| **Topiramate** |
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| **Purpose** | This procedure provides instructions for performing the TOPIRAMATE PROCEDURE on serum in Children’s Minnesota Laboratory.The ARK Topiramate Assay is a homogeneous enzyme immunoassay 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. |
| **Policy Statements** | This procedure applies to all personnel who run the Abbott Architect c4000 in Children’s St. Paul Laboratory. Refer to the [Abbott Architect Operations Manual](https://starnet.childrenshc.org/References/labsop/chem/operator/abbott-architect-operations-manual.pdf) for details on correct operation of the analyzer. |
| **Principle** | ARK Topiramate Assay is a homogeneous enzyme immunoassay based on competition between drug in the specimen and Topiramate epitope labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. As the latter binds antibody, enzyme activity decreases. In the presence of drug from the specimen, enzyme activity increases and is directly proportional to the drug concentration. Active enzyme converts the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance. Endogenous serum G6PDH does not interfere with the results because the coenyzme NAD functions only with the bacterial enzyme used in the assay. |
| **Clinical Significance** | Topiramate (2,3:4,5-Di-O-isopropylidene-β-D-fructopyranose sulfamate) is an anti-convulsant drug approved for use as adjunctive therapy in the treatment of epilepsy and is often prescribed as mono-therapy or as one component of a multiple anti-epileptic drug therapy.Topiramate is used alone or with other medications to treat certain types of seizures in people who have epilepsy. Topiramate is also used with other medications to control seizures in people who have Lennox-Gastaut syndrome (a disorder that causes seizures and developmental delays). Topiramate is used to treat patients who continue to have seizures even when they take other antiseizure medications. Topiramate is also used to prevent migraine headaches but not to relieve the pain of migraine headaches when they occur. Topiramate is in a class of medications called anticonvulsants. It works by decreasing abnormal excitement in the brain.Hyperchloremic, non-anion gap, metabolic acidosis (i.e., decreased serum bicarbonate below the normal reference range in the absence of chronic respiratory alkalosis) is associated with Topiramate treatment. This metabolic acidosis is caused by renal bicarbonate loss due to the inhibitory effect of Topiramate on carbonic anhydrase. Changes in several clinical laboratory values (increased creatinine, BUN, alkaline phosphatase, total protein, total eosinophil count and decreased potassium) have been observed in a clinical investigational program in very young (<2 years) pediatric patients who were treated with adjunctive Topiramate for partial onset seizures.Topiramate treatment produced a dose-related increased shift in serum creatinine from normal at baseline to an increased value at the end of 4 months treatment in adolescent patients (ages 12-16 years) who were treated for another indication in a double-blind, placebo-controlled study.Topiramate treatment with or without concomitant valproic acid (VPA) can cause hyperammonemia with or without encephalopathy. |
| **Analyzer** | Abbott Architect c4000 |
| **Sunquest Test Codes** | **TOPX** Topiramate (Topamax, Topiragen) |
| **Sample** | Preferred specimenLithium heparinplasma, **NO GEL.** Refer to specimen collection procedures for collection of diagnostic blood specimens. Sodium heparin, Potassium EDTA**,** and serum **NO GEL** are acceptable specimen types, however, using the same specimen matrix for individual patients is preferred. **Patient** **Preparation:** A steady state, trough (pre-dose) sample is generally accepted as most consistent for therapeutic drug monitoring of Topiramate. Time of blood draw since last dose should be noted.**Minimum volume:** 0.2 mL**Stability:** 2-8 °C / 7 days, <-10 °C / 1 month. **Rejection criteria**: Unlabeled specimens, tubes containing gel, any sample other than serum, EDTA plasma or heparinized plasma as noted above.**Preparation:** Serum or plasma should be physically separated from cells as soon as possible with a maximum limit of **two hours** from the time of collection. Specimens should be free of particulate matter. Whole blood specimens should be centrifuged according to Specimen Processing procedures prior to analysis. See Processing Procedure Manual.Transfer serum or plasma to a properly labeled sample cup or tube. Special sample cups/tubes are not required, as the Architect c4000 has a level detect mechanism which negates the need for specialized containers. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.* Do not use gel separators.
* Do not induce foaming and avoid repeated freezing and thawing
* Fibrin, red blood cells, and other particulate matter may cause an erroneous result. Ensure adequate centrifugation
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| **Materials** |

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| **Supplies** | **Reagent** | **Preparation** | **Stability** |
| * Abbott Architect 55 mL reagent cartridge
* Abbott Architect 20 mL reagent cartridge,
 | ARK Topiramate Assay – 5015-0001-00**R1 Reagent – Antibody/Substrate**1 X 28 mL**R2 Reagent – Enzyme**1 X 14 mL | * ARK Topiramate Assay reagents are provided liquid, ready to use and may be used directly from the refrigerator
* Reagents R1 and R2 will be transferred to analyzer-specific reagent containers prior to use.
* Avoid cross-contamination of R1 and R2
* Do not freeze.
* Do not expose to temperatures above 32°C.
 | 2–8°C/ expiration date printed on the label when stored upright and tightly capped. Improper storage of reagents can affect assay performance |
| ARK Topiramate **Calibrator** – 5015-0002-00 | * Calibrators are ready to use.
* Mix each level by gentle inversion before dispensing.
* Squeeze 2 - 4 drops of each level into individual sample cups.
 | 2–8°C/ expiration date printed on the label when stored tightly capped |

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| **Risk and Safety** | For *In Vitro Diagnostic* Use. For prescription use only.Reagents **R1** and **R2** are provided as a matched set and should not be interchanged with reagents from different lot numbers.Handle all patient specimens as if they were potentially infectious. |
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| **Preparing and Loading the Reagents** | **STEP**  | **ACTION**  |
|  |  | Label the outside of the ARK Diagnostics Topiramate kit with the following information * Expiration date of the kit
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|  |  | Preparing Reagent R1:1. Obtain a small 55mL cartridge
2. Label cartridge with Assay name, Lot number, R1 and date made
3. Pour 14mL of R1 into the small 55mL cartridge
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|  |  | Preparing Reagent R21. Obtain a 20 mL Architect cartridge
2. Label cartridge with Assay name, Lot number, R2 and date made
3. Pour 7mL of R2 into the 20mL Cartridge
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|  |  | Refer to the operating procedure, CH5.107 Abbott Architect c4000 Operating Procedure, for instructions on how to configure and load the reagent in the following sections **Configuration of Non-barcoded Reagents and Diluents** and**Loading Non-barcoded Reagents** |
|  |  | **ALINITY C** |
|  |  | Obtain an empty, black Alinity cartridge |
|  |  | Pour the entire R1 bottle (28 mL) into the larger of the two black bottles of the empty cartridge |
|  |  | Pour the entire R2 Bottle (14 mL) into the smaller of the two black bottles of the Alinity cartridge |
|  |  | Utilizing the Operating Procedure, print a 1D barcode, ensuring the correct lot number and expiration date are used, and affix the barcode to the side of the cartridge as indicated in the depiction. Load the cartridge on the RSM. |
| **Calibration** |

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| Assay Range: | 1.5 - 54.0 mcg/mL |
| Reference Material: | ARK Topiramate Calibrators A, B, C, D, E, and F |
| Suggested Calibration Levels: | A 0.0 mcg/mL B 2.0 mcg/mL C 4.0 mcg/mL D 8.0 mcg/mL E 24.0 mcg/mL F 60.0 mcg/mL  |
| Calibration Scheme: | Six levels in duplicate |
| Calibration Frequency: | * Whenever a new lot number of reagents is used
* Whenever indicated by quality control results
* Whenever required by standard laboratory protocols
* Once every 960 hours
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| Assigned Coefficients: | C0 0.000 C1 1.000 |
| Analytical Measuring Range | 1.5 – 54.0 mcg/mLThe AMR is verified with each calibration using 6 levels of calibrator that span the full reportable range every 960 hours or more frequently as described above. |
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| **STEP**  | **ACTION**  |
|  | For Abbott Architect c4000 only: Two sample carriers with sequential numbers are required. Example N350 and N351A = rack N350 position 1B = rack N350 position 2C = rack N350 position 3D = rack N350 position 4E = rack N350 position 5F = rack N351 position 1 |
|  | Refer to the calibration sections of the Abbott Operating Procedures for programming and accepting calibrations. |

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| **Quality Control** | ARK Topiramate Controls * **LOW** (2.5 mcg/mL) 1 X 4 mL (Level 1 in URT)
* **MID** (10.0 mcg/mL) 1 X 4 mL (Level 2 in URT)
* Use each lot as a set

**Frequency:** * Two levels of controls must be run every 24 hours
* After loading a new Flex™ reagent cartridge
* After calibration
* After any major maintenance/ repairs have been performed on the analyzer
* When indicated by QC results

**Storage and Stability**: Unopened: 2°- 8°C. Use prior to expiration date on containerOpen: until expiration date on label when stored tightly capped at 2°- 8°C**Procedure**Controls are ready to use. Mix each level by gentle inversion before dispensing.Waste 1 drop and then squeeze sufficient volume (~40μL/drop) into individual sample cups.**Acceptable ranges:** * Non-Bio-Rad controls will utilize manufacturer ranges and 2 SD Westgard rules.
* New lots of Bio-Rad controls should be run for 20 days in parallel with the current lot whenever possible prior to switching to the new lot.
* Refer to the [Westgard Rules in Chemistry procedure](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.18-westgard-rules-in-chemistry.pdf) for current Westgard rules in place for each analyte.
* **Acceptable ranges are current in Unity Real Time only.** Quality Control results must be rejected in Sunquest when the results cross the interface.
* In the event of a QC failure, refer to the [Unity Real Time QC Review, General User](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.17-unity-real-time-qc-review-general-user.pdf) and navigate to the QC Troubleshooting section.
* Do not load or release patients until QC is acceptable in Unity Real Time.
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| **Interferences** | Interference studies were conducted by ARK, Inc. using CLSI/NCCLS Protocol EP7-A2 as a guideline.*Drug Interference*Topiramate-selective antibody did not cross-react with other anti-epileptic or co-administered drugs 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. Measurement of Topiramate resulted in ≤10% error in the presence of drug compounds at the levels tested.Clinically high concentrations of potentially interfering substances in serum with known levels of Topiramate (approximately 5 and 20 mcg/mL) were evaluated. Each sample was assayed using the ARK Topiramate Assay, along with a serum control of Topiramate. Measurement of Topiramate resulted in ≤10% error in the presence of interfering substances at the levels tested. Refer to the product insert for additional information.**Specificity***Metabolites*Metabolites of Topiramate are found primarily in urine of patients being administered Topiramate therapy. ARK Topiramate Assay serum and plasma results are unlikely to be affected by metabolism of Topiramate drug, since plasma levels of metabolites are usually not clinically significant.See the product insert for a list of substances for which no significant interference was noted. |
| **Reference Range** | **2 to 25 mcg/mL** A therapeutic range for Topiramate has not been well established. The therapeutic range on a trough sample for seizure control is 2 to 25 mcg/mL. An inconsistent correlation exists between levels of circulating Topiramate to toxicity, adverse effect or clinical efficacy. Topiramate drug concentrations should be used in conjunction with information available from clinical evaluations and other diagnostic procedures. Clinicians should carefully monitor patients during therapy and dosage adjustments. Pharmacokinetics may vary widely, particularly with co-medications, age, and/or compromised renal function. Multiple samples over time may be needed to determine steady-state concentrations for individual patients. |
| **Critical Values** | None defined. |
| **Limitations** | Linear range of detection: **1.5 to 54.0 mcg/mL**The instrument reporting system contains flags and comments to provide the user with information regarding instrument processing errors, instrument status information and potential errors in open channel method results. Refer to your Abbott Architect® Operator’s Guide for the meaning of report flags and comments. Any report containing flags and/or comments must be resolved prior to reporting.  |
| **Dilutions** | * **Above 54.0 mcg/mL**:
* Dilute results with “assay range” appended.
	+ Prepare a 1:2 maximum dilution with the zero calibrator (CAL A), to obtain results within the assay range.
	+ Label diluted sample with “label foot” or Accession number, and dilution factor.
	+ Program dilution factor in c4000. The analyzer software calculates the result using the dilution factor entered, so the result has been corrected for dilution.
	+ Document dilutions and calculations on the manual dilution log and have results checked prior to reporting.
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| **Result Reporting** | * Results between **1.5 to 54.0 mcg/mL** without error messages are released
* Results below **1.5 mcg/mL**: report as < **1.5 mcg/mL** instead of the numerical value.
* Results >**54.0 mcg/mL** are reported as the numerical result following a maximum dilution of 1:2
* Results that exceed the assay range following the maximum dilution are reported as >**108.0 mcg/mL**.
* To convert results from mcg/mL Topiramate to μmol/L Topiramate, multiply mcg/mL by 2.95.
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| **Specimen Storage** | Promptly stopper tested specimen and store upright in specimen rack. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 7 days in specimen storage freezer. |
| **Backup Method** | * Abbott Architect c4000: if the Abbott Alinity c becomes inoperable for an extended period of time and a patient sample is received, load reagent, calibrate as necessary and run quality controls prior to testing patient samples.
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| **References** | 1. ARK Diagnostics, Inc. package insert for the ARK Topiramate Assay, ARK Diagnostics, Inc., 48089 Fremont Blvd, Fremont, CA 94538, USA, 1600-0105-00 Rev 05, Printed in USA, Revised February 2017
2. ARK Diagnostics, Inc. package insert for the ARK Topiramate Calibrator, ARK Diagnostics, Inc., 48089 Fremont Blvd, Fremont, CA 94538, USA, 1600-0137-00 Rev 03, Printed in USA, Revised August 2012
3. ARK Diagnostics, Inc. package insert for the ARK Topiramate Control, ARK Diagnostics, Inc., 48089 Fremont Blvd, Fremont, CA 94538, USA, 1600-0138-00 Rev 03, Printed in USA, Revised August 2012
4. Epilepsia, Sunday, December 3, 2006 Poster Session II 7:30 a.m.–4:30 p.m. Article first published online: 30 OCT 2006
5. http://www.nlm.nih.gov/medlineplus/druginfo/meds/a697012.html
6. Torrent Pharma Inc., 5380 Holiday Terrace, Suite 40, Kalamazoo, Michigan 49009. 8029987 Revised August 2011
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Linda Lichty | 10/29/2013 | New test |
| 2 | Erin Bartos | 12/16/2016 | Added #10 to preparing the flex |
| 3 | Stephen Gripentrog | 5/1/2019 | Removed Sunquest QC information, added Unity Real Time QC information. |
| 4 | Stephen Gripentrog, Erin Bartos | 10/15/2019 | Revamped for switch to Abbott Architect from Dimension RXL. |
| 5 | Erin Bartos | 10/28/2020 | Added Alinity c |
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