| **Lamotrigine** |
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| **Purpose** | This procedure provides instructions for performing LAMOTRIGINE ON ABBOTT INSTRUMENTATION on plasma or serum in Children’s Minnesota Laboratory.The ARK Lamotrigine Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of lamotrigine in human serum or plasma on automated clinical chemistry analyzers. The results obtained can be used as an aid in management of patients treated with lamotrigine. |
| **Policy Statements** | This procedure applies to all personnel who operate the Abbott Alinity c or Architect c4000 systems. Refer to the Abbott Alinity or Architect Operations Manuals for proper instrument operation. |
| **Principle** | ARK Lamotrigine Assay is a homogeneous enzyme immunoassay based on competition between drug in the specimen and lamotrigine 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** | Lamotrigine (LAMICTAL®, 3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine) is an anti-convulsant 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.In a retrospective review of the records of children and adolescents treated with lamotrigine monotherapy between 2001 and 2006, data was collected including demographics, seizure type, etiology of seizures, age at onset of seizures and at initiation of lamotrigine treatment, number of antiepileptic drugs (AEDs) prior to lamotrigine, dose of lamotrigine, length of follow-up, treatment response, and adverse events. Lamotrigine has been shown to confer broad-spectrum, well-tolerated control of epilepsy. Monotherapy is preferable over polytherapy because of better compliance, fewer adverse events, less interactions, lower teratogenicity and lower cost. The aim of the study was to evaluate the efficacy and safety of lamotrigine monotherapy on seizure control in a cohort of children and adolescents with epilepsy. In conclusion, lamotrigine was effective and well-tolerated as monotherapy in children and adolescents for both focal and generalized epilepsies. |
| **Analyzer** | **St. Paul Primary: Abbott Alinity ci (SALIC)****St. Paul Backup: Abbott Architect c4000 (ARCH4) (Reagent will not be routinely loaded on the c4000. In case of extended Alinity c downtime, load reagent, calibrate, and quality control prior to testing patient samples.)** |
| **Sunquest Test Codes** | **LAMO** Lamotrigine, (Lamictal) |
| **Sample** | Preferred specimen:Lithium heparin plasma **NO GEL** Refer to specimen collection procedures for collection of diagnostic blood specimens. Sodium heparin, Potassium EDTA plasma**, or** 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 Lamotrigine. Time of blood draw since last dose should be noted.**Minimum volume:** 200 µL**Stability:** 2-8 °C / 7 days, <-10 °C / 1 month. Specimens were shown to withstand 3 freeze-thaw cycles when stored at -20°C**Rejection criteria**: Unlabeled specimens, samples other than acceptable tube types listed above, specimens collected using gel separators.**Preparation:** **Preparation:**1. Whole blood specimens should be centrifuged following complete clot formation, according to Specimen Processing procedures prior to analysis.
2. 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.
3. Lipemic samples should be ultrafuged.
4. Specimens should be free of particulate matter.
5. Transfer plasma/serum to a properly labeled Siemens SSC nested on a bar-coded pilot tube. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.
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| **Reagents** |

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| **Reagents** | **Stability** | **Preparation** |
| **ARK Lamotrigine Assay Reagent R1 –Antibody/Substrate****5023-0001-00**1 X 28 mL | **Unopened:** 2–8°C, upright and tightly closed, expiration date printed on the label Do not freeze reagents. Avoid prolonged exposure to temperatures above 32°CImproper storage of reagents can affect assay performance | Liquid, ready to use, may be used directly from the refrigerator |
| **Reagent R2–Enzyme**Lamotrigine labeled with bacterial G6PDH1 X 14 mL |
| **ARK Lamotrigine Calibrator Kit** **5023-0002-00** | **Unopened**: 2-8°C, date on vial**Opened**: 2-8°C and tightly capped, 12 months or date on vial | Ready to use. Mix by gentle inversion before dispensing.  |

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| **Calibration** |  |
| Assay Range: | 0.85 to 40.00 mcg/mL |
| Reference Material: | ARK Lamotrigine Calibrators |
| Suggested Calibration Levels: | A | 0.00 mcg/mL |
| B | 1.00 mcg/mL |
| C | 3.00 mcg/mL |
| D | 9.00 mcg/mL |
| E | 18.00 mcg/mL |
| F | 40.00 mcg/mL |
| Calibration Scheme: | Six levels in duplicate. Verify the calibration with 2 levels of QC |
| 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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| Analytical Measuring Range | 0.85 to 40.00 mcg/mLThe AMR is verified with each calibration using 6 levels of calibrator that span the full reportable range. Further studies are not necessary. |
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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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|  | Architect c4000: |
| **Preparing and Loading the Reagent** | **STEP**  | **ACTION**  |
|  | Label the outside of the ARK Diagnostics Lamotrigine kit with the following information Expiration date of the kit |
|  |  | Preparing Reagent R1:1. Obtain a small 55mL cartridge
2. Label cartridge with Assay name, Lot number, R1 and date made

Pour 14mL of R1 into the small 55mL cartridge |
|  |  | Preparing Reagent R21. Obtain a 20mL cartridge
2. Label cartridge with Assay name, Lot number, R2 and date made

Pour 7mL of R2 into the 20mL Cartridge |
|  |  | Refer to the 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 Reagent** |
|  | Alinity c:

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| **STEP**  | **ACTION**  |  |
| 1. | Obtain an empty, black Alinity cartridge. |  |
| 2. | Pour the entire R1 bottle (28 mL) into the larger of the two bottles of the cartridge |  |
| 3. | Pour the entire R2 bottle (14 mL) into the smaller of the two bottles of the cartridge |  |
| 4. | Print a 1D barcode in the instrument software according to the Alinity operating procedure. Affix the barcode to the outside of the cartridge according to the depiction. Load the cartridge onto the Alinity c RSM as directed. |  |

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| **Calibration** |

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| Assay Range: | 0.85 -40.00 mcg/mL |
| Reference Material: | ARK Lamotrigine Calibrators A, B, C, D, E, and F |
| Suggested Calibration Levels: | A 0.0 mcg/mL B 1.0 mcg/mL C 3.0 mcg/mL D 9.0 mcg/mL E 18.0 mcg/mL F 30.0 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 |
| Assigned Coefficients: | C0 0.000 C1 1.000 |
| Analytical Measuring Range | 0.85 – 40.0 mcg/mLThe AMR is verified with each calibration using 6 levels of calibrator that span the full reportable range. Further studies are not necessary |

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| **Quality Control** | **ARK™ Lamotrigine Controls**LOW (2.00 mcg/mL) Level 1 in URTMID (12.00 mcg/mL) Level 2 in URTUse 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 that a repeat is required by previous QC results

**Storage and Stability**: **Unopened**: 2°- 8°C. Use prior to expiration date on container**Open:** 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** | *Drug Interference*Interference studies were conducted by Ark using CLSI/NCCLS Protocol EP7-A2 as a guideline. Clinically high concentrations of potential interfering substances in serum with known levels of lamotrigine (approximately 3 and 15 μg/mL) were evaluated. Each sample was assayed using the ARK Lamotrigine Assay, along with a serum control of lamotrigine. Measurement of lamotrigine resulted in ≤10% error in the presence of interfering substances at the levels tested. See product insert for more information.*Specificity*Lamotrigine’s major metabolite, medications that may be routinely co-administered with lamotrigine and other anti-epileptic drugs were tested to determine whether these compounds affect the quantitation of lamotrigine concentrations using the ARK Lamotrigine Assay. High levels of these compounds were spiked into serum pools containing low (3 mcg/mL) and high (15 mcg/mL) therapeutic levels of lamotrigine. The samples were analyzed and the lamotrigine concentrations of samples containing interferent were compared to the serum control.*Metabolites*Lamotrigine is metabolized predominantly by UDP-glucuronyltransferase to form a pharmacologically inactive metabolite, 2-N-glucuronide. Lamotrigine-2-N-methyl has been detected in human plasma by HPLC and capillary electrophoresis. Other minor metabolites, lamotrigine-2-N-oxide, and lamotrigine-5-N-glucuronide have been proposed. Lamotrigine-2- N-glucuronide, Lamotrigine-2-N-methyl and Lamotrigine-2-N-oxide metabolites were tested for cross-reactivity. These metabolites were spiked into two separate samples each containing low and high lamotrigine concentrations of 3 and 15 μg/mL, respectively.*Drug that Cross-Reacts*Cross-reactivity of the antibody to trimethoprim at the following concentration was tested. A high concentration was spiked into normal human serum with known levels of lamotrigine (approximately 3 and 15 mcg/mL) and assayed along with a serum control of lamotrigine. The results are shown below.**Care should be taken when interpreting ARK Lamotrigine results if trimethoprim is also being administered to the patient.***Drug Interference*Lamotrigine-selective antibody did not cross-react with most other anti-epileptic or coadministered drugs tested. Due to structural similarities with lamotrigine, high trimethoprim levels may interfere. A high concentration of each compound was spiked into normal human serum with known levels of lamotrigine (approximately 3 and 15 μg/mL) and assayed along with a serum control of lamotrigine. Measurement of lamotrigine resulted in ≤10% error in the presence of drug compounds at the levels tested. |
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| **Reference Range** | **2 to 20 mcg/mL (Medtox)**A correlation exists between lamotrigine serum level and tolerability, independent of the use of other AEDs. Adverse effects requiring a dose change are uncommon with the most frequently encountered lamotrigine concentrations (<10 mcg/mL) and occur in only 7.4% of patients at levels obtained during the majority of clinical trials (<5 mcg/mL). An initial target range of 1.5 to 10 mcg/mL is suggested, although higher levels, up to more than 20 mcg/mL, are often tolerated and can lead to additional efficacy in refractory patients. |
| **Critical Values** | None defined. |
| **Limitations** | Linear range of detection: 0.85 to 40.00 **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 the Abbott Architect Operations Manual for the meaning of report flags and comments. Any report containing flags and/or comments must be resolved prior to reporting. |
| **Dilutions** | **Above 40.00** **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 (2) in the Abbott Analyzer software, according to manufacturer’s instructions.
	+ Document dilutions and calculations, and have results checked prior to reporting.
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| **Result Reporting** | * Results between **0.85 to 40.00 mcg/mL** without error messages are released
* Results below **0.85 mcg/mL**: report as < **0.85 mcg/mL** instead of the numerical value
* Results >**40.00 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 >**80.0 mcg/mL**.
* To convert results from **mcg/mL** (μg/mL) lamotrigine to μmol/L lamotrigine, multiply μg/mL by 3.90
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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** | If the Alinity c analyzer in St. Paul becomes inoperable for an extended period of time and a sample is received, load reagent on the c4000 Architect, calibrate, and run QC prior to reporting patient samples. |
| **References** | 1. Ark ™ Lamotrigine Assay package insert, ARK Diagnostics, Inc., 1190 Bordeaux Drive, Sunnyvale, CA 94089 USA, Printed in USA, Revised February 2017, 1600-0179-00 Rev 04
2. Ark ™ Lamotrigine Calibrator package insert, ARK Diagnostics, Inc., 1190 Bordeaux Drive, Sunnyvale, CA 94089 USA, Printed in USA Revised February 2017, 1600-0180-00 Rev 04
3. Ark ™ Lamotrigine Control package insert, ARK Diagnostics, Inc., 1190 Bordeaux Drive, Sunnyvale, CA 94089 USA, 1600-0181-00 Rev 04, February 2017
4. Making Sense of Lamotrigine Serum Levels, Bassel W Abou-Khalil, M.D., American Epilepsy Society, Epilepsy Currents, v.5(3); 2005 May, PMC1198624
5. Efficacy and safety of lamotrigine monotherapy in children and adolescents with epilepsy, European Journal of Paediatric Neurology, Volume 13, Issue 2, March 2009, Pages 141–145
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Linda Lichty | January 21, 2016 | New test |
|  | Linda Lichty | March 7, 2016 | QC section |
|  | K. Brown/S. Gripentrog | May 23, 2017 | Removed High control from quality control. |
|  | Stephen Gripentrog | May 1, 2019 | Updated QC to reflect Unity Real Time.  |
|  | Stephen Gripentrog, Erin Bartos | 10/15/19 | Changed procedure to Abbott Architect c4000 |
|  | Erin Bartos, Elauteria Earnhardt | June 1, 2020 | Changed analyzer to Alinity ci with Architect c4000 as backup method |
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