| **Phenobarbital** |
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| **Purpose** | This procedure provides instructions PHENOBARBITAL ON ABBOTT INSTRUMENTATION. The Alinity c Phenobarbital assay is for in vitro diagnostic use for the quantitative measurement of phenobarbital 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 Phenobarbital assay is a homogeneous particle-enhanced turbidimetric inhibition immunoassay (PETINIA) used for the analysis of phenobarbital 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 phenobarbital antibody reagent. The phenobarbital-coated microparticle reagent is rapidly agglutinated in the presence of the anti-phenobarbital 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 particles. When a sample containing phenobarbital 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 phenobarbital concentration and the lowest agglutination rate at the highest phenobarbital 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 |
| **Clinical Significance** | Phenobarbital was introduced in 1912 for the treatment of epilepsy, particularly for controlling focal motor or sensory seizures and grand mal seizures. Phenobarbital is bound to both plasma and tissue proteins. Monitoring serum concentrations of phenobarbital has been shown to improve patient therapy by providing physicians with a tool for adjusting dosage. In addition, because of the narrow therapeutic index and wide inter-individual variability in the rate of phenobarbital metabolism and clearance, the determination of blood levels of phenobarbital for patients receiving therapy is important. |
| **Analyzer** | **Minneapolis: Abbott Alinity ci (Sunquest method code: MACC)****St. Paul: Abbott Alinity c (Sunquest method code:SALIC)**Backup: Opposite campus |
| **Sunquest Test Codes** | **PHB** |
| **Specimen** | Sample: Plasma or serum (with or without gel barrier)**Preferred:** Lithium Heparin**Alternative:** SST, Sodium Heparin, EDTA, 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:** 7 days**2 to 8°C:** 6 months**-20°C:** 6 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** 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. **Prior to loading the reagent onto the analyzer, gently invert the cartridge 5 times.** AVOID BUBBLES and remove any that have formed prior to loading on the analyzer.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 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*** |
| Alinity c Phenobarbital Reagent Kit  | 09P8520 | **Store at:** 2 to 8°C**Unopened:** Until expiration Date**On-board**: 40 days |
| Alinity c TDM Multiconstituent Calibrator Kit | 08P7403 | **Store at:** 2 to 8°C**Unopened:** Until manufacturer’s printed expiration date**Opened expiration:** 60 days when stored tightly capped at 2 to 8°C. Return immediately to refrigerated storage. The instrument tracks how long the calibrator has been onboard, and will not allow calibrator to be used if it has been at room temperature (onboard) for longer than 24 total hours. This includes time the rack has been onboard the RSM. |

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| **Risk and Safety** | **CAUTION:** This product contains human-sourced and/or potentially infectious components. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human-sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be 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 human-sourced material used in and is nonreactive for HBsAg, HIV-1 RNA, HCV RNA, anti-HIV-1/HIV-2, and anti-HCV.The following warnings and precautions apply to: **R1** and **R2**Contains sodium azide.Contact with acids liberates very toxic gas.No special disposal requirements 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: | 2 to 80 µg/mL |
| Reference Material: | Abbott Alinity c TDM Multiconstiuent Calibrator |
| Suggested Calibration Levels: | See package insert for exact levels; this is an estimate:Cal 1: 0Cal 2: 5Cal 3: 10Cal 4: 20Cal 5: 40Cal 6: 80 |
| Calibration Scheme: | 6 Levels every 14 days, after major instrument repairs or maintenance, and whenever quality controls indicate need for calibration. |
| Calibration Frequency: | 14 Days |
| AMR | AMR is verified with every calibration and performed at an interval of 14 days or less, as indicated by quality control results. |

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| **-Quality Control** | 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**, which is the expiration of the least stable analyte in use at Children’s Laboratory in this quality control material (estradiol). **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: Trough Level 15-40 µg/mL**Therapeutic phenobarbital concentrations vary significantly depending on the individual patient. A range of 15–40 mcg/mL for peak drug levels indicates effective levels for many patients; however, some individuals are best treated at concentrations outside this range. Concentrations greater than 50 mcg/mL are often associated with toxic symptoms. |
| **Critical Values** | **>60 µg/mL**Critical values must be called and documented according to Critical Values policies. |
| **Limitations** | In studies by Abbott Diagnostics, no interferences have been found with testing numerous endogenous and potentially interfering drug substances. None have been greater than 10% at the levels tested. See the package insert for more information. |
| **Dilutions** |

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| Max Auto Dilution: | 1:2.02 |
| Maximum Manual Dilution: | None |
| Diluent: | Onboard diluent |
| Manual 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 2, do not report the result. Rerun and/or investigate for other possible causes of error. |

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| **Result Reporting** | * Results between 2 and 60 without error messages are released. Results >60 are reported as critical values.
* Results below 2 without error messages are reported as < 2 µg/mL
* Results > 80 should be diluted using the onboard automated 1:2.02 dilution. Release results without error messages following this dilution.
* Results > 161.6 following automated dilution are reported as > 162.
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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 Phenobarbital Reagent Kit Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised July 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 24, 2020 | New Procedure for Abbott analyzers |
| 1 | Erin Bartos | October 28, 2020 | Corrected Mpls Alinity instrument, added AMR, calibrators, dilution, reference interval, critical values, interferences, references, and major editing. |