| **25-OH Vitamin D** | |
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| **Purpose** | This procedure provides instructions for performing 25-OH VITAMIN D on the Abbott Architect i1000SR. |
| **Policy Statements** | This procedure applies to all personnel responsible for performing testing on the Abbott Architect i1000SR. |
| **Principle** | The 25-OH Vitamin D assay is a quantitative delayed one-step competitive immunoassay to determine the presence of vitamin D in human serum and plasma using Chemiluminescent Microparticle immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex. The sample, assay diluent and paramagnetic anti-vitamin D coated microparticles are combined. 25-OH vitamin D present in the sample is displaced from the vitamin D binding protein and binds to anti-vitamin D coated microparticles, forming an antigen antibody complex. After incubation, a conjugate containing acridinium-labeled vitamin D is added to the reaction mixture and binds to unoccupied binding sites of the anti-vitamin D coated microparticles. After further incubation and washing, Pre-Trigger and Trigger Solutions are added to the reaction mixture. The resulting Chemiluminescent reaction is measured as relative light units (RLUs). There is a relationship between the amount of 25-OH vitamin D in the sample and the RLUs detected by the Architect iSystem optics. Results are calculated automatically based on the previously established calibration curve. |
| **Clinical Significance** | Vitamin D is a fat-soluble steroid prohormone mainly produced photochemically in the skin from 7-dehydrocholesterol. Two forms of vitamin D are biologically relevant - vitamin D3 (Cholecalciferol) and vitamin D2 (Ergocalciferol). Both vitamins D3 and D2 can be absorbed from food, with vitamin D2 being an artificial source, but only an estimated 10-20% of vitamin D is supplied through nutritional intake. Vitamins D3 and D2 can be found in vitamin supplements. Vitamin D is converted to the active hormone 1,25-(OH)2-vitamin D (Calcitriol) through two hydroxylation reactions. The first hydroxylation converts vitamin D into 25-OH vitamin D and occurs in the liver. The second hydroxylation converts 25-OH vitamin D into the biologically active 1,25-(OH)2-vitamin D and occurs in the kidneys as well as in many other cells of the body. Most cells express the vitamin D receptor and about 3% of the human genome is directly or indirectly regulated by the vitamin D endocrine system. The major storage form of vitamin D is 25-OH vitamin D and is present in the blood at up to 1,000 fold higher concentration compared to the active 1,25-(OH)2-vitamin D. 25-OH vitamin D has a half-life of 2-3 weeks vs. 4 hours for 1,25-(OH)2-vitamin D. Therefore, 25-OH vitamin D is the analyte of choice for determination of the vitamin D status.  Epidemiological studies have shown a high global prevalence of vitamin D insufficiency and deficiency. Risk factors for vitamin D deficiency include low sun exposure, malnutrition, some malabsorption syndromes, and liver or kidney diseases. The measurement of vitamin D status provides opportunities for preventive and therapeutic interventions. Vitamin D deficiency is a cause of secondary hyperparathyroidism and diseases resulting in impaired bone metabolism (like rickets, osteoporosis, and osteomalacia). The Architect 25-OH Vitamin D assay is standardized against NIST SRM 2972 (National Institute of Standards & Technology Standard Reference Material 2972). |
| **Instrument** | **PRIMARY METHOD:** Abbott Architect  Backup Method**:** Mayo Medical Laboratories. Order 25HDN |
| **Sunquest Test Code** | VDT |
| **Specimen** | Serum Separator Tube (SST) preferred. Also acceptable: Lithium Heparin with or without gel, Sodium Heparin, K2EDTA or K3 EDTA plasma.  **Recommended Draw volume** : 1.2 mL (minimum 0.5 mL)  **Minimum processed volume:** 150 µL of serum or plasma  **Stability:** Room Temperature ≤ 72 hours, 2 – 8 °C ≤ 12 Days, ≤-20°C for up to one year.  **Rejection criteria:** Unlabeled tube, Unacceptable sample type, grossly hemolyzed samples (>500 mg/dL), lipemic samples (>500mg/dL) that cannot be cleared via ultracentrifugation  **Preparation:**   1. Serum samples should be centrifuged following complete clot formation, according to Specimen Processing procedures prior to analysis. 2. Remove serum or plasma from the clot, red blood cells, or separator gel. 3. Lipemic samples should be ultrafuged. 4. Specimens should be free of particulate matter. 5. Transfer serum or plasma to a properly labeled sendout tube. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time. |
| **Reagents** | |  |  |  |  | | --- | --- | --- | --- | | ***Product Description*** | ***Product Code*** | ***Stability*** | | | 25 OH-Vitamin D Reagent | 5P02 | **Store at:** 2 – 8 °C  **Unopened/Opened:** Manufacturer expiration date.  **On-board:** 21 Days | | 25 OH-Vitamin D Calibrator | 5P02-02 | **Store at:**  2 – 8 °C  **To Use**: Gently mix after removal from fridge  **Unopened**: Manufacturer expiration date.  **Opened**: Store at 2 – 8 °C and store for 90 days. | | Multiassay Diluent | 7D82-50 | Refer to Supply Status on Analyzer | | Pre-Trigger Solution | 06E23-65 | Refer to Supply Status on Analyzer | | Trigger Solution | 06C55-60 | Refer to Supply Status on Analyzer | | Wash Buffer | 06C54-58 | Refer to Supply Status on Analyzer | | Reaction Vessels | 07C15 (-02 or -03) | N/A | | Liquichek Specialty Immunoassay Controls | Level 1 - 364  Level 2 - 365  Level 3 - 366 | **Unopened storage:** ≤-20°C  **To Use:** Thaw at Room Temperature  **Once Opened, Store:** 2-8°C  **Stability:** 30 Days | |
| **Risk and Safety:** | Contains sodium azide. Avoid contact with skin and eye. Causes serious eye irritation. Wear gloves. Contact with acids liberates very toxic gas. Recap reagents and dispose of in appropriate Hazardous Waste Container. |
| Calibration/ Verification/AMR | |  |  | | --- | --- | | Analytical Measuring Range: | 3.4 – 155.9 ng/mL | | Reference Material: | 25-OH Vitamin D Calibrator | | Suggested Calibration Levels | A – 0.0 ng/mL  B – 4.0 ng/mL  C – 10.0 ng/mL  D – 30.0 ng/mL  E – 75.0 ng/mL  F – 160.0 ng/mL | | Verification Scheme: | n=6 | | Verification Frequency: | * Every 30 Days * For each new lot of reagent * After major maintenance or service, if indicated by quality control results * As indicated in laboratory quality control procedures | | AMR | Verification of AMR is accomplished with each calibration.   * Cal Verification and AMR verification are performed at least once every six (6) months. | |  | | | |
| **Quality Control** | Bio-Rad Liquichek Specialty Immunoassay Levels 1,2 and 3  **Frequency:** Three levels each day of use.  **Stability:** 30 Days at 2-8°C.  **Preparation**: Allow vials to thaw at room temperature, not more than 1 hour.  Do not let QC sit for more than 20 minutes at room temperature when aliquoting daily QC.  **Sunquest Control names:** Level 1 = C-LQSI1, Level 2 = C-LQSI2, Level 3= C-LQSI3  **Acceptable ranges:**   * Ranges are current in Sunquest and the instrument. Refer to the Quality Control in Chemistry procedure for QC exception codes. * If a control value is outside the confidence interval, the determination must be repeated. If the repeat determination confirms the deviation, a new reference curve should be established. * Do not release patient results until the cause of deviation has been identified and corrected. Document ALL troubleshooting actions in Sunquest or in the Architect i1000SR Instrument Maintenance Log notes under the current day's maintenance log. * When a new lot of assayed control is received, validate the manufacturer’s insert range by running the new lot in parallel with the current lot for a recommended 20 days, and confirming that the results obtained are within the stated range. |
| **Interferences** | * Triglycerides >500mg/dL and Hemolysis > 500 mg/dL cause falsely low values. Lipemic samples should be cleared using ultracentrifugation, and grossly hemolyzed samples should be rejected. * Published data has shown that patients undergoing hemodialysis may show a negative bias when measured with various automated 25-OH vitamin D assays when compared to LC/MS. * Patients receiving vitamin D2 supplementation, results that are subtherapeutic should be confirmed with another method, such as LC-MS/MS, before being used for patient management. * Specimens from patients who have received preparations of rabbit monoclonal antibodies for diagnosis or therapy may contain human anti-rabbit antibodies (HARA). Such specimens may show either falsely elevated or depressed values when tested with assay kits such as ARCHITECT 25-OH Vitamin D that employ rabbit monoclonal antibodies * Rheumatoid factor (RF) in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays * Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference, and anomalous values may be observed. |
| **Reference Range** | |  |  | | --- | --- | | **Vitamin D Status** | **25-OH Vitamin D** | | Deficient | <10 ng/mL | | Insufficiency | 10-30 ng/mL | | Sufficiency | 30-100 ng/mL | | Toxicity | >100 ng/mL | |
| **Critical Values** | None specified |
| **Limitations** | The instrument reporting system contains error messages to warn the operator of specific malfunctions. Refer to Operator’s Manual for troubleshooting specific error messages. Also refer to Interferences. |
| **Dilutions** | Samples with a 25-OH Vitamin D value exceeding 155.9 ng/mL will be diluted using a manual dilution.  Maximum Manual Dilution factor: 1:2   1. Add 100 μL of the sample to 100 μL of Architect 25-OH Vitamin D Calibrator A. 2. The operator must manually program the sample with the manual dilution factor. (See [Abbott Architect Operating Procedure](https://starnet.childrenshc.org/References/labsop/chem/procedure/ch5.106-abbott-architect-operating-procedure.pdf) for details about manually programming a patient sample) 3. The analyzer will use this dilution factor to calculate the concentration of the patient sample before dilution, and report the result. |
| **Result Reporting** | * Results between 3.4 – 155.9 ng/mL without an error messages are released * Results less than 3.4 are reported as < 3.4 rather than the numerical value * Results greater than 155.9 ng/mL are manually diluted 1:2 with Calibrator A. Results of 1:2 dilution are reported. * Results greater than 311.8 ng/mL are reported as >311.8 ng/mL rather than the numerical value |
| **Specimen Storage** | Specimens are stored in the Minneapolis Chemistry freezer. Specimens are stored for 14 days before being discarded. |
| **References** | 1. Abbott Architect reagent cartridge insert sheet Abbott Laboratories, Abbott Park, IL, 60064. Revised Date August 2016 2. Abbott Architect calibrator insert sheet Abbott Laboratories, Abbott Park, IL 60064. Revised September 2016. 3. Abbott Architect Safety Data Sheet, Abbott Diagnostics, Abbott Park, IL 60064. Revised 2015-07-30. 4. Bio-Rad Liquichek Specialty Immunoassay Control Product Insert, Bio-Rad Laboratories, Irvine, CA 92618 September 2017. |
| **Historical Record** | |  |  |  |  | | --- | --- | --- | --- | | **Version** | **Written/Revised By** | **Effective Date** | **Summary of Revisions** | | 1 | Kelsi Brown/ Erin Bartos | 4/24/2018 | New Procedure | |  |  |  |  | |  |  |  |  | |