DXC (CALC) CALCIUM

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PURPOSE

To provide instructions for the quantitative determination of Calcium on the DXC 600/800.

PRINCIPLE

ISE Electrolyte Buffer reagent and ISE Electrolyte Reference reagent, when used in conjunction with UniCel® DxC 600/800 System(s) and SYNCHRON® Systems AQUA CAL 1 and 2, are intended for the quantitative determination of Calcium concentration in human serum, plasma or urine.

BACKGROUND

Clinical Significance

Calcium measurements are used in the diagnosis and treatment of parathyroid disease, a variety of bone diseases, chronic renal disease and tetany (intermittent muscular contractions or spasms). Urinary calcium measurement is used in the differential diagnosis of absorptive hypercalciuria and hypercalciuria caused by hyperparathyroidism, hyperthyroidism, Paget`s disease or "renal leak" type of calciuria as seen in renal tubular acidosis.

Methodology

The SYNCHRON® System(s) determines total calcium concentration by indirect potentiometry utilizing a calcium ion selective electrode in conjunction with a sodium reference electrode. In principle, a calcium ion selective electrode measures un-bound free calcium ions in solution. Total calcium can only be calculated from free calcium when the molar ratio between free and total calcium concentrations is constant. This constant molar ratio is achieved by the buffered solution which contains strong calcium complexing agents. A precise volume of sample (40 microliters) is mixed with the buffered solution. The ratio used is one part sample to 33 parts buffered solution. The high molar strength buffer is used to establish a constant activity coefficient for calcium ions, calibrating the electrode to concentration values.

Chemical Reaction

$$E = \text{Constant} + (\text{slope})(\log[Ca^{2+}])$$

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RELATED DOCUMENTS

R-PO-CH-0810	Quality Control Program General Laboratory
R-PO-CH-0809	Quality Control Westgard Rules Statistics
R-PR-AD-0540	Specimen Rejection/Cancellation Protocol
J-F-CH-0820	DXC 800 Controls
J-F-CH-0826	DXC 800 Calibrators
J-F-CH-1940	DXC 800 Analytical Measurement Range
M-F-CH-0820	DXC 600 Controls
M-F-CH-0826	DXC 600 Calibrators
M-F-CH-1940	DXC 600 Analytical Measurement Range

SPECIMEN

Type of Specimen

Biological fluid samples should be collected in the same manner routinely used for any laboratory test. Freshly drawn serum, plasma or properly collected urine (random/timed) are the specimens of choice. Acceptable anticoagulants are listed in the PROCEDURAL NOTES section of this chemistry information sheet. Whole blood is not recommended for use as a sample.

Specimen Storage and Stability

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.

2. Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.

3. It is recommended that urine assays be performed within 2 hours of collection. For timed specimens, the collection container is to be kept in the refrigerator or on ice during the timed period. Urine should be acidified with 10 mL of 6N HCl added to the container before collection begins.

Sample Type	Volume	Sample Stability
Plasma/Serum/Urine	0.5mL	• Separate serum from cells within 2 hours. • Room Temp 8 hours • Refrigerated 48 hours • Frozen 3 months. • Urine: Analyze within 2 hours or keep on ice; no preservative required • Timed Urine: Add 25 mL of 6N HCl to container before collection

Criteria for Unacceptable Specimens

See Specimen Rejection/Cancellation Protocol

Sample Volume

A filled 0.5 mL sample cup is the optimum volume. For optimum primary sample tube volumes in primary tube samples and minimum volumes, refer to the Primary Tube Sample Template for your system.

REAGENTS

Contents

Each kit contains the following items:
Two Electrolyte Buffer Reagent Bottles (2 x 2 L)
Two Electrolyte Reference Reagent Bottles (2 x 2 L)

Volume per Test

Serum or Plasma or Urine

Sample Volume	40uL
ISE Electrolyte Buffer	1.27mL
ISE Electrolyte Reference	3.23(no par of sample dilution)

Reactive Ingredients

Tris	230 mmol\L
Sodium	7mmol|L
Potassium	0.2mmol\L
Chloride	5 mmol|L
Carbon Dioxide	1.5 mmol\L
Calcium	0.1mmol|L

Also non-reactive chemicals necessary for optimal system performance

Reagent Preparation

No preparation is required.

Acceptable Reagent Performance

The acceptability of a reagent is determined by successful calibration and by ensuring that quality control results are within your facility's acceptance criteria.

Reagent Storage and Stability

- ISE Electrolyte Reference reagent stored unopened at room temperature is stable until the expiration date printed on the bottle label. Once opened, the reagent is stable at room temperature for 30 days, unless the expiration date is exceeded.
- ISE Electrolyte Buffer reagent stored unopened at room temperature is stable until the expiration date printed on the bottle label. Once opened, the reagent is stable at room temperature for 30 days, unless the expiration date is exceeded.
- For any electrolyte reagents frozen in transit, completely warm to room temperature and mix thoroughly by gently inverting bottle at least 20 times to redissolve salts into solution.

CALIBRATION

Calibrator Required
SYNCHRON® Systems AQUA CAL 1 and 2

Calibrator Preparation

No preparation is required.

Calibrator Storage and Stability

- Unopened calibrators should be stored at +2°C to +8°C until the expiration date printed on the calibrator bottle. Once opened, the calibrators are stable at room temperature for 30 days.
- Repetitive refrigeration of the aqueous calibrators may facilitate crystal formation. Once removed from refrigerated storage, these calibrators should remain at room temperature.

Calibration Information

1. The system must have a valid calibration in memory before controls or patient samples can be run.

2. Under typical operating conditions the CALC assay must be calibrated every 12 hours or with each new bottle of reagent and also with certain parts replacement or maintenance procedures, as defined in the UniCel DxC 600/800 System *Instructions for Use* (IFU) manual.

3. For detailed calibration instructions, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

4. The system will automatically perform checks on the calibration and produce data at the end of calibration. In the event of a failed calibration, the data will be printed with error codes and the system will alert the operator of the failure. For information on error codes, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

Traceability

For Traceability information refer to the Calibrator instructions for use.

QUALITY CONTROL

See DXC 600/800 Controls document

STEPS

1. If necessary, load the reagent onto the system.

2. After reagent load is completed, calibration is required.

3. Program samples and controls for analysis.

4. After loading samples and controls onto the system, follow the protocols for system operation.

For detailed testing procedures, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual

CALCULATIONS

SYNCHRON® System(s) perform all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

ANTICOAGULANT TEST RESULTS

If plasma is the sample of choice, the following anticoagulants were found to be compatible with this method based on a study of 20 healthy volunteers:

Anticoagulant	Level Tested for In Vitro Interference	Average Plasma-Serum Bias (mg/dL)
Ammonium Heparin	14 Units/mL	No Significant Interference within 0.4 g/dL or 4%
Lithium Heparin	14 Units/mL	No Significant Interference within 0.4 g/dL or 4%
Sodium Heparin	14 Units/mL	No Significant Interference within 0.4 g/dL or 4%

PERFORMANCE CHARACTERISTICS

Reference Range

Sample Type	Range	Critical Low	Critical High
Serum / plasma	8.5-10.5 mg/dL	<6.5 mg/dL	>12.0 mg/dL
Urine 24 hours	0 – 240 mg/24 hrs	N/A	N/A
Urine random	N/A	N/A	N/A

Analytic Range

The SYNCHRON® System(s) method for the determination of this analyte provides the following analytical range:

Sample Type	Conventional Units	S.I. Units
Serum or Plasma	2.0 – 20.0 mg/dL	0.5 – 5.0 mmol/L
Urine	2.0 – 30.0 mg/dL	0.5 – 7.5 mmol/L

Samples with concentrations exceeding the high end of the analytical range should be diluted with saline and reanalyzed.

Reporting results outside of analytical range

Lower limit of range: serum / plasma	2 mg/dL	Result below 2, report as <2mg/dL
Upper limit of range: serum / plasma	20.0 mg/dL	Result >20, dilute, starting at X2, with Nerl H20 and reanalyze
Lower limit of range: urine	2 mg/dL	Result below 2, report as <2mg/dL
Upper limit of range: urine	30.0 mg/dL	Result >30, dilute, starting at X2, with Nerl H20 and reanalyze

Sensitivity

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for CALC determination is 2.0 mg/dL (0.5 mmol/L).

LIMITATIONS

1. If urine samples are cloudy or turbid, it is recommended that they be centrifuged before transfer to a sample cup.

2. For each serum calcium measurement, the sodium concentration is used in the calculation. If sodium is not calibrated or the result is suppressed, a nominal value for sodium is used.

3. For each urine calcium measurement, the sodium and potassium concentrations are used in the calculation of the calcium concentration. If the sodium or potassium chemistries are not calibrated or the sodium or potassium results are suppressed, the calcium value will be suppressed when a urine sample is analyzed.

4. Recovery of aqueous calibrators or linearity standards, may exhibit a recovery bias since the calcium algorithms have been optimized to compute recovery of patient samples.

5. Urine Proficiency Survey samples should not be acidified.

Interferences

The following substances were tested for interference with this methodology:

Substance	Source	Level Tested	Observed Effect
Bilirubin (unconjugated)	Bovine	30 mg/dL	NSI
Hemoglobin	RBC hemolysate	500 mg/dL	NSI
Lipemia	Intralipid[d]	500 mg/dL	NSI
Aluminum	Aluminum Nitrate	20 mg/dL	-0.2 mg/dL
Bromide	Lithium bromide	1 mmol/L	+1.5 mg/dL
Methicillin	NA[e]	10,000 µg/mL	-0.2 mg/dL
Methylbenzethonium	NA	0.2 mg/dL	-0.2 mg/dL

1. Serum or plasma from patients receiving EDTA therapy may yield depressed calcium values.

2. Flint glass containers contain calcium and should not be used to store samples.

3. Lipemic samples with visual turbidity >3+, or with a Lipemia Serum Index >10, should be ultracentrifuged and the analysis performed on the infranate.

4. Refer to References (10,11,12) for other interferences caused by drugs, disease and preanalytical variables.

ADDITIONAL INFORMATION

For more detailed information on UniCel DxC Systems, refer to the appropriate system manual.

REFERENCES

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DOCUMENT APPROVAL **Purpose of Document / Reason for Change:**			
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