| **Calcium in Plasma/Serum or Urine** |
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| **Purpose** | This procedure provides instructions for performing CALCIUM IN PLASMA/SERUM OR URINE. |
| **Policy Statements** | This procedure applies to all personnel who run the Siemens Dimension Vista 500 and the Dimension RxL MAX. |
| **Principle** | The calcium method is a modification of the calcium o-cresolphthalein complexone (OCPC) reaction originally reported by Schwartzenbach, et al. Stern and Lewis later adapted this reaction to a colorimetric calcium assay. Connerty and Briggs demonstrated the use of 8-quinolinol to reduce magnesium interference, and Sarkar and Chauhan reported the use of this procedure for serum calcium without protein precipitation. This method incorporates the use of 8-quinolinol to reduce magnesium interference and glycine buffer for pH control.Calcium reacts with OCPC to form a purple complex. The amount of complex thus formed is proportional to the calcium concentration and is measured using a bichromatic (577, 540 nm) endpoint technique. Magnesium ions, which also form a colored complex with OCPC, are removed from the reaction by complexation with 8-quinolinol. |
| **Clinical Significance** | Although 99% of the calcium of the body is contained in the bones and teeth, it is the calcium content of blood that is of most importance to the physician. In the blood essentially all of the calcium exists in one of three forms: 1) as free calcium ion (Ca ++), 50%; 2) bound to protein (primarily albumin), 45%; and 3) complexed with certain organic compounds, mainly citrate, 5%. The ionized fraction is the most important, but direct measurement has proved to be difficult. The percentage of total calcium that is in the ionized form is known to depend upon the amount of protein present and the pH of the blood. High protein levels and elevated pH tend to reduce the relative amount of ionized calcium. Within limits, the serum calcium varies inversely with the phosphorus concentration.Calcium ion is important in the transmission of nerve impulses, in the maintenance of normal muscle contractility, as a cofactor in certain enzyme reactions, and in the coagulation of the blood. A substantial reduction in calcium ion concentration results in a state of neuromuscular excitability known as tetany. In the extreme state, the muscles are contracted continuously. Higher than normal concentrations of calcium, on the other hand, result in loss of normal neuromuscular excitability and muscle weakness Relative constancy of serum calcium concentration must be maintained. Bone serves as a reservoir for this purpose, releasing calcium when required to prevent hypocalcemia and trapping calcium to prevent excessively high levels. The uptake and release of calcium from the bone is under the control of the parathyroid hormone.Calcium concentrations in disease may be either lower or higher than normal. Normal is highest in children and falls slightly throughout life. Variations in serum calcium may be due to disease of the parathyroid glands, disease of bone, defective absorption of calcium from the intestine, kidney disease, and various other causes. Low Serum Calcium is found in hypoparathyroidism and in Pseudohypoparathyroidism, a rare inherited disease. |
| **Analyzer** | **PRIMARY METHOD:** Siemens Dimension Vista 500**SECONDARY (BACKUP) METHOD:** Siemens Dimension RxL MAX |
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| **Sunquest Test Codes** |

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| CA | Calcium in plasma or serum in mg/dL |
| UCAL | Calcium in urine in mg/dL |
| UCAQ/UCA | Quantitative urine calcium, timed collection in mg/collection |
| CCRA | Calcium Creatinine Ratio |

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| **Specimen** | Plasma (lithium heparin) preferredSerum (no gel) Urine (on Vista 500 only): Timed (24 hour) or Random collection no preservatives**Minimum volume:** 0.2 mL**Stability:** 2-8 °C / 48 hours, < -20°C / 6 months**Rejection criteria:** Unlabelled specimens.**Preparation:**1. Timed urine collections are measured for total volume, and the collection date and time recorded for the start and end of the collection. Enter this information into Sunquest in CVIS by ordering the test PV on the same accession number.
2. Centrifuge all urine calcium specimens prior to testing. Complete testing as soon as possible after collection.
3. Whole blood specimens should be centrifuged according to Specimen Processing procedures prior to analysis. See Processing Procedure Manual.
4. Serum should be separated from red cells and analyzed promptly.
5. Transfer serum, plasma or prepared urine sample to a properly labeled Siemens SSC nested on a bar-coded pilot tube. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.
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| **Reagents** | **PRIMARY METHOD:**

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| CA Flex Reagent Cartridge (Vista) | K1023 | **Store at:** 2 - 8 °C.**Unopened:** Refer to carton for expiration date of individual unopened reagent cartridges. **On-board:** Sealed wells on the instrument are stable for 90 days.**Open well stability:** 21 days for wells 1 - 12. |
| CHEM 1 CALFrozen liquid. | KC110 | **Store at:** <-15°C**Unopened:** Refer to carton for expiration date.**Preparation:** Allow vial to thaw and equilibrate to room temperature for 1 hour. Before use, gently invert calibrator at least 10 times to ensure the contents are mixed thoroughly. DO NOT VORTEX.**On-Board:** Once the vial stopper is punctured, assigned values are stable for 7 days when stored on board the Dimension Vista® System |

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|  | **SECONDARY (BACKUP) METHOD:**

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| CA Flex Reagent Cartridge (RXL) | DF23A | **Store at:** 2 - 8 °C.**Unopened:** Refer to carton for expiration date of individual unopened reagent cartridges. **On-board:** Sealed wells on the instrument are stable for 30 days.**Open well stability:** 3 days for wells 1 - 6. |
| CHEM 1 CAL (RXL)Lyophilized, see package insert for preparation instructions. | DC18B | **Store at:** 2 - 8 °C.**Unopened:** Refer to carton for expiration date.**On-board:** Assigned values are stable for 24 hours after reconstitution when vials are stoppered and stored at 2 - 8 °C. |

**OTHER REAGENTS:*** 6 M HCL to acidify urine. Prepare 6 M HCL by slowly adding 6.2 mLs of 38% HCL to 3.8 mLs purified H2O
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| **Calibration** | **PRIMARY METHOD:**

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| Assay Range (AMR): | 5.0 – 15.0 mg/dL |
| Reference Material: | CHEM 1 CAL (Cat. No. KC110)  |
| Suggested Calibration Levels: | 5.3, 15.1 mg/dL |
| Calibration Scheme: | Two levels, n=5 |
| Calibration Frequency: | •For each new lot of Flex® reagent cartridges•Every 90 days for any one lot.• After major maintenance or service, if indicated by QC results• As indicated in laboratory quality control procedures• When required by government regulations |
| Analytical Measuring Range | 5.0-15.00 mg/dL |

**SECONDARY METHOD:**

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| Assay Range (AMR): | 5.0 – 15.0 mg/dL |
| Reference Material: | CHEM 1 Calibrator (Cat. No. DC18B)  |
| Suggested Calibration Levels: | 7, 10, 14.0 mg/dL |
| Calibration Scheme: | Three levels in triplicate |
| Calibration Frequency: | •For each new lot of Flex® reagent cartridges•Every 60 days for any one lot.• After major maintenance or service, if indicated by QC results• As indicated in laboratory quality control procedures• When required by government regulations |
| Assigned Coefficients: | C0 1.000 C1 0.090 |
| Analytical Measuring Range: | 5.0 – 15.0 mg/dL |

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| **Analytical Measuring Range (AMR)** | **PRIMARY METHOD:*** Cal Verification and AMR verification are performed at least once every six (6) months.
* Touch Advanced 🡪 Calibrations 🡪 Calibrations by Lot, select method “Calcium” and “Order a Linearity Study”
* See iGuide “Calibration by Lot” for more information.

**SECONDARY METHOD:**Verification of AMR is accomplished with each calibration. |
| **Quality Control**  | **Plasma/Serum****PRIMARY METHOD**: BioRad MultiQual Levels 1 & 3, contained in Vista vials. **Sunquest Control names:** C-MQ1, C-MQ3**SECONDARY METHOD**: Bio-Rad Liquichek™ Unassayed Chemistry Control, Levels 1 & 2. **Sunquest Control names:** C-X1, C-X2 |
|  | **Urine****PRIMARY METHOD**: Biorad Liquichek® **Urine Chemistry** Control Levels 1 & 2 for use with urine samples. Transfer 2.5 mL’s into each of four (4) Vista Vials. See iGuide or Vista Job Aid for instructions.**Sunquest Control names:** Urine Level 1 = C-UR1, Level 2 = C-UR2**SECONDARY METHOD**: Biorad Liquichek® **Urine Chemistry** Control Levels 1 & 2 for use with urine samples.**Sunquest Control names:** Urine Level 1 = C-UR1, Level 2 = C-UR2**Quality Control Frequency:** * Two levels each day of use

**Quality Control Stability:** Refer to the current lot product insert**Acceptable ranges:** * Ranges are current in Sunquest and the instrument. Refer to the Quality Control Procedure for QC exception codes.
* If a control value is outside the confidence interval, the determination must be repeated. If the repeated 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
* When a new lot of control is received, validate the manufacturer’s insert range by running the new lot in parallel with the current lot, and confirming that the results obtained are within the stated range
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| **Calculations** | **UCAQ** = Ca in mg/24 Hrs = Ca (mg/dL) x 10 dL/L x Total Volume in mL 1000**CCRA** = Urine Calcium/Creatinine ratio = Ca (mg/dL) Creat (mg/dL)Timed Urine calculations are performed by the Laboratory computer system when all necessary information is present. Enter the measured urine calcium value in mg/dL, collection time in hours, and the volume.Note: Calcium results < 1.0 mg/dL will not calculate when entered into the above equations. Answer the final result with the Sunquest comment code UNCA for “unable to calculate”. |
| **Interferences** | No interference was found for:* Commonly used drugs.
* HIL:
	+ Hemoglobin (free) up to 1000 g/dL
	+ Bilirubin (conjugated or unconjugated) up to 60 mg/dL
	+ Lipemia up to 600 mg/dL

Interfering substances:* Interference due to magnesium is negligible at magnesium levels normally encountered in human serum. A maximum positive interference of 0.7 mg/dL occurs at a magnesium level of 7 mg/dL.
* EDTA when present at 200 mg/dL and potassium oxalate when present at 500 mg/dL depresses the CA result to less than the assay range of the method.
* Calcium values may be falsely decreased in the presence of gadolinium-containing contrast agents such as Omniscan.™ Samples for serum calcium determination should be drawn 24 hours after administration of Omniscan™ to avoid interference.
* Bilirubin (unconjugated) of 80 mg/dL decreases calcium at 6.4 mg/dL by 11%
* Lipemia (Intralipid®) of 600 mg/dL tripped a test report message.
* Refer to the product insert for a list of substances that have been shown to have no measurable effect on the CA result at typical concentrations.
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| **Reference Range** | Serum/Plasma:

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| Age | Calcium  |
| 0-7 days | 7.0 – 12.0 mg/dL |
| 8 days – 12 months | 8.0 – 11.0 mg/dL |
| 1-18 years | 9.0 – 10.8 mg/dL |
| >18 years | 8.6 – 10.6 mg/dL |

Urine:25-150 mg/24 hrsDIET DEPENDENTLow Calcium intake <5 mg/24 hrsAvg calcium Intake 50-150 mg/24 hrsHigh calcium intake 10-300 mg/24 hrsThe reference values are for a 24-hour collection. Random specimens and timed specimens collected for other than a 24-hour time period are reported in units of mg/dL, for which reference values are not established.Calcium/Creatinine Ratio

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| Up to age 16 | < 0.20 |
| Adult | < 0.11 |

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| **Critical Values** | Plasma/Serum: <6.0 or >14.0 mg/dL.Critical values must be called according to the Critical Limit Test Value Policy. |
| **Limitations** | Linear range of detection: 5.0 – 15.0 mg/dLThe instrument reporting system contains flags and comments to provide the user with information regarding instrument processing errors, instrument status information and potential errors in calcium results. Refer to your Dimension Vista or Dimension RXL Operator’s Guide for the meaning of report flags and comments. Any report containing flags and/or comments should be addressed according to your laboratory’s procedure manual and not reported. |
| **Dilutions** |

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| **CA (PRIMARY METHOD)** |
| **Initial Dilution:** | None |
| **Max Manual Dilution / Diluent:** | Serum/Plasma: 1:2, CLRW (Clinical Laboratory Reagent Water)Urine: 1:10, CLRW |
| **Surplus Rack:** | Serum/Plasma: Samples with results >15.0 mg/dL are repeated on a higher dilution (1:2).Urine: Samples with results >15.0 mg/dL are repeated on a higher dilution (1:2.5) |
| **Limited Rack:** | Serum/Plasma: Samples with results >15.0 mg/dL should be repeated as an Add-On Test with a Special Dilution of 1:2.Urine: Samples with results >15.0 mg/dL should be repeated as an Add-On Test with a Special Dilution of 1:2.5. |
| **Urine Dilutions:** | **If the result is “above assay range” after an instrument performed 1:2.5 dilution**:* Make appropriate dilutions with Purified Water to obtain result within the assay range. Determine optimum dilution by dividing result obtained by 15 (the assay range) and rounding up to the next whole number (max 1:10)
* Label diluted sample with “label foot” or Accession number, and dilution factor.
* Program Manual Dilution Factor in Vista. Reassay. Resulting readout is corrected for dilution.

**Below 5.0 mg/dL**:* Dilute urine sample with high urine control (UR2) using equal parts (1:2).
* Enter dilution factor of 2 in Vista in Manual Order Entry. Reassay. Resulting readout is corrected for dilution.
* Run an aliquot of UR2 at the same time.
* In Sunquest, when prompted for “spiked” value, enter UR2 result.
* The result is automatically calculated.
* For manual calculation: Subtract the value of UR2 from this readout to determine urine calcium.
* Document dilutions and calculations, and have results checked prior to reporting.
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| **Dilutions (continued)** |

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| **CA (SECONDARY METHOD)** |
| **Initial Dilution:** | None |
| **Max Manual Dilution / Diluent:** | Serum/Plasma: 1:2, CLRW (Clinical Laboratory Reagent Water)Urine: 1:10, CLRW |
| **Auto Dilution:** | Serum/Plasma Urine: Samples with results >15.0 mg/dL are autodiluted by the instrument.Results with “dilution” appended are reportable. |
| **Urine Dilutions:** | **If the result is “assay range/diluted” after an instrument performed autodilution**:* Make appropriate dilutions with Purified Water to obtain result within the assay range. Determine optimum dilution by dividing result obtained by 15 (the assay range) and rounding up to the next whole number (max 1:10)
* Label diluted sample with “label foot” or Accession number, and dilution factor.
* Program Manual Dilution Factor in RXL. Reassay. Resulting readout is corrected for dilution.

**Below 5.0 mg/dL**:* Dilute urine sample with high urine control (UR2) using equal parts (1:2).
* Label diluted sample with “label foot” or Accession number, and dilution factor.
* Enter dilution factor of 2 in RXL. Reassay. Resulting readout is corrected for dilution.
* Run an aliquot of UR2 at the same time.
* In Sunquest, when prompted for “spiked” value, enter UR2 result.
* The result is automatically calculated.
* For manual calculation: Subtract the value of UR2 from this readout to determine urine calcium.
* Document dilutions and calculations, and have results checked prior to reporting.
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| **Result Reporting** | * Results between 5.0 – 15.0 mg/dL without error messages are released
* Serum/Plasma results below 5.0 mg/dL: report as < 5.0 mg/dL.
* Results >15.0 mg/dL are reported following a maximum dilution of 1:2 (plasma) or 1:10 (urine)
* Urine results >1.0 mg/dL and < 5.0 mg/dL: The Laboratory Information System calculates urine calcium on timed and random collections, as well as the Calcium/Creatinine ratio when all necessary information is present. Report the numerical value. See “Calculations” for results < 1.0 mg/dL.
* Urine results < 1.0 mg/dL: Report as <1.0 mg/dL.
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| **Specimen Storage** | Promptly stopper tested specimen and store upright in specimen rack. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 7 days in specimen storage freezer. |
| **References** | 1. CA Flex reagent cartridge insert (RXL), PN 717123.002 – Version O, Rev 2016-06-26
2. CA Flex reagent cartridge insert (Vista) PN 781023.001 – Version F, Rev 2014-07-28
3. Jacobs & DeMott Laboratory Test Handbook, Lexi-Comp, Inc, Hudson, OH, 5th Edition, 2001
4. Biorad Multiqual Assayed Chemistry Control Product Insert, Bio-Rad Laboratories, Irvine, CA
5. Biorad Liquichek Unassayed Chemistry Control Product Insert, Bio-Rad Laboratories, Irvine, CA
6. Biorad Liquichek Urine Chemistry Control Product Insert, Bio-Rad Laboratories, Irvine, CA
7. [Ann Clin Biochem.](https://www.ncbi.nlm.nih.gov/pubmed/19729500) 2009 Nov;46(Pt 6):484-7. doi: 10.1258/acb.2009.009027. Epub 2009 Sep 3
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Unknown |  | Calcium on Dimension AR, Initial Version |
|  | Deane L Riedel | August 2000 |  |
|  | L. Lichty | March 19, 2003 | Calcium on Dimension RxL |
|  | L. Lichty | 6/15/2005 | Calcium on Dimension RxL MAX |
|  | L. Lichty | 1/18/06 | Calcium on Dimension RxL and MAX |
|  | L. Lichty | 01/01/2007 |  |
|  | D. Helfinstine | April 1, 2011 | New Format, Updated package insert information. Renumbered from CH 3.16 |
|  | L. Lichty | December 3, 2012 | Add HCL to reagents. |
|  | L. Lichty | December 10, 2013 | Conversion to mg/dL for reporting unitsUpdate product insert. |
|  | L. Lichty | December 17, 2013 | Siemens CLSI CA for Vista Procedure (07.24.2013) |
|  | D. Helfinstine | May 12, 2014 | Revised for Vista, primary/secondary designation, updated IFUs, dilutions. |
|  | Erin Bartos | July 10, 2017 | Removed acidification of urine. Added centrifugation of every urine prior to testing, and running urine as soon as possible after collection. |
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