| **Phenytoin** |
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| **Purpose** | This procedure provides instructions for performing PHENYTOIN on ABBOTT INSTRUMENTATION. Phenytoin is also known by the brand name Dilantin. The Alinity c Phenytoin assay is used for in vitro diagnostic use for the quantitative measurement of phenytoin in human serum or plasma on the Alinity c analyzer. The measurements obtained are used in the diagnosis and treatment of phenytoin overdose and in monitoring levels of phenytoin to help ensure appropriate therapy. |
| **Policy Statements** | This procedure applies to all personnel responsible for operating the Minneapolis Alinity c at Children’s Minnesota Laboratory. |
| **Principle** | The Phenytoin assay is a liquid ready-to-use, homogeneous enzyme immunoassay. The method uses specific antibodies to detect phenytoin in the sample, with minimal cross-reactivity to various over-the-counter, structurally related compounds. The method is based on the competition for a fixed amount of specific antibody binding sites between enzyme [glucose-6-phosphate dehydrogenase (G6PDH)]-labeled phenytoin, and phenytoin contained in the sample. In the absence of phenytoin from the sample, the specific antibody binds the G6PDH-labeled phenytoin and causes a decrease in enzyme activity. If phenytoin is present in the sample, it occupies the antibody binding sites, which allows the G6PDH-labeled phenytoin to interact with the substrate, resulting in enzyme activity. This phenomenon creates a direct relationship between the phenytoin concentration in sample and enzyme activity. By measuring the enzyme’s ability to convert nicotinamide adenine dinucleotide (NAD) to NADH, its activity is determined spectrophotometrically at 340 nm.**Methodology**: Enzyme Immunoassay (Enzymatic)For additional information on system and assay technology, refer to the Alinity ci-series Operations Manual, Section 3. |
| **Clinical Significance** | Monitoring phenytoin concentrations, along with careful clinical assessment, is the most effective means of improving seizure control, reducing the risk of toxicity, and minimizing the need foradditional anticonvulsant medication for the following reasons:**•** Phenytoin concentrations correlate better with pharmacologic activity than dosage does because of individual differences in absorption, metabolism, disease states, concomitant medication, and compliance. Concentration monitoring helps physicians individualize dosage regimens.**•** The hepatic enzyme system for metabolizing phenytoin can become saturated within the drug’s therapeutic range. When this occurs, small dosage alterations can lead to unexpected drug accumulation and clinical toxicity.**•** Phenytoin is safe and effective only in a narrow range of concentrations. |
| **Analyzer** | **Minneapolis: Abbott Alinity c (Sunquest method code: MACC)****BACKUP:** Abbott Alinity c (Sunquest method code: MALIC) |
| **Sunquest Test Codes** | **DIL** |
| **Specimen** | Sample: Plasma or Serum (with or without gel barrier) **Preferred:** Lithium Heparin**Alternative:** SST, Sodium Heparin, K2 EDTA, Sodium Citrate (consider dilution effect of liquid anticoagulant), Sodium Fluoride/potassium oxalate**Minimum sample volume:** 0.6 mL blood, 0.2 mL serum/plasma**Stability when separated from cells/gel:** **20 to 25°C:** 2 days**2 to 8°C:** 1 month**-20°C**: 5 months**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. 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 1 hour before use to allow bubbles that may have formed to dissipate. **Immediately prior to loading on the analyzer, gently invert the 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 c.* 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*** |
| Alinity c Phenytoin Reagent Kit  | 08P5420 | **Store at:** 2 to 8°C**Unopened:** Until manufacturer’s printed expiration date**On-board**: 40 days |
| Abbott Alinity c TDM Multiconstituent Calibrator Kit | 08P7403 | **Store at:** 2 to 8°C**Unopened:** Until manufacturer’s printed expiration date**Opened expiration:** 60 Days |

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| **Risk and Safety** | 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** Contains sodium azide. Contact with acids liberates very toxic gas. Dispose of contents / container in accordance with local regulations. *The following warnings and precautions apply to*: **R2** Contains tromethamine hydrochloride and sodium azide. Causes mild skin irritation. Contact with acids liberates very toxic gas.Special disposal requirements not indicated.Safety data sheets (MSDS/SDS) available on [Children’s Intranet](https://starnet.childrenshc.org/emergency-and-safety/) |
| **Calibration** |

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| Assay Range: | 1.8 to 40.0 µg/mL |
| Reference Material: | Abbott Alinity c TDM Multiconstituent Calibrator Kit |
| Suggested Calibration Levels: | Approximate values:CAL 1: 0CAL 2: 2.5CAL 3: 5.0CAL 4: 10.0CAL 5: 20.0CAL 6: 40.0 |
| Calibration Scheme: | 6 Levels, Spline data reduction method |
| Calibration Frequency: | 7 days, every new lot, as required after instrument maintenance, field service request, or as indicated by quality control results.  |
| AMR | AMR is verified with every calibration. |

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| **Quality Control** | **QC Material:** Bio-Rad Liquichek Immunoassay Plus 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). **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 substances yielded clinically significant interference in Abbott studies.The compound **fosphenytoin**, at a level of 60 μg/mL, caused a 41.1% change in drug concentration when tested in the presence of 15 μg/mL phenytoin. Utilize other methods, such as mass spec, to determine phenytoin in affected patients. |
| **Reference Intervals** | **Therapeutic range**: 10 - 20 µg/mLPeak concentrations above 20 μg/mL are often associated with toxicity. For effective treatment, some patients may require serum levels outside these ranges. Therefore, the expected range is provided only as a guide, and individual patient results should be interpreted in light of other clinical signs and symptoms. |
| **Critical Values** | **>30 µg/mL**Critical results must be called according to the Critical Values policy. |
| **Limitations** | Factors that can influence the relationship between phenytoin serum or plasma concentrations and clinical response include the type and severity of seizures, age, general state of health, and use of other drugs.The concentration of phenytoin in serum or plasma depends on the time of the last drug dose; mode of administration; concomitant drug therapy; sample condition; time of sample collection; and individual variations in absorption, distribution, biotransformation, and excretion. These parameters must be considered when interpreting results. |
| **Dilutions** |

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| Max Auto Dilution: | 1:4 |
| Maximum Manual Dilution: | Not specified |
| 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 1.8, do not report the result. Rerun and/or investigate for other possible causes of error. |

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| **Result Reporting** | * Results between 1.8 and 30.0 µg/mL without error messages are released
* Results below 1.8 without error messages are reported as < 1.8 µg/mL.
* Results > 40.0 should be diluted using the onboard automated 1:4 dilution. Release results without error messages following this dilution; report according to critical values policy
* Results > 160 following automated 1:4 dilution are reported as > 160.0 µg/mL and called according to the critical values policy.
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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 14 days in specimen storage freezer. |
| **References** | 1. Abbott Alinity c Phenytoin Reagent Kit Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised July 2018
2. Abbott Alinity c Phenytoin 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 24, 2020 | New Procedure for Abbott analyzers |
| 1 | Erin Bartos | October 28, 2020 | Added assay number for title, fixed instruments, added AMR, calibrators, references, reference interval, dilutions, limitations, interferences, etc for new analyzer. |