**Intended Use**
System reagent for the quantitative determination of Calcium concentrations in human serum and urine on Beckman Coulter AU analyzers.

**Summary**
Measurement of calcium is used in the diagnosis and treatment of parathyroid disease, a variety of bone diseases, chronic renal disease and tetany (intermittent muscular contractions or spasms).

Although more than 99% of body calcium exists in bones and teeth, it is the calcium in blood which is of most concern clinically. The bones serve as a reservoir to maintain relative constancy of serum calcium by releasing calcium when required to prevent hypocalcemia and trapping calcium to prevent excessively high levels of serum calcium. The uptake and release of calcium from bone is under the control of parathyroid hormone.

The percentage of ingested calcium absorbed decreases as the dietary calcium content increases, and so the amount absorbed can remain relatively constant. The slight increase in absorption that occurs on a high-calcium diet is reflected in an increased renal excretion.

Serum calcium exists in three forms: 1) free calcium ion, Ca\(^{2+}\), 50%, 2) protein bound calcium, 45% and 3) complexed calcium, mainly with citrate, 5%. The ionized calcium is physiologically most significant but has proven difficult to assay directly. It may be estimated from total calcium given a knowledge of the protein content and pH of the blood which strongly affect the level of ionized calcium. Levels of calcium are roughly inversely proportional to phosphorus levels.

Calcium ions are important in the transmission of nerve impulses, as a cofactor in several enzyme reactions, in the maintenance of normal muscle contractility, and in the process of coagulation. A significant reduction in calcium ion concentration results in muscle tetany. A higher than normal concentration of calcium ions produces lowered neuromuscular excitability and muscle weakness along with other more complex symptoms.

In disease, calcium concentration may be either higher or lower than normal. Normal levels are highest in children and decline gradually throughout life. Variations in serum calcium may be due to disease of the parathyroid gland, bone disease, defective absorption of calcium from the intestine, kidney disease, multiple myeloma and various other abnormalities.

**Methodology**
This Calcium oCPC procedure is based on calcium ions (Ca\(^{2+}\)) reacting with \(o\)-cresolphthalein complexone in an alkaline solution to form an intense violet colored complex which maximally absorbs at 577 nm.\(^3\) 8-Hydroxyquinoline is added to remove interference by magnesium and iron.\(^4\) In this method the absorbance of the Ca-oCPC complex is measured bichromatically at 570/660 nm. The resulting increase in absorbance of the reaction mixture is directly proportional to the calcium concentration in the sample.

\[
\text{Ca}^{2+} + \text{oCPC} \rightarrow \text{Ca-oCPC Complex (Violet)}
\]

**System Information**
For AU400/400®/480, AU600/640/680 and AU2700/5400 Beckman Coulter Analyzers.

**Reagents**
Final concentration of reactive ingredients:
- Ethanolamine (pH 10.6) 0.375 mol/L
- \(o\)-Cresolphthalein-complexone 82.0 \(\mu\)mol/L
- 8-Hydroxyquinoline 7.16 mmol/L
- Hydrochloric acid 27.75 mmol/L

**Precautions**
1. For in vitro diagnostic use.
2. WARNING! CORROSIVE! Avoid ingestion or contact with skin or eyes. In case of external contact, immediately rinse affected area with plenty of water for 15 minutes. Obtain medical attention immediately for eye contact or ingestion.

**Preparation of Reagents**
The Calcium oCPC Reagents are ready for use. No preparation is required.

**Storage and Stability**
1. The unopened reagents are stable until the expiration date printed on the label when stored at 2 – 25\(^\circ\)C.
2. Opened reagents are stable for 30 days when stored in the refrigerated compartment of the analyzer.

**Indications of Deterioration**
Visible signs of microbial growth, turbidity or precipitate, or any change in reagent color may indicate degradation and warrant discontinuance of use.

**Specimen Collection and Preparation**
Serum sample, free from hemolysis, is the recommended specimen. Separate serum from red cells as soon as possible. Timed, 24-hour collection is recommended for urine specimens.\(^{5}\)
Calcium oCPC

Sample Storage and Stability
Serum calcium is stable for at least 24 hours at 15-25°C, approximately 1 week at 2-8°C and up to 1 year at ≤ 20°C. Urine calcium can be kept in solution by adding 10 mL of 6M HCL to the collection container before a 24 hour specimen is collected. The urine should be kept well mixed during the collection period.5

Interfering Substances
DO NOT use the following anticoagulants in collecting blood for use in this test: EDTA, Sodium Citrate, Sodium Fluoride or Oxalate.

Results of laboratory studies6 show that the following substances interfere with this calcium determination.
The criteria for no significant interference is recovery within 10% of the initial value.

Bilirubin: No significant interference up to 40 mg/dL Bilirubin
Hemolysis: No significant interference up to 500 mg/dL Hemolysate

* Intralipid, manufactured by KabiVitrum Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc., makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young7 for a compilation of reported interferences with this test.

Procedure
A complete list of test parameters and operational procedure can be found in the User’s Guide appropriate to the analyzer.

Materials Provided
Calcium oCPC Reagent

Materials Required But Not Provided
Urine Calibrator (Cat # DR0090)
Chemistry Calibrator (Cat # DR0070)

Stability of Final Reaction Mixture
The Beckman Coulter AU analyzer automatically computes every determination at the same time interval.

Calibration
The frequency of calibration is daily. Calibration of this procedure is accomplished by use of the Chemistry Calibrator (Cat # DR0070), which is traceable to the National Institutes of Standards and Technology (NIST) Standard Reference Material (SRM)909b. Calibration for urine specimens is accomplished by the use of Urine Calibrator (Cat # DR0090).

Recalibration of this test is required when any of these conditions exist:
1. A reagent lot number has changed or there is an observed shift in control values.
2. A fresh bottle of reagent is used for testing.
3. Major preventative maintenance was performed on the analyzer or a critical part was replaced.

Quality Control
During operation of the Beckman Coulter AU analyzer at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration, and after specific maintenance or troubleshooting steps described in the appropriate User’s Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory’s standard procedure. Appropriate qualified urine controls should be established and utilized during urine analysis.

Results
No further calculations are required. Results are automatically printed out for each sample in mg/dL. For SI units (mmol/L) the results must be multiplied by 0.25.

Dynamic Range
The Calcium oCPC procedure is linear from 0 to 18 mg/dL for serum determinations and 0 to 40 mg/dL for urine determinations. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically by utilizing the AUTO REPEAT RUN.

Note: Care should be taken when interpreting calcium results from patients who have received gadolinium containing contrast media within the previous 24 hours especially if the patient has impaired renal function.10,11,12 Such samples should be assayed using non-colorimetric techniques e.g. ion selective electrodes or emission spectroscopy. If non-colorimetric assays are unavailable, samples should be drawn prior to administration of such contrast media.

Expected Values

Serum 8
8.6 - 10.2 mg/dL

Urine 5
100 - 300 mg/day

Urine calcium values may vary considerably and are only meaningful if the patient is kept on a low-calcium, neutral-ash diet for 3 days before collection. Low output is 50 to 100 mg/day.5

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics
The following data was obtained using the Calcium oCPC Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

Precision14
Estimates of precision, based on CLSI recommendations9, are consistent with typical performance. The within run precision for serum or urine samples will typically be less than 0.25 mg/dL or 3.0% of the mean and the total precision will typically be less than 0.33 mg/dL or 5.0% of the mean.

Assays of control sera were performed and the data reduced following the CLSI guidelines above:
Calcium oCPC

Serum

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<th>N = 80</th>
<th>Within Run</th>
<th>Total</th>
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<tbody>
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<td>Mean, mg/dL</td>
<td>SD</td>
<td>CV%</td>
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<tr>
<td>7.38</td>
<td>0.06</td>
<td>0.80</td>
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<tr>
<td>11.61</td>
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<td>0.78</td>
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<td>10.40</td>
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Urine

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<tr>
<th>N = 80</th>
<th>Within Run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, mg/dL</td>
<td>SD</td>
<td>CV%</td>
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<tr>
<td>5.86</td>
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<td>9.39</td>
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<td>12.56</td>
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Method Comparison

Patient samples were used to compare this Calcium oCPC Reagent. The table below demonstrates representative performance on the AU analyzer.

<table>
<thead>
<tr>
<th>Y Method</th>
<th>AU600/640/640*</th>
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<tbody>
<tr>
<td>X Method</td>
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<tr>
<td>Slope</td>
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<tr>
<td>Intercept</td>
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<tr>
<td>Correlation Coeff. (r)</td>
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<td>No. of Samples (n)</td>
<td>79</td>
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<tr>
<td>Range (mg/dL)</td>
<td>2.3-18.0</td>
</tr>
</tbody>
</table>

Sensitivity

Typical change in absorbance for 1 mg/dL of calcium is 12 mAbsorbance.

References

8. Beckman Coulter Inc. data on samples collected from 200 blood donors in North Texas.
14. Data is on file for specific AU analyzers.

Manufactured by: Beckman Coulter, Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA