|  |  |
| --- | --- |
| **Lead (Pb)** | |
| **Purpose** | This procedure provides assay instructions for performing LEAD (Pb) measurement on the Agilent Technologies 240Z Graphite Furnace. |
| **Policy Statements** | This procedure is intended for all Chemistry personnel responsible for testing samples for Blood Lead Levels on the Agilent Technologies 240Z Graphite Furnace. |
| **Principle** | Graphite Furnace Atomic Absorption Spectrometry (GFAAS): Lead is determined in blood by GFAAS. Measurement is based on the amount of light absorbed at 283.3 nm from a hollow-cathode lamp source by ground state atoms of lead in the gas phase. Blood samples, blood-based quality control materials, and blood-based Pb standards are diluted 1:10 with a solution containing ammonium dihydrogen phosphate modifier, Triton X-100, and reagent grade water. The lead content is determined using a graphite furnace atomic absorption spectrometer equipped with a Zeeman Continuum background system operated under stabilized temperature platform furnace conditions. |
| **Clinical Significance** | Lead poisoning is a chronic disease, due to cumulative intake of lead, the course of which may or may not be punctuated by acute symptomatic episodes. If such episodes occur, it is usually during periods of active overexposure. The clinical signs and symptoms of lead poisoning are nonspecific; therefore, a lead measurement, preferably a venous blood lead measurement, is essential for diagnosis. Clinical symptoms of cumulative lead poisoning generally begin with irritability progressing to loss of appetite, change in personality, increased irritability, and abdominal pain. These manifestations are generally seen starting at blood lead concentrations of approximately 50 mcg/dL. If the disease is not recognized at this stage, blood lead levels may well increase above 100 mcg/dL, and then the clinical presentation in children is usually with signs of increased intracranial pressure.  Studies in young children show that 40-50% of dietary lead is absorbed, and that about one-half the amount absorbed is retained. Approximately 99% of the lead in blood is bound to red blood cells. The remaining 1% in plasma serves as an intermediate in transporting lead from the erythrocytes to other body compartments. |
| **Instrument** | Agilent Technologies 240Z Graphite Furnace Atomic Absorption Spectrometer (GFAAS) |
| **Test Code** | **PB** blood lead in whole blood |
|  |  |
| **Materials** | **Reagents** |
|  | * Argon Gas Ultra High Purity (Puritan Bennett Gas Supply C0.) * Triton x-100 T-9284 Ultra (Sigma) * Ammonium Phosphate Monobasic HPLC Grade (Fisher Scientific) A685-500 * Concentrated Nitric Acid * Reagent Grade Water Millipore Water Purification System AFS-8D * Blood Lead Standards WSLH (Wisconsin State Laboratory of Hygiene) * Blood Lead Controls Lyphochek (BIO-RAD) 561,562,563 * SRM Lead in Blood NIST 9556 |
|  | **Supplies** |
|  | * HCL Lead Lamp Agilent PN 5610124800 * 2 mL polyethylene cups Agilent PN 9910028200 * Press overfit caps, small Elkay Co. 1270090100 * GTA partitioned tube (10 ea) Pyrolytic Agilent PN 63-100012-00 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Materials (cont)** | **Equipment** | | | |
|  | * Agilent AA 240Z Graphite Furnace System with Hollow Cathode Lamp SN EL06013809 * Tube Cam * Agilent GTA 120 Graphite Tube Atomizer SN EL06013120 * PSD 120 Autosampler SN EL06013168 * Dell Pentium Computer and Monitor BWGSC91 * Lytron Kodiak Recirculating Chiller 794711-03 * Bar-code Scanner * Automated pipette system with dual syringes 503A (Hamilton Co.) | | | |
|  |  | | | |
| **Sample** | Specimen Collection  * An aliquot of 100 µL whole blood obtained by venipuncture is the only reliable specimen for the confirmation, diagnosis, or medical management of lead poisoning. * Whole blood obtained by capillary puncture is acceptable for screening purposes provided it is obtained with proper and thorough hand-washing technique. If venous blood cannot be collected a capillary sample will be accepted with the restriction that an elevated level must be confirmed with a venous sample. * Specimens collected in Becton Dickinson Vacutainer and Microtainer Brand tubes that contain the anticoagulants EDTA or Lithium Heparin are acceptable. Tubes should be filled to their designated capacity when possible. * Specimens must not contain clots. Reject all clotted specimens. Do not separate cells from plasma.   **Specimen Stability:** 3 weeks refrigerated at 2-8 º C.  **Sample Preparation:**  Dilute all whole blood standards, controls, and patient specimens (100ul) with the specimen diluent (900ul) using the Hamilton hand-held auto-dilutor.   * 1. Mix whole blood samples on a vortex and place pipette tip below the surface.   2. Press the thumb switch to take up sample (whole blood is difficult to pipette so the slowest speed has been selected) and wait for both syringes to complete their tasks.   3. Remove pipette tip from the sample, wipe outside with a Kimwipe tissue, poise pipette tip over a 2 ml cup and press the switch to deliver.   4. Wait until the reagent syringe has delivered all its contents.   5. These dilutions are stable for 1 week tightly capped and refrigerated. | | | |
| **Reagent Preparation** | **Specimen Diluent/Blank Solution:** Place 2 grams Ammonium Phosphate Monobasic into a 1000mL acid-washed flask, Add approximately 500mL reagent grade water and mix until dissolved. Add 5 mL Triton x-100 and dilute to volume with reagent grade water. Add 1ml concentrated Nitric Acid. Mix well (it may take overnight to completely dissolve). Solution is stable for four months at room temperature. This solution is used to dilute standards, controls, and patient specimens with the 503A Hamilton dilutor.  **Auto Sampler Rinse Solution:** Partially fill a 1000mL acid-washed flask with reagent grade water. Add 1 mL Triton X-100 and 0.5mL concentrated Nitric Acid (10 drops). Dilute to volume with reagent grade water and mix well. The solution is stable for 6 months at room temperature. This solution is used in the reservoir bottle of the PSD. Always keep the reservoir bottle at least half full of rinse solution. | | | |
| **Calibration** | * Perform calibration by selecting 3 peer verified proficiency samples of known concentrations that span the reportable range. * A blank value, or zero, is run on the sample diluent as the first sample. This value is expressed as an absorbance determined by peak height and is subsequently subtracted from each sample analyzed. The calibrators are all run and are plotted as absorbances on a signal graph. The 3-calibrator set is run every 20 samples. The calibrator is resloped by running the middle calibrator and checking its absorbance against the original slope every 10 samples. * Every time a run commences the critical mass is checked for each calibrator. The critical mass (also known as the characteristic mass) is defined as the mass of analyte (in pg.) required to produce an integrated absorbance of 0.0044 seconds, and is calculated using the blank-corrected, integrated absorbance value (Ai) for a standard of known concentration (ug/dL) and the volume injected (uL) into the furnace.   **Analytical Measuring Range for Blood Lead:** 1-50mcg/dL  Calibration and Analytical measuring range verification are performed in each run using standards that span the reportable range. | | | |
| **Quality Control** | Three levels of assayed whole blood control materials are used to monitor the precision of each run of blood lead analysis within the clinical range.   * **Lyphochek Whole Blood Control** purchased from BIO-RAD is prepared from human whole blood with pure chemicals and preservatives added. * **Storage and Stability**: Lyphochek Whole Blood Control will be stable until the expiration date when stored unopened @ 2-8 °C. Once the control is reconstituted blood lead control vials are stable @ 2-8 °C for 14 days, or @ -10 to –20 °C for 30 days. * **Preparation**: Reconstitute each vial with 2mLs reagent grade water. Replace the stopper, and allow the control to stand for at least 20 minutes, swirling occasionally. Before sampling, gently swirl the vials to ensure homogeneity. * **Sunquest control names**:   + C-LO is the level 1 control   + C-MEDC is the level 2 control   + C-HI is the level 3 control   The controls are assayed and evaluated the same as patient samples. Enter results into Sunquest and answer QC failures with action comments. For more information on entering, evaluating, and accepting QC results, refer to the Quality Control in Chemistry procedure. | | | |
| **Calculations** | **Characteristic/Critical Mass:** = concentration in ug/dL x volume in uL (15) x 0.0044  Integrated absorbance | | | |
| **Reference Range** | <5 mcg/dL  Since the environment introduces blood lead concentrations in humans, there is no normal or even “safe” level. This reference value is based on the 97.5th percentile of the BLL distribution among children 1–5 years old in the United States (currently 5 μg/dL) using data generated by the National Health and Nutrition Examination Survey (NHANES).  CDC will update the reference value every four years using the two most recent NHANES surveys  The intent of the new reference range is to shift the focus to primary prevention of lead exposure, by reducing or eliminating dangerous lead sources in children’s environments *before* they are exposed  Experts recommend chelation therapy when a child has a test result of greater than or equal to 45 micrograms per deciliter of lead in blood | | | |
| **Critical Values** | >30 mcg/dL, Call Critical values according to Children’s Laboratory Critical Results Policies. | | | |
| **Dilutions** | Blood lead results greater than the highest standard must be diluted to bring the result into the reportable range. Maximum Dilution: 1:3     1. Do not use the auto-sampler dilution to report patient resutls 2. Make a new dilution of the blood lead specimen. 3. Dilute that sample with the sample diluent 1:2 (100uL of sample and 100 uL of sample diluent) to bring the result into the reportable range using MLA pipettes and tips and a new sample cup. 4. Multiply the result by the diluting factor. | | | |
| Limitations | Sensitivity and Precision can be used to determine the instrumental detection limit. In furnace AAS this is defined as the concentration equivalent to three times the standard deviation of the blank measurement. This value is rounded to 1 mcg/dL for routine reporting purposes.  Only well-mixed whole blood may be used for these determinations. Clotted specimens or centrifuged specimens must not be used. Specificity The assay is specific for elemental lead in whole blood. | | | |
| **Result Reporting** | Print and save Agilent patient, control, and calibration result sheets. Record lead results for controls and patients to the Sunquest worksheets rounding the decimal values for the patient samples to the nearest whole number.   * Results below the assay range are reported as <1 mcg/dL * Results > 50 mcg/dL are reported following proper dilution   **Computer Entry:**  MEM (manual result entry)   1. In Sunquest, use function MEM. 2. Use worksheet PB. 3. Enter patient’s accession # and results. 4. When results are displayed on the screen, press “D” to display the previous results for comparison. 5. Press “M” to modify or remove the result (enter -), or to append a comment (-MIQ English text code.) 6. When the results are ready to be sent, press “A” to accept. 7. The next available result comes up for review and resulting.   **MDH Notification:**   1. Fax venous blood lead results of **15ug/dL** or above to MDH 651-201-4909 **within 2 working days**. 2. All blood lead results with the patients’ demographics are sent electronically by CSV file to MN Department of Health, HRA Blood Lead Surveillance, P0 Box 64975, St. Paul, MN 55164-0975 within one month of analysis. 3. Minnesota Statutes, section 144.9502, Childhood Lead Poisoning Prevention Act, requires medical laboratories to report all blood lead analyses and related information to the Minnesota Department of Health. 4. New guidelines for Minneapolis require MDH to notify a local health agency of blood lead levels > 5 in order to provide education to the family.   **Corrected Reports:**  MDH should be notified of all corrected reports by faxing the corrected results to 651-201-4909. | | | |
| **Specimen Storage** | Patient specimens in their primary tubes are kept refrigerated for 1 week after analysis. | | | | |
| **References** | 1. Graphite Tube Atomizer- GTA-110/GTA/120 Operation Manual, Agilent Publication # 8510118500, June 2004 2. Agilent AA140/240/280 series including Zeeman Operation Manual, Agilent Publication # 8510154700, May 2004 3. Programmable Sample Dispenser (PSD120) Operation Manual, Agilent Publication # 8510121800, April 2004 4. Agilent Techniques of Graphite Furnace AA Spectroscopy Course Manual, 8/28/2002. 5. Minnesota Department of Health, Lead Program, PO Box 64975, St Paul, MN, 612-201-4610, 12/2005. 6. NCCLS Approved Guideline C40-A, Analytical Procedures for the Determination of Lead in Blood and Urine Volume 21 No. 9, June 2001. 7. CDC Response to Advisory Committee on Childhood Lead Poisoning Prevention Recommendations in “*Low Level Lead Exposure Harms Children: A Renewed Call of Primary Prevention*”, 6/7/12 (reference range). | | | |
|  |  | | | |
| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Unknown |  | Initial Version |
|  | J. Murrow | 10/1996 | Replaces Version 1 |
|  | J. Murrow | 9/3/2003 | Revised Version 2 |
|  | J. Murrow | 8/3/05 | BLLRS QC omitted, no longer available |
|  | L. Lichty | 9/19/2005 | Added maintenance criteria. |
|  | J. Murrow, L. Lichty | 3/16/2006 | Instrument replacement |
|  | L. Lichty | 9/16/2009 | Added PSD precision specification |
|  | D. Helfinstine/L. Lichty | April 1, 2011 | New format. Removed operating procedure. Vendor name change from “Varian” to “Agilent Technologies”. Updated language. Renumbered from CH 6.09 |
|  | L. Lichty | May 13, 2011 | Revised Reporting section, fax corrected reports to MDH |
|  | L. Lichty | March 12, 2013 | Changed reference range to CDC and MDH level |
|  | Kelsi Brown | June 27, 2017 | Biennial Review |
|  |  | Erin Bartos | June 29, 2018 | Clarified directions for dilutions |
|  |  | | | |