QMS® Topiramate (TPM)

Thermo SCIENTIFIC

IVD **For In Vitro Diagnostic Use Only**

Rx Only

REF **0374140**
10017218

This Quantitative Microsphere System (QMS) package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Intended Use
The QMS Topiramate assay is intended for the quantitative determination of topiramate in human serum or plasma on automated clinical chemistry analyzers.

The results obtained are used in the diagnosis and treatment of topiramate overdose and in monitoring levels of topiramate to help ensure appropriate therapy.

Summary and Explanation of the Test
Topiramate (2,3:4,5-Di-*O*-isopropylidene-ß-D-fructopyranose) is an anticonvulsant drug approved for use in the treatment of epilepsy and is often prescribed as monotherapy or as one component of a multiple anti-epileptic drug therapy.[1]

Principles of the Procedure
The QMS Topiramate assay is a homogeneous particle-enhanced turbidimetric immunoassay. The assay is based on competition between drug in the sample and drug coated onto a microparticle for antibody binding sites of the topiramate antibody reagent. The topiramate-coated microparticle reagent is rapidly agglutinated in the presence of the anti-topiramate antibody reagent and in the absence of any competing drug in the sample. The rate of absorbance change is measured photometrically. When a sample containing topiramate is added, the agglutination reaction is partially inhibited, slowing down the rate of absorbance change. A concentration-dependent classic agglutination inhibition curve can be obtained with maximum rate of agglutination at the lowest topiramate concentration and the lowest agglutination rate at the highest topiramate concentration.

Reagents
Reagent Kit

QMS Topiramate, **REF** 0374140, 10017218 is supplied as a liquid, ready-to-use, two-reagent kit that contains:

REF 0374140	**REF** 10017218
R1 Reagent 1 1 x 22 mL	**R1** Reagent 1 1 x 19 mL
R2 Reagent 2 1 x 16 mL	**R2** Reagent 2 1 x 14 mL

Reactive Ingredients

INGRED	Ingredient	Concentration
R1	Anti-topiramate Polyclonal Antibody (Sheep)	<5.0%
	Sodium Azide	≤0.09%
R2	Topiramate-coated Microparticles	<1.0%
	Sodium Azide	≤0.09%

Reagent Handling and Storage
- **R1** and **R2** Ready for Use.
- Before use, invert several times, avoiding the formation of bubbles.
- Remove air bubbles, if present in the reagent cartridge, with a new applicator stick. Alternatively, allow the reagent to sit at the appropriate storage temperature to allow the bubbles to dissipate. To minimize volume depletion, do not use a transfer pipette to remove the bubbles.
- When either the **R1** or the **R2** reagent cartridge becomes empty, replace both cartridges and verify calibration with at least two levels of controls according to the established Quality Control requirements for your laboratory. If control results fall outside acceptable ranges, recalibration may be necessary.
- In the case of accidental spill, clean and dispose of material according to your laboratory's SOP, local, state, and country regulations, with consideration that the material contains potentially infectious materials.
- In the case of damaged packaging on arrival, contact your technical support representative (contact details listed at the end of this package insert).

⚠ **CAUTION:** Reagent bubbles may interfere with proper detection of reagent level in the cartridge, causing insufficient reagent aspiration that could impact results.

2°C 8°C The unopened reagents are stable until the expiration date when stored at 2 to 8°C. **Do not freeze reagents or expose them to temperatures above 32°C.**

Warnings and Precautions
Precautions for Users
- For in vitro diagnostic use.
- Do not mix materials from different kit lot numbers.
- The reagents contain ≤0.2% bovine serum albumin (BSA). Avoid contact with skin and mucous membranes. Avoid inhalation. May cause topical or respiratory allergic reaction. Flush affected areas with copious amounts of water. In case of accident by inhalation, remove to fresh air and keep at rest.

DANGER: QMS Topiramate (TPM) assay contains ≤5.0% Drug-specific antibody, ≤3.5% IgM, & ≤0.2% Bovine serum albumin (BSA).
H317 - May cause allergic skin reaction.
H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Avoid breathing mist or vapor. Contaminated work clothing should not be allowed out of the workplace. Wear protective gloves/eye protection/face protection. In case of inadequate ventilation wear respiratory protection. If on skin: Wash with plenty of soap and water. IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. If skin irritation or rash occurs: Get medical advice/attention. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. Wash contaminated clothing before reuse. Dispose of contents/container to location in accordance with local/regional/national/international regulations.

⚠ **CAUTION:** This product contains human sourced and/or potentially infectious components. Components sourced from human blood have been tested and found to be nonreactive for HBsAg, anti-HIV 1/2, and anti-HCV. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, it is recommended that all human sourced materials be considered potentially infectious and handled with appropriate biosafety practices.

Specimen Collection and Handling
The following specimen collection tubes may be used for the QMS Topiramate assay:

	Glass	Plastic
Serum	• No Additive	• Serum Separator Tube (gel) • No Additive
Plasma	• EDTA (K_3)	• EDTA (K_2) • Lithium Heparin • Plasma Separator Tube with Lithium Heparin (gel) • Sodium Heparin

Other specimen collection tubes have not been validated for use with the QMS Topiramate assay. Follow the manufacturer's processing instructions for serum or plasma collection tubes.

- Inadequate centrifugation of the specimen may cause an erroneous result.
- Ensure specimens are free of fibrin, red blood cells, and other particulate matter.
- Remove the plasma or serum from the cells, clot, or gel as soon as possible after collection. Some gel separator tubes may not be suitable for use with therapeutic drug monitoring assays; refer to information provided by the tube manufacturer.[2]
- Specimens removed from the cells, clot, and gel may be stored up to one week at 2 to 8°C. If testing will be delayed more than one week, specimen should be stored frozen (≤-10°C) prior to being tested. Specimen frozen up to two weeks showed no performance differences from fresh samples. Care should be taken to limit number of freeze-thaw cycles.

Procedure
Materials Provided
- QMS Topiramate Reagents, **REF** 0374140, 10017218

Materials Required but not Provided
- QMS Topiramate Calibrators, **REF** 0374173
  CAL A-F: 1 × 1.0 mL each
- QMS Topiramate Controls, **REF** 0374181
- Levels 1-3: 1 x 2.0 mL each

Assay Procedure
For a detailed description of how to run and calibrate an assay, refer to the instrument specific operations manual.

Specimen Dilution Procedures
Use QMS Topiramate CAL A (0.0 µg/mL) to manually dilute samples outside the linearity of the assay.

Manual Dilution Protocol

A manual dilution can be performed on patient samples with topiramate concentrations reported as greater than 32.0 µg/mL by making a dilution of the specimen with QMS Topiramate CAL A (0.0 µg/mL) before pipetting the sample into the sample cup. The dilution must be performed so the diluted test results read greater than the assay sensitivity of 1.5 µg/mL. The concentration reported must be multiplied by the manual dilution factor to obtain the final sample concentration.

$$\text{Final Sample Concentration} = \text{Reported Concentration} \times \text{Manual Dilution Factor}$$

$$\text{Manual Dilution Factor} = \frac{\text{(Volume of Sample + Volume of CAL A)}}{\text{Volume of Sample}}$$

Calibration

The QMS Topiramate assay must be calibrated using a full calibration (6-point) procedure. To perform a full calibration, test the QMS Topiramate Calibrators A, B, C, D, E, and F in duplicate.

Calibration is required with each new lot number. Verify the calibration curve with at least two levels of controls according to the established Quality Control requirements for your laboratory. If control results fall outside acceptable ranges, recalibration may be necessary.

Note: Topiramate CAL A is the calibration blank for this assay.

Quality Control

As appropriate, refer to your laboratory Standard Operating Procedure(s) and/or Quality Assurance Plan for additional quality control requirements and potential corrective actions. All quality control requirements should be performed in conformance with local, state, and/or federal guidelines or accreditation requirements.

Recommended control requirements for the QMS Topiramate assay:

- A minimum of two levels of controls spanning the medical decision range should be run every 24 hours.
- If more frequent control monitoring is required, follow the established Quality Control procedures for your laboratory.
- If quality control results do not fall within an acceptable range defined by your laboratory, patient values may be suspect and corrective action should be taken.

Results

The result unit for the QMS Topiramate assay can be reported as µg/mL or µmol/L. To convert results from µg/mL topiramate to µmol/L topiramate, multiply µg/mL by 2.95.

As with all analyte determinations, the topiramate value should be used in conjunction with information available from clinical evaluations and other diagnostic procedures.

Result Error Codes

Some results may contain Result Error Codes. Refer to the instrument-specific operations manual for a description of the error codes.

Limitations of the Procedure

Interfering heterophile antibodies occur at a low frequency in the general population. These antibodies can cause autoagglutination of the microparticle reagent leading to undetected erroneously low results.

For diagnostic purposes, interfering heterophile antibodies occur at low frequency in the general population. These antibodies can cause auto-agglutination of the microparticle reagent leading to erroneous results that may be unexpectedly low or unexpectedly high. An erroneous result could lead to incorrect patient management; incorrect patient management could potentially cause serious injury or death. Test results should not be used in isolation to make patient management decisions. Results should always be assessed in conjunction with the patient's medical history, clinical examinations, and other clinicopathological findings. An alternative test method should be used to confirm results when results are inconsistent with clinical expectations.

See the SPECIMEN COLLECTION AND HANDLING and SPECIFIC PERFORMANCE CHARACTERISTICS sections of this package insert.

Expected Values

A therapeutic range for topiramate has not been well established. Some reports in the literature suggest a target range for steady-state concentrations of 2 to 25 µg/mL.[3] Inconsistent correlation exists between levels of circulating topiramate to toxicity, adverse affect or clinical efficacy.[3] Therefore, monitoring topiramate concentration in patients is warranted.

Topiramate drug concentrations should not be the only means of therapeutic drug management. The assay should be used in conjunction with information available from clinical evaluations and other diagnostic procedures. Clinicians should carefully monitor patients during therapy initiation and dosage adjustments. It may be necessary to obtain multiple samples to determine expected variations of optimal (steady-state) concentrations for individual patients.

Specific Performance Characteristics

Sensitivity

Limit of Quantitation (LOQ)

The LOQ of the QMS Topiramate assay is defined as the lowest concentration for which acceptable inter-assay precision and recovery is observed (often considered ≤±20% CV with ≤±15% recovery). The LOQ was determined to be 1.5 µg/mL.

Assay Range

The range of the assay is 1.5 µg/mL to 32.0 µg/mL. Report results below this range as <1.5 µg/mL.

Accuracy

An accuracy-by-recovery was performed by adding high purity topiramate drug into human serum negative for topiramate. Initially, a serum stock of 32.00 µg/mL topiramate was prepared gravimetrically by adding topiramate to human serum. The stock concentrate was then volumetrically added to human serum negative for topiramate, representing drug concentrations across the assay range. Each sample was assayed in triplicate on an automated clinical chemistry analyzer. The results were averaged and compared to the target concentration and percent recovery calculated. Results are shown below.

$$\%\ \text{Recovery} = \frac{\text{Mean recovered concentration}}{\text{Theoretical concentration}} \times 100$$

Theoretical Concentration (µg/mL)	Mean Recovered Concentration (µg/mL)	% Recovery
32.00	32.48	101.5
24.00	24.50	102.1
16.00	16.74	104.6
8.00	8.35	104.4
6.40	6.61	103.3
3.20	3.47	108.4
2.56	2.67	104.3
1.92	2.11	109.9
1.60	1.65	103.1
1.28	1.33	103.9

Mean percent recovery: 104.6

Linearity

Linearity studies were performed by diluting a high patient pool to concentrations across the assay range. The patient pool was adjusted in order to obtain a 20 to 30% value above the desired reportable range as suggested in NCCLS Protocol EP6-A.[4] The dilutions were made with QMS Topiramate Calibrator A (blank calibrator). Linearity at specific dilutions was considered acceptable if the percent difference was ±10% between the predicted 1st and 2nd order regressed values. Results are shown below.

Estimated Value (µg/mL)	Dilution Factor	Results (µg/mL)	1st Order Predicted Results	2nd Order Predicted Results	Percent Difference (Acceptance Criteria: ±10%)
35.0	0.8750	36.57	36.76	36.78	-0.04%
30.0	0.7500	31.87	31.52	31.53	0.00%
20.0	0.5000	20.86	21.05	21.04	0.06%
15.0	0.3750	15.89	15.82	15.80	0.09%
10.0	0.2500	10.54	10.58	10.57	0.10%
5.0	0.1250	5.28	5.35	5.34	0.06%
3.0	0.0750	3.11	3.25	3.25	-0.02%
2.0	0.0500	2.22	2.20	2.21	-0.13%
1.5	0.0375	1.68	1.68	1.68	-0.25%
1.2	0.0300	1.43	1.37	1.37	-0.36%

Method Comparison

Correlation studies were performed using NCCLS Protocol EP9-A2.[5] Results from the QMS Topiramate assay were compared with results from a commercially available FPIA Immunoassay. The topiramate concentrations ranged from 1.56 µg/mL to 30.72 µg/mL. Results of the Passing-Bablok[6] regression analysis for the study are shown below.

Slope	0.962
y-intercept	0.228
Correlation Coefficient (R^2)	0.986
Number of Samples	148

Precision

Precision was determined as described in NCCLS Protocol EP5-A2.[7]

A tri-level human serum based commercial control containing topiramate was used in the study. Each level of control was assayed in duplicate twice a day for 20 days. Each of the runs per day was separated by at least two hours. The within run, between day, total SD, and percent CVs were calculated. Results are shown below.

			Within Run		Between Day		Total	
Sample	N	Mean (µg/mL)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	80	2.94	0.08	2.77	0.06	2.10	0.12	4.22
2	80	10.14	0.18	1.83	0.23	2.34	0.34	3.37
3	80	25.69	0.82	3.23	0.73	2.87	1.14	4.44

Acceptance criteria: <10% total CV

Interfering Substances

Interference studies were conducted using NCCLS Protocol EP7-A2[8] as a guideline. Clinically high concentrations of the following potential interferents were added to serum with known levels of topiramate (approximately 5 and 20 µg/mL). Each sample was assayed using the QMS Topiramate assay, along with a serum control of topiramate. All substances resulted in ≤ ±10% error in detecting topiramate. The results are shown below.

Interfering Substance	Interferent Concentration
Albumin	12 g/dL
Bilirubin	70 mg/dL
Cholesterol	250 mg/mL
Gamma-Globulin	12 g/dL
HAMA type 1*	normal human level
HAMA type 2*	normal human level
Hemoglobin	1000 mg/dL
Heparin	185.5 USP/mL
Rheumaoid Factor	500 IU/mL
Triglycerides	825 mg/dL
Uric Acid	30 mg/dL

*HAMA = human anti-mouse antibodies

Specificity

Cross-reactivity was tested for the known metabolites of topiramate. Other medications routinely administered with topiramate were also tested to determine whether these compounds affect the quantitation of topiramate concentrations using the QMS Topiramate assay. High levels of these compounds were spiked into serum pools containing low and high therapeutic levels of topiramate. The samples were analyzed and the topiramate concentrations of samples containing interferent were compared to the control serum.

Metabolites

Metabolites of topiramate are found primarily in urine of patients being administered topiramate therapy.[9,10] They are not however seen at clinically significant levels in plasma or serum. The QMS topiramate assay serum and plasma results are unlikely to be affected by metabolism of topiramate drug. The following metabolite was tested for cross-reactivity.

Metabolite	Metabolite Concentration (µg/mL)	Low Concentration	High Concentration	Low Concentration	High Concentration
		Percent Cross-Reactivity		Percent Interference	
9-Hydroxy topiramate	4.00	19.75	14.50	18.33	2.55
	8.00	22.63	12.50	44.03	4.49
	32.00	15.56	18.25	137.19	30.62

Drug Interference

Studies using the QMS Topiramate assay were conducted to examine if any of the commonly administered compounds have any effect on the recovery of topiramate concentration. A high concentration of each compound was spiked into normal human serum with known levels of topiramate (approximately 5 and 20 µg/mL) and assayed along with a serum control of topiramate. All compound resulted in ≤±10% error in detecting topiramate. The results are shown below.

Compound	Compound Concentration (µg/mL)	Compound	Compound Concentration (µg/mL)
Acetaminophen	31	Lamotrigine	45
Acetozolamide	40	Levetiracetam	124
Alprazolam	2.0	Methysergide	5.2
Amitriptyline	1.0	Metoprolol	5.25
Acetylsalicylic acid	67	Nadolol	121
Atenolol	10.33	Naproxen	509
Caffeine	60	Nimodipine	75
Carbamazepine	30	Nortriptyline	1.0
Chlorthalidone	64	Phenelzine	14.38
Clonazepam	0.18	Phenobarbital	40
Clorazepate	2.0	Primidone	40
Diazepam	5.1	Protriptyline	1.03
Dichlorphenamide	32	Salicylic Acid	598
Ethosuxamide	252	Sulfanilamide	1500
Famotidine	0.97	Tolbutamide	642
Felbamate	243.33	Valproic Acid	100.67
Flurazepam	17.5	Verapamil	1.6
Furosemide	3.7	Viagabatrin	112
Gabapentin	93	Zonisamide	122
Hydrochlorothiazide	6.0		

Drugs that Cross-React

The cross-reactivity of the antibody to ibuprofen, phenytoin and tiagabine at the following concentrations were tested. A high concentration of each compound was spiked into normal human serum with known levels of topiramate (approximately 5 and 20 µg/mL) and assayed along with a serum control of topiramate. The results are shown below.

Compound	Concentration (µg/mL)	Low Concentration	High Concentration	Low Concentration	High Concentration
		Percent Cross-Reactivity		Percent Interference	
Ibuprofen	500	0.09	0.19	11.38	4.39
Phenytoin	20	5.48	2.25	28.14	4.86
Tiagabine	250	0.49	-0.50	29.60	5.35

Care should be taken when interpreting QMS Topiramate results if any of the above compounds are being administered to the patient.

Bibliography

1. OrthoMcNeil Pharmaceutical, Inc. *Topamax*® monograph, Raritan, NJ Revision Date August 2004.
2. Dasgupta A, Dean R, Saldana S. et.al. Absorption of therapeutic drugs by barrier gels in serum separator blood collection tubes. *Am J Clin Pathol* 1994:101(4):456-64.
3. Johannessen SI, Battino, D, Berry DJ. et.al. Therapeutic Drug Monitoring of the Newer Antiepileptic Drugs. *Ther Drug Monit.* 2003;(25):347-63.
4. Tholen DW, Kroll M, Astles, JR et.al. *Evaluation of the linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline* (EP6-A). Wayne, PA. The National Committee for Clinical Laboratory Standards, 2003.
5. Krouwer, JS, Tholen DW, Garber, CC, et.al. *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline*-Second Edition (EP9-A2). Wayne, PA: The National Committee for Clinical Laboratory Standards, 2002.
6. Bablok W, Passing H, Bender R, Schneider B. 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;26(11):783-90.
7. Tholen DW, Kallner A, Kennedy JW, et.al. *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline*-Second Edition (EP5-A2). Wayne, PA: The National Committee for Clinical Laboratory Standards, 2004.
8. McEnroe RJ, Burritt MF, Powers DM, et.al. *Interference Testing in Clinical Chemistry: Approved Guideline*-Second Edition (EP7-A2). Wayne, PA: The National Committee for Clinical Laboratory Standards, 2005.
9. Britzi M, Sobak S, Isoherranen N. et.al. Analysis of Topiramate and its Metabolites in Plasma and Urine of Healthy Subjects and Patients with Epilepsy by Use of a Novel Liquid Chromatography-Mass Spectrometry Assay. *Ther Drug Monit.* 2003;(25):314-22.
10. Britzi M, Perucca E, Soback S. et.al. Pharmacokinetic and Metabolic Investigation of Topiramate Dispostion in Healthy Subjects in the Absence and in the Presence of Enzyme Induction by Carbamazepine. *Epilepsis* 2005;(46):378-84.

Glossary:
http://www.thermofisher.com/symbols-glossary

Microgenics Corporation
46500 Kato Road
Fremont, CA 94538 USA
US Customer and
Technical Support:
1-800-232-3342

EC REP
B·R·A·H·M·S GmbH
Neuendorfstrasse 25
16761 Hennigsdorf, Germany

For insert updates go to:
www.thermoscientific.com/diagnostics

Other countries:
Please contact your local Thermo Fisher Scientific representative.

0155227-J-EN
2017 12

Thermo SCIENTIFIC