (29 CFR 1910.1030). *Federal Register*. December 06, 1991;56:64004; amended April 13, 1992;57:12717; July 01, 1992;57:29206; February 13, 1996;61:5507.
4. World Health Organization. *Laboratory Biosafety Manual*. 2nd ed. Geneva: World Health Organization; 1993.
5. Jacobs DS, DeMott WR, Grady HJ, Horvat RT, Huestis DW, Kasten BL Jr. *Laboratory Test Handbook*. 4th ed. Hudson, Ohio: Lexi-Comp Inc; 1996:577–578.

13 Symbols Key



For technical assistance, call Siemens Healthcare Diagnostics:
1-800-227-8994 in the USA
1-800-264-0083 in Canada
Outside the USA and Canada, call your local Siemens representative.

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