| **Levetiracetam** |
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| **Purpose** | This procedure provides instructions for performing LEVETIRACETAM PROCEDURE in Children’s Minnesota Laboratory on Abbott instrumentation.The ARK Levetiracetam Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of levetiracetam in human serum or plasma on automated clinical chemistry analyzers. Levetiracetam concentrations can be used as an aid in management of patients treated with levetiracetam. |
| **Policy Statements** | * This procedure applies to all personnel who run the Abbott Architect c4000 and Alinity c analyzers in St Paul Laboratory
* All components of individual ARK Levetiracetam Assay reagent kits must be used together, and not shared between kits.
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| **Principle** | ARK Levetiracetam Assay is a homogeneous immunoassay based on competition between drug in the specimen and levetiracetam 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** | Levetiracetam (KEPPRA®, (S)-α-ethyl-2-oxo-1-pyrrolidine acetamide) is an anti-convulsant drug approved for use as adjunctive therapy in the treatment of epilepsy.KEPPRA is indicated as adjunctive therapy in the treatment of partial onset seizures in adults and children 1 month of age and older with epilepsy.KEPPRA is indicated as adjunctive therapy in the treatment of myoclonic seizures in adults and adolescents 12 years of age and older with juvenile myoclonic epilepsy.KEPPRA is indicated as adjunctive therapy in the treatment of primary generalized tonic-clonic seizures in adults and children 6 years of age and older with idiopathic generalized epilepsy.In a study published in the journal Epilepsia, nine out of fifteen patients (60%) showed a good response to levetiracetam with reduction in seizure frequency. Four out of fifteen patients (26.7%) became seizure free and 5/15 (33.3%) patients had fewer seizure frequency with therapy. Five out of fifteen patients (33.3%) had no response to therapy.Of those with generalized tonic clonic epilepsy, 4/11 (36.4%) had become seizure free, 3/11 (27.2%) had a decrease in seizure frequency, and 3/11 (27.2%) showed no response.Adverse effects occurred in four children (26.6%) including behavioral change (1/15), tiredness (1/15), decrease appetite (1/15), and reason unknown (1/15) (child was seen as an in-patient and followed at an outlying hospital).Treatment with levetiracetam resulted in reduction of seizure frequency in 60% of the patients included in this study. Levetiracetam was efficacious and safe for use in children with idiopathic generalized epilepsy with few, mild side effects reported. |
| **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** | LEVET: Levetiracetam (Keppra®) |
| **Specimen** | Serum (**no gel**). Refer to specimen collection procedures for collection of diagnostic blood specimens. Lithium heparin, sodium heparin, and Potassium EDTA plasma are acceptable specimen types, however, since using the same specimen matrix for individual patients is preferred, serum is the preferred specimen type.**Patient** **Preparation:** A steady state, trough (pre-dose) sample is generally accepted as most consistent for therapeutic drug monitoring of Levetiracetam. 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, other than serum or heparinized plasma. Specimens collected using gel separators.**Preparation:** **Process the blood as soon as possible after collection, since hydrolysis of levetiracetam may occur in the prolonged presence of whole blood.** 1. Complete clot formation should take place before centrifugation.
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. Specimens should be free of particulate matter.
4. Whole blood specimens should be centrifuged according to Specimen Processing procedures prior to analysis. See Processing Procedure Manual.
5. Transfer serum or plasma to a properly labeled sample cup or tube. 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 to preserve the integrity of the specimen from the time it is collected until the time it is assayed. |
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| **Materials** |

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| **Reagent** | **Preparation** | **Stability** |
| **ARK Levetiracetam Assay** – 5024-0001-00**Reagent – Antibody/Substrate**1 X 28 mL**Reagent – Enzyme**1 X 14 mL | ARK Levetiracetam Assay reagents are provided liquid, ready to use and may be used directly from the refrigerator 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. |
| **ARK Levetiracetam** **Calibrator** – 5024-0002-00 | Calibrators are ready to use. Mix each level by gentle inversion before dispensing. Squeeze 2 - 4 drops into individual sample cups for each level. | 2–8°C/ expiration date printed on the label when stored tightly capped |

* Improper storage of reagents can affect assay performance
* Do not mix reagents for ARK Levetiracetam between different kits. Use all reagents within the same kit lot together, or discard.
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| **Risk and Safety** | For *In Vitro Diagnostic* Use. For prescription use only.Reagents and 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 /Loading the Reagent Cartridges** | **STEP**  | **ACTION** **Architect c4000** |
|  |  | Label the outside of the ARK Diagnostics Levetiracetam 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 20mL 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 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** |
|  |  | Obtain an empty, black Alinity cartridge. |
|  |  | Pour the entire R1 bottle (28 mL) into the larger of the two bottles of the cartridge |
|  |  | Pour the entire R2 bottle (14 mL) into the smaller of the two bottles of the cartridge |
|  |  | 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. |
| **Calibration** |

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| Assay Range: | 2.0 to 100.0 μg/mL |
| Reference Material: | ARK Levetiracetam Calibrators A, B, C, D, E, and F |
| Suggested Calibration Levels: | **A** 0.0μg/mL 1 X 2 mL**B** 5.0 μg/mL 1 X 2 mL**C** 12.5 μg/mL 1 X 2 mL**D** 25.0 μg/mL 1 X 2 mL**E** 50.0 μg/mL 1 X 2 mL**F** 100.0 μg/mL 1 X 2 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 | 2.0 to 100.0 μg/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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| **STEP**  | **ACTION**  |
|  | Two racks with sequential numbers are required for Architect c4000.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 Operating Procedures for directions on calibrations. |

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| **Quality Control** | ARK Levetiracetam Controls* **LOW** (7.5 μg/mL) 1 X 4 mL
* **HIGH** (75.0 μg/mL) 1 X 4 mL (Level 2 in URT)

Use each lot as a set “MID” control is not used.**Frequency:** * Two levels of controls must be run every 24 hours
* After loading a new set of reagents
* 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.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 of control before dispensing.Squeeze sufficient volume (~40μL/drop) into individual sample cups for each level.**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. |
| **Interferences** | Interference studies were conducted using CLSI/NCCLS Protocol EP7-A2 as a guideline.*Drug Interference*Levetiracetam-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 Levetiracetam. Measurement of levetiracetam resulted in ≤10% error in the presence of drug compounds at the levels tested.**Specificity***Metabolites*Levetiracetam is hydrolyzed to its major metabolite 2-pyrrolidone-N-butyric acid (ucb L057) and two minor metabolites. The metabolite ucb L057 was tested for cross-reactivity and demonstrated <10% interference and cross reactivity.See the product insert for a list of substances for which no significant interference was noted. |
| **Reference Range** | **5 – 45 mcg/mL** Levetiracetam drug concentrations should be used in conjunction with information available from clinical evaluations. Circulating levels of levetiracetam may be affected by compliance, renal function, pregnancy, drug-drug interactions and timing of the sample draw. |
| **Critical Values** | None defined. |
| **Limitations** | Linear range of detection: **2.0 to 100.0 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 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 100.0 mcg/mL**:
* Dilute results with “assay range” appended.
	+ Make appropriate dilution with the zero calibrator (CAL A), to obtain results within the assay range. Determine optimum dilution by dividing C4000 result obtained by 100 (the measuring range) and rounding up to the next whole number. The maximum dilution is 1:3.
	+ Label diluted sample with “label foot” or Accession number, and dilution factor.
	+ Program dilution factor in c4000. Resulting readout is corrected for dilution.
	+ Document dilutions and calculations, and have results checked prior to reporting.
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| **Result Reporting** | * Results between **2.0 to 100.0 mcg/mL** without error messages are released
* Results below **2.0 mcg/mL**: report as < **2.0 mcg/mL** instead of the numerical value.
* Results >**100.0 mcg/mL** are reported as the numerical result following a maximum dilution of 1:3
* Results that exceed the assay range following the maximum dilution are reported as >**300.0 mcg/mL**.
* To convert results from mcg/mL levetiracetam to μmol/L levetiracetam, multiply mcg/mL by 5.88
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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. |
| **References** | 1. ARK Diagnostics, Inc. package insert for the ARK Levetiracetam Assay, ARK Diagnostics, Inc., Sunnyvale, CA 94089 USA, 1600-0169-00 Rev 01, Printed in USA, Revised November 2009
2. ARK Diagnostics, Inc. package insert for the ARK Levetiracetam Calibrator, ARK Diagnostics, Inc., Sunnyvale, CA 94089 USA, 1600-0170-00 Rev 01, Printed in USA, Revised November 2009
3. ARK Diagnostics, Inc. package insert for the ARK Levetiracetam Control, ARK Diagnostics, Inc., Sunnyvale, CA 94089 USA, 1600-0171-00 Rev 01, Printed in USA, Revised November 2009
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. Medtox Laboratories Test Catalogue, **MEDTOX Scientific, Inc.** 402 West County Road D, St. Paul, MN 55112, Phone: (800) 832-3244, (651) 636-7466
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Linda Lichty | 7/16/2012 | New test |
|  | Linda Lichty | 10/8/2012 | Set reference range, defined QC |
|  | Linda Lichty | 6/28/13 | Add policy, do not mix kit components |
|  |  | Erin Bartos | 12/16/2016 | Added #10 to preparing the flex |
|  |  | Stephen Gripentrog, Erin Bartos | 10/15/19 | Converted procedure to reflect Abbott Architect c4000.  |
|  |  | Erin Bartos | 10/28/2020 | Added alinity c |