| **Valproic Acid** |
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| **Purpose** | This procedure provides instructions for performing VALPROIC ACID on ABBOTT INSTRUMENTATION. The Alinity c Valproic Acid assay is used for the quantitative in vitro measurement of valproic acid in human serum or plasma on the Alinity c analyzer. |
| **Policy Statements** | This procedure applies to all personnel responsible for operating the Abbott Alinity c at Children’s Minnesota Laboratory. |
| **Principle** | The Alinity c Valproic Acid assay is a homogeneous particle-enhanced turbidimetric inhibition immunoassay (PETINIA) used for the analysis of valproic acid in serum or plasma. The assay is based on competition between drug in the sample and drug coated onto a microparticle, for antibody binding sites of the valproic acid antibody reagent. The valproic acid-coated microparticle reagent is rapidly agglutinated in the presence of the anti-valproic acid antibody reagent and in the absence of any competing drug in the sample. The rate of absorbance change is measured photometrically, and is directly proportional to the rate of agglutination of the microparticles. When a sample containing valproic acid 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 valproic acid concentration and the lowest agglutination rate at the highest valproic acid concentration.**Methodology:** Particle-enhanced turbidimetric inhibition immunoassay (PETINIA)For additional information on system and assay technology, refer to the Alinity ci-series Operations Manual, Section 3. |
| **Clinical Significance** | Valproic acid (2-propylpentanoic acid; Depakene) is a broad-spectrum anticonvulsant drug used solely or in combination with other anticonvulsant drugs for the treatment of absence seizures. It also has demonstrated effectiveness in the management of generalized tonic-clonic and myoclonic seizures, as well as atypical absence, simple and complex partial, and mixed grand mal and petit mal seizures. The capability of treating many types of seizures with a single anticonvulsant has resulted in the widespread use of valproic acid, particularly in children in whom tonic-clonic and myoclonic seizures are most prevalent. Valproic acid has proven effective in the treatment of many patients otherwise refractory to other anticonvulsant treatments. Most patients receiving valproic acid do not develop a tolerance to its anticonvulsant effects. |
| **Analyzer** | **Minneapolis: Abbott Alinity c (Sunquest method code: MACC)****St. Paul: Abbott Alinity c (Sunquest method code: SALIC)**Backup: Opposite campus |
| **Sunquest Test Codes** | **VALP** |
| **Specimen** | Sample: Plasma or Serum (with or without gel barrier)**Preferred:** Lithium Heparin**Alternative:** SST, Sodium Heparin, K2 and K3 EDTA**•** To confirm that an adequate dose has been prescribed, specimens for the Alinity c Valproic Acid assay should be drawn at trough levels, just prior to a dose. The trough concentration is most indicative of the therapeutic value of valproic acid.**Minimum sample volume:** 0.6 mL blood, 0.2 mL serum/plasma**Stability when separated from cells/gel:** **20 to 25°C:** Not specified**2 to 8°C:** 48 hours**-20°C**: 7 days**Rejection criteria:** Unlabeled tube, sample type other than serum or acceptable plasma**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. Specimens should be free of particulate matter.
4. Transfer serum or plasma directly to a properly labeled pilot tube.
5. Architect and Alinity systems utilize a specimen level detect mechanism, so special racks specific to tube-type are not required.
6. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.
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| **Reagents** | **Reagent Handling** Reagents are shipped refrigerated or on wet ice/cold packs. Upon receipt, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate. If a reagent cartridge is dropped, place in an upright position for 8 hour before use to allow bubbles that may have formed to dissipate. **Prior to loading, gently invert cartridge 5 times. Check to ensure there are no bubbles.**Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results. Use a pipette to remove all bubbles prior to loading on the Alinity or Architect system.* Do not use reagents beyond the expiration date.
* Do not pool reagents within a kit or between kits.
* Do not use components from one lot with components from another lot.
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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Abbott Alinity c Valproic Acid Reagent Kit  | 09P9220 | **Store at: 2 to 8°C****Unopened:** Until manufacturer’s printed expiration date**On-board**: 54 days |
| Abbott Alinity c TDM Multiconstituent Calibrator | 08P7403 | **Store at:** 2 to 8°C**Unopened:** Until manufacturer’s printed expiration date**Opened expiration:** 60 days when stored tightly capped in the refrigerator |

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| **Risk and Safety** | **CAUTION:** This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.The following warnings and precautions apply to: **R1** **WARNING:** Contains bis-tris propane\* and sodium azide. Causes mild skin irritation. Contact with acids liberates very toxic gas. **Response:** If skin irritation occurs: Get medicaladvice / attention.The following warnings and precautions apply to: **R2** Contains sodium azide. Contact with acids liberates very toxic gas.Special disposal not required.Safety data sheets (MSDS/SDS) available on [Children’s Intranet](https://starnet.childrenshc.org/emergency-and-safety/) |
| **Calibration** |

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| Assay Range: | 13 to 150 µg/mL |
| Reference Material: | Abbott Alinity c TDM Multiconstituent Calibrator |
| Suggested Calibration Levels: | CAL 1: 0CAL 2: 12.5CAL 3: 25CAL 4: 50CAL 5: 100CAL 6: 150 |
| Calibration Scheme: | 6 Levels, spline data reduction method |
| Calibration Frequency: | 27 Days and with every new lot. Calibration may be required after maintenance to critical parts or after field service procedures have been performed. |
| AMR | AMR is verified with each calibration.  |

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| **Quality Control** | Bio-Rad Liquichek™ Immunoassay Plus Control Levels 1 and 3**Frequency:** Two levels each day of use**Stability:** Stable until the expiration date when stored frozen between -20 and -40°C. Once thawed, opened, and stored tightly capped at 2 to 8°C, this product is stable for 5 days in Minneapolis (due to estradiol in this control) and 14 days in St. Paul. **Preparation**: This product should be treated the same as patient specimens and run in accordance with the instructions accompanying the instrument, kit, or reagent being used. * To thaw the product, allow it to stand at room temperature (18° to 25°C) until completely thawed but no longer than one (1) hour.
* After thawing, the product **MUST** be gently swirled and inverted several times to ensure homogeneity.
* For optimal analyte stability in the thawed state, promptly return to 2 to 8°C storage after each use and minimize the time at room temperature to no more than 20 minutes daily.
* **Before each use**, gently swirl the contents until homogeneous with no visible signs of precipitate.

**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 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** | **Hemolysis, Icterus & Lipemia (HIL) Index Values:**

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At HIL levels at or above the specified cutoff value, append the appropriate comment AFTER visually confirming presence of interferent: -HP for “Hemolysis present, may affect results.” -BIN for “Bilirubin Interference”-LINT for “Lipid Interference”No endogenous substance interference has been discovered. |
| **Reference Intervals** | Therapeutic range: 50 - 100 µg/mLManufacturer notes:There is no precise relationship between serum valproic acid levels and control of seizures, although most patients require at least a serum level of 50 μg/mL (346.5 μmol/L) for effective therapy. A therapeutic range of 50 to 100 μg/mL (346.5 to 693 μmol/L) has been suggested for valproic acid. Due to great individual differences in dosage requirements to achieve efficacious therapy, determination of valproic acid serum concentrations is required to direct effective therapy. Refer to the drug manufacturer’s package insert or the Physicians’ Desk Reference (PDR) for proper drug dosage and for valproic acid measurement sampling times.Valproic acid modulates the action of various other common antiepileptic drugs. It inhibits the non-renal clearance of phenobarbital, resulting in elevated phenobarbital levels. It competes with phenytoin for protein-binding sites. The free phenytoin concentration remains approximately the same, but the total phenytoin in the plasma decreases. Because the free phenytoin concentration remains unchanged, the pharmacological effect is retained. Other common anti-epileptic drugs that induce hepatic oxidative enzymes result in increased valproic acid clearance; this increased clearance rate requires a higher dose to maintain effective therapeutic levels. |
| **Critical Values** | > 200 µg/mLCritical values must be called and documented according to the critical values policy. |
| **Limitations** | * Sodium citrate and sodium fluoride anticoagulants were tested and found to be unacceptable.
* To confirm that an adequate dose has been prescribed, specimens for the Alinity c Valproic Acid assay should be drawn at trough levels, just prior to a dose. The trough concentration is most indicative of the therapeutic value of valproic acid.
* In very rare cases, patient samples may contain heterophile antibodies, which may produce low results with the Alinity c Valproic Acid assay. 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.
* As with all analyte determinations, the valproic acid value should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.
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| **Dilutions** |

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| Max Auto Dilution: | 1:4 and 1:8 |
| Maximum Manual Dilution: | None |
| Diluent: | Onboard Diluent |
| Instrument Dilution: | Follow Abbott [Alinity Operator’s Manual](https://starnet.childrenshc.org/References/labsop/chem/operator/alinity-ci-series-operations-manual.pdf) instructions for programming automated dilutions. The system will automatically calculate the concentration of the sample and report the result. If a diluted sample result is less than the lower value of the measuring interval of 13, do not report the result. Rerun and/or investigate for other possible causes of error. |

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| **Result Reporting** | * Results between 13 and 150 without error messages are released
* Results below 13 without error messages are reported as < 13.
* Results > 150 should be diluted using the onboard automated 1:4 dilution. Results greater than 600 following this dilution should be further diluted using the automated 1:8 dilution. Release results without error messages following either dilution.
* Results > 1200 following automated dilution are reported as > 1200 µg/mL.
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| **Specimen Storage** | Promptly stopper tested specimen and store upright in a specimen rack. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 7 days in specimen storage freezer. |
| **References** | 1. Abbott Alinity c Valproic Acid Reagent Kit Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised October 2018
2. Abbott Alinity c TDM Multiconstituent Calibrator Package Insert, Abbott Diagnostics Division, Abbott Park, IL USA. Revised July 2018
3. Bio-Rad Liquichek Immunoassay Plus Package Insert, Bio-Rad Laboratories, Irvine CA USA.
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Elauteria Earnhardt | April 28, 2020 | New Procedure for Abbott analyzers |
| 1 | Erin Bartos | October 28, 2020 | Corrected analyzer for Mpls, Added correct QC material, changed sample aliquot tube type, added AMR, ref interval, references, dilutions, interferences and limitations. |