In Vitro Diagnostics
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This package insert must be read carefully prior to use.

Valproic acid assay kit
(Classification No.: 30404000)

Nanopia TDM Valproic Acid

General Precautions

1. This product is for in vitro diagnostic use, and must not be used for any other purposes.
2. Clinicians should make a comprehensive clinical decision based on assay results in conjunction with clinical symptoms and other examination results.
3. This product should be used only as directed in this package insert. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.
4. If the reagent accidentally comes in contact with eyes and/or mouth, rinse immediately with ample water as first aid, and consult the doctor if required.
5. Carefully read the operating instructions for each type of automated analyzers prior to using this product. Parameters for each type of analyzers are available, and can be requested from SEKISUI MEDICAL CO., LTD. if required.
6. Perform a quality control test prior to assay to ensure accuracy.

Description (Kit Components)

Component: Ingredients
VPA Antibody Solution 1: Anti-valproic acid mouse monoclonal antibody
VPA Latex Reagent 2: Valproic acid-coated latex

Intended Use

Measurement of valproic acid in serum or plasma

Valproic acid (2-propylpentanoic acid) is an antiepileptic drug that was developed relatively recently. It is mainly used for the treatment of primary seizures and secondary generalized seizures. Valproic acid is effective for primary generalized seizures, especially myoclonic seizures that tend to be induced by photic or sonic stimulation. It is also known to be effective for the treatment of non-convulsive generalized seizures and affective seizures such as partial seizures in children. Valproic acid is coadministered with other antiepileptic drugs. However, recent studies have shown that valproic acid is also effective as monotherapy.[1),2)]

At the therapeutic range, 90% or more of valproic acid in the bloodstream is bound to plasma proteins, especially albumin. The free valproic acid concentration is generally correlated with the total concentration of the drug. It has also been reported that the concentration of valproic acid in the cerebrospinal fluid is correlated with the total or free plasma valproic acid concentration at the therapeutic range. However, free valproic acid increases at higher concentrations due to saturation of binding. Because valproic acid competes with other antiepileptic drugs for binding to albumin, the free valproic acid concentration is higher when it is coadministered with other antiepileptic drugs than when it is used alone. Similarly, valproic acid competes with salicylic acid and free fatty acids for binding to albumin. Various metabolites of valproic acid are produced by β-oxidation, ω-oxidation, or conjugation. Some of these metabolites have anticonvulsant activity, while others are known to cause adverse reactions.

It has been reported that the risk of valproic acid causing adverse reactions is lower than with any other standard antiepileptic drug. General adverse reactions to valproic acid include gastrointestinal symptoms such as nausea and vomiting. As serious adverse reactions, symptoms related to the central nervous system have been reported, including tremor, clouding of consciousness, and coma. These reactions are often noted during coadministration of valproic acid with other antiepileptic drugs. The above-mentioned adverse reactions are dependent on the blood level of valproic acid. While rare, adverse reactions such as hepatic failure, Reye's syndrome (acute encephalopathy), pancreatitis, and thrombocytopenia are known to occur independently of the blood level.

It is well known that the time to reach the maximum blood concentration and the biological half-life of valproic acid show inter-individual variations, because the volume of distribution, metabolism, and elimination of this drug differ among patients. It has also been reported that these parameters show considerable variation depending on the dosage form of this drug. Metabolism of valproic acid is also profoundly affected by coadministration with other antiepileptic drugs. Therefore, monitoring the blood level of valproic acid is important to determine the appropriate dosage.

Assay Principle

1. Assay Principle

When a certain amount of anti-valproic acid antibody is added and reacted with a sample, consumption of the antibody depends on its content in the sample. When valproic acid-coated latex is added, residual anti-valproic acid antibody reacts with the latex and forms aggregates. Since the extent of aggregation depends on the valproic acid concentration in the sample, the valproic acid concentration can be determined by measuring aggregation as the change of absorbance.

Sample (valproic acid) + Anti-valproic acid antibody ⟶ Antigen-antibody reaction

Unreacted anti-valproic acid antibody + Valproic acid-coated latex ⟶ Aggregation by antigen-antibody reaction

2. Features

1) Because a highly specific monoclonal antibody is used, this product shows excellent sensitivity and accuracy.

2) Liquid reagents, ready-to-use.
3) Applicable to various automated analyzers.

Procedural Precautions **

1. Properties of Samples and Sampling Methods

1) Samples
Serum and plasma (heparin plasma, EDTA plasma and citrated plasma) may be used.

2) Storage of samples
If the isolated serum or plasma sample cannot be tested on the same day, specimens should be stored as follows:
2–8°C: for tests within 7 days
≤ -20°C: for tests within 4 weeks
Bring samples to room temperature (15–30°C) before use.

3) Caution must be exercised, because a separating agent, etc. in the blood collection tube may affect assay values.[3)]

4) Sampling should be performed after removing insoluble matter from the sample.

2. Interfering substances

1) Assay results are not affected by free bilirubin (up to 20 mg/dL), conjugated bilirubin (up to 20 mg/dL), hemoglobin (up to 500 mg/dL), ascorbic acid (up to 50 mg/dL), formazin turbidity (up to 2500 FTU), or rheumatoid factors (up to 450 IU/mL).

2) Because mouse antibody is used in the assay, artifactual elevation of results may occur if the sample contains human anti-mouse antibody. In this case, perform re-measurement by another method.

3) Cross-reactivity
The following table summarizes the cross-reactivity with the valproic acid-like substances and other drugs.

Compound	Concentration tested (μg/mL)	Cross-reactivity (%)
2-Phenyl-2-ethylmalonamide	1000	0.5
2-Propylglutaric acid	500	10.6
Carbamazepine	1000	0.5
Carbamazepine-10,11-epoxide	1000	0.6
Clonazepam	1000	0.5
Diazepam	2000	0.3
Phenobarbital	2000	0
Phenytoin	400	1
Primidone	1000	0.2
Salicylic acid	1000	0.6

3. Others

1) Always use TDM Calibrator for Nanopia for calibration.

2) Precautions for assay range
If the concentration of a target substance in the sample exceeds the measurement range, dilute the sample with a separately sold diluent (manufactured by SEKISUI MEDICAL CO., LTD.), and perform re-measurement.

Dosage/Administration (Assay Procedure)

1. Preparation of reagents

Reagent (1): VPA Antibody Solution 1 is ready to use.
Reagent (2): VPA Latex Solution 2 is ready to use.
Before using this product, gently invert the VPA Latex Reagent 2 bottle to mix it thoroughly, and check that there are no bubbles.

2. Assay Procedure

This product is compatible with various types of automated analyzer. An example of the assay procedure is indicated below.

Sample 2 μL + Reagent (1) 150 μL → 37°C 270 sec → Reagent (2) 50 μL → 37°C 53 sec → Measurement (Absorbance I※)
→ 37°C 107 sec → Measurement (Absorbance II※)
→ Calculation of concentration

※Absorbance I and II: Absorbance at 600 nm
Calibration material: TDM Calibrator for Nanopia (Manufacture's assigned value)

Assessment of Assay Results **

1. Reference Standard Range

The therapeutic rage of valproic acid has been reported to be 50-100 μg/mL. In general, the concentration over 200 μg/mL is considered as the toxic zone, but the symptoms of adverse reaction may occur within the zone of therapeutic concentrations.[4)] Therefore, interpretation of assay results should also be based on the patient's clinical findings and other examination results.
It is recommended that the blood concentration of valproic acid should be measured in a sufficient number of samples for statistical analysis and that its therapeutic range should be determined by each medical institution.

2. There may be reactions or interfering reactions with non-target substances. If assay results appear to be unreliable, repeat the measurement (if necessary, after dilution) or try another analytical methods.

Performance **

1. Sensitivity

1) The change of absorbance of the standard solution (0 μg/mL) per minute is 0.15–0.30.

2) The ratio between the change of absorbance per minute with the standard solution (0 μg/mL) and that with another standard solution (12.5 μg/mL) is 68–95%.

2. Accuracy: 80–120 % of the expected assay value

3. Within-run Reproducibility:
Coefficient of variation ≤ 10 %
(Test methods used for 1.–3. are in-house methods.)

4. Measurement Range[5)]: (On Hitachi 7170S automated analyzer)
12–150 μg /mL

5. Correlation[5)]

1) Serum N=66 r=0.998 y=1.08x–0.29
Control method: Approved in vitro diagnostic (enzyme immunoassay)

2) Plasma N=101 r=0.994 y=1.04x+2.77
Control method: Approved in vitro diagnostic (enzyme immunoassay)

6. Standard Material
Valproic Acid (U.S. Pharmacopoeia)

Precautions for Use or Handling

1. Precautions for Handling (to Ensure Safety)

1) All samples used in the test should be handled as a material possibly infected with HIV, HBV, HCV, or other viruses. To prevent infection, use disposable gloves and avoid mouth pipetting during the test.
2) Sodium azide is added as an antiseptic agent in the VPA Antibody Solution 1 and VPA Latex Reagent 2. Therefore, if the reagent comes in accidentally contact with eyes, mouth or skin, rinse immediately with ample water as first aid, and consult the doctor if required.

2. Precautions for use

1) This product should be stored as directed, without freezing. Freezing can deteriorate the reagents, which can produce inaccurate results. Therefore, avoid using the reagents which have been previously frozen.
2) Do not use expired reagents. Use of such reagents cannot guarantee the reliability of measurement values.
3) Do not replenish the reagents.
4) Do not mix materials from different kit lot numbers.
5) Do not perform the assay under direct sunlight

3. Precautions for Disposal

1) Before disposal, used samples and their containers must be immersed in sodium hypochlorite solution at a concentration of greater than 0.1% for longer than 1 hour or autoclaved at 121ºC for 20 minutes.
2) To prevent infections from spilled samples or solutions containing samples, wipe the spilled area thoroughly with disinfectants such as sodium hypochlorite solution at a concentration of greater than 0.1%.
3) The reagents and treated samples should be discarded as medical waste or industrial waste according to the waste disposal regulations.
4) The reagents should be disposed of in accordance with the Water Pollution Control act or related regulations.
5) Sodium azide has been added as an antiseptic agent in the VPA Antibody Solution 1 and VPA Latex Reagent 2. It can react with lead or copper pipes to produce the highly explosive metal azide. Therefore, the reagent should be flushed with large amounts of water during disposal.

4. Other precautions
Do not use the containers for other purposes.

Storage and Shelf Life

1. Storage temperature: 2–8°C
2. Shelf life: 20 months from the date of manufacture (The expiration date is printed on the outer package.)

Packaging

Name		Package
Nanopia TDM Valproic Acid	VPA Antibody Solution 1	1 × 30 mL
	VPA Latex Reagent 2	1 × 10 mL

Constituent reagents are available in other configurations. For further details please contact SEKISUI MEDICAL CO., LTD.

References **

1) Wilder B. J., Rangel R. J. : Review of valproate monotherapyin the treatment of generalized tonic−clonic seizures.Am. J. Med. 84（Suppl 1A）: 7−13, 1988.
2) Chadwick D. W. : Valproate monotherapy in the management of generalized and partial seizures. Epilepsia 28 （Supplement 2）: S12−S17, 1987.
3) Sawada T. et. al.: J Med Pharm Sci, 51(1), 131−141, 2004.
4) The Japanese Society of Therapeutic Drug Monitoring. [Guideline for therapeutic drug monitoring (TDM) for antiepileptic drug 2018]. Tokyo: Kanehara Shuppan; 2018, pp. 14-15, pp. 57-64.
5) In house data, SEKISUI MEDICAL CO., LTD.

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