| **25-OH Vitamin D** |
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| **Purpose** | This procedure provides instructions for performing 25-OH VITAMIN D on the Abbott Architect i1000SR and Abbott Alinity i. The 25-OH Vitamin D assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of 25-hydroxyvitamin D (25-OH vitamin D) in human serum and plasma and is to be used as an aid in the assessment of vitamin D sufficiency. |
| **Policy Statements** | This procedure applies to all personnel responsible for performing testing on the Abbott Architect i1000SR or the Abbott Alinity i (ci) in Minneapolis Laboratory. |
| **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 and Alinity 25-OH Vitamin D assay is standardized against NIST SRM 2972 (National Institute of Standards & Technology Standard Reference Material 2972). |
| **Instrument** | **PRIMARY METHOD:** Abbott Alinity i (MACI)Backup Method**:** Abbott Architect i1000SR |
| **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 (>500 mg/dL) that cannot be cleared via ultracentrifugation**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** |

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Abbott Architect 25 OH-Vitamin D Reagent | 5P02-25 | **Store at:** 2 – 8 °C**Unopened/Opened:** Manufacturer expiration date. Store in upright position. If cartridge does not remain upright, gently invert the cartridge 10 times and place in an upright position for 1 hour before use.**On-board:** 21 Days |
| Abbott Architect 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. |
| Abbott Alinity c 25 OH-Vitamin D Reagent Kit | 08P4522 | **Store at:** 2 – 8 °C**Unopened:** Manufacturer expiration date. Store in upright position. If cartridge does not remain upright, gently invert the cartridge 10 times and place in an upright position for 1 hour before use.**On-board:** 21 Days. **Opened**: Until expiration dateStore in upright position. If cartridge does not remain upright during storage, discard the cartridge.Do not reuse original reagent caps or replacement caps due to the risk of contamination and potential to compromise reagent performance. |
| Abbott Alinity c 25 OH-Vitamin D Calibrator Kit | 08P4501 | **Store at: 2 – 8 °C****To Use:** Gently mix after removal from fridge**Unopened:** Manufacturer expiration date.**Opened:** Manufacturer expiration date- Store at 2 – 8 °C. Store tightly capped with new replacement cap.Return to refrigerated storage after use. |
| Liquichek Specialty Immunoassay Controls | Level 1 - 364Level 2 - 365Level 3 - 366 | **Unopened storage:** ≤-20°C**To Use:** Thaw at Room Temperature**Once Opened, Store:** 2-8°C**Stability:** 30 Days |

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| **Reagent Handling** | **Architect reagent Handling:****•** Do not use reagent kits beyond the expiration date.• Do not pool reagents within a kit or between kits.• Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. For microparticle mixing instructions, refer to the PROCEDURE, Assay Procedure section of this package insert.• Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.• To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.• Once a septum has been placed on an open reagent bottle, do not invert the bottle as this will result in reagent leakage and may compromise assay results.For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.Reagent Storage: Do not freeze. May be used immediately after removal from 2-8°C storage.When stored and handled as directed, reagents are stable until the expiration date.**Alinity reagent handling:**Upon receipt, gently invert the unopened reagent kit by rotating it over and back for a full 180 degrees, 5 times with green label stripe facing up and then 5 times with green label stripe facingdown. This ensures that liquid covers all sides of the bottles within the cartridges. During reagent shipment, microparticles can settle on the reagent septum. Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.• After mixing, 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.• 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 mayadversely affect results.For a detailed discussion of reagent handling precautions during system operation, refer to the Alinity ci-series Operations Manual, Section 7.Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it isrecommended that they be stored in their original trays or boxes to ensure they remain upright.For information on unloading reagents, refer to the Alinity ci-series Operations Manual, Section 5. |
| **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.Vitamin D Reagent Kit Pretreatment Reagents 1 & 2 contain methanol. Dispose of in the Methanol waste satellite container located near the manual chemistry bench. If empty, place in regular trash. |
| Calibration/ Verification/AMR | **Alinity c** and **Architect i1000**:

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| Analytical Measuring Range: | 3.5 – 154.2 ng/mL |
| Reference Material: | Alinity c or Architect 25-OH Vitamin D Calibrator |
| Suggested Calibration Levels | A – 0.0 ng/mLB – 4.0 ng/mLC – 10.0 ng/mLD – 30.0 ng/mLE – 75.0 ng/mLF – 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
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| AMR | Verification of AMR is accomplished with each calibration. |
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| **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.**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** | * 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.
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| **Reference Range** |

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| **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 |

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| **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. When testing samples from patients whose predominant form of vitamin D is vitamin D2, such as 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.Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions.If the 25-OH vitamin D results are inconsistent with clinical evidence, additional testing is ecommended.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 the Abbott 25-OH Vitamin D assays that employ rabbit monoclonal antibodies. Additional information maybe required for diagnosis.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 canbe prone to this interference, and anomalous values may be observed. Additional information may be required for diagnosis.Rheumatoid factor (RF) in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Additional information may be required for diagnosis.The Alinity i 25-OH Vitamin D assay is susceptible to interference effects from triglycerides at >500 mg/dL. A triglyceride concentration of 800 mg/dL resulted in -13.8%, -10.2%, and -17.5% bias in results for 25-OH vitamin D concentration at approximately 20 ng/mL, 30 ng/mL, and 40 ng/mL 25-OH vitamin D, respectively. |
| **Dilutions** | Samples with a 25-OH Vitamin D value exceeding 154.2 ng/mL will be diluted using a manual dilution.Maximum Manual Dilution factor: 1:21. Add 100 μL of the sample to 100 μL of Architect or Alinity 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) and [Alinity Operating Procedure](https://starnet.childrenshc.org/References/labsop/chem/assays/ch-6.96-vitamin-d.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.
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| **Result Reporting** | * Results between 3.5 – 154.2 ng/mL without an error messages are released
* Results less than 3.5 are reported as < 3.5 rather than the numerical value
* Results greater than 154.2 ng/mL are manually diluted 1:2 with Calibrator A. Results without error flags after 1:2 dilution are reported.
* Results greater than 308.4 ng/mL are reported as >308.4 ng/mL rather than the numerical value
* Results < 20 ng/mL will automatically repeat. Do not report until repeat is resulted
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| **Specimen Storage** | Specimens are stored in the Minneapolis Chemistry freezer 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. Abbott Alinity i 25-OH Vitamin D Reagent Kit Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL 60064 USA. Revised February 2018
5. Abbott Alinity i 25-OH Vitamin D Calibrator Kit Package Insert, Abbott Diagnostics Division, Abbott Park, IL 60064 USA. Revised February 2018
6. Bio-Rad Liquichek Specialty Immunoassay Control Product Insert, Bio-Rad Laboratories, Irvine, CA 92618 September 2017.
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| **Historical Record** |

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| **Version** | **Written/Revised By** | **Effective Date** | **Summary of Revisions** |
| 1 | Kelsi Brown/ Erin Bartos | 4/24/2018 | New Procedure |
| 2 | Erin Bartos | 11/2/18 | Updated repeat if <20 |
| 3 | Erin Bartos | October 28, 2020 | Added Alinity i references. |

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