Brown Clinic Northridge
511 14th Ave. NE
Watertown, SD

Brown Clinic
506 First Ave. SE
Watertown, SD

BROWN CLINIC
LABORATORY PROCEDURE MANUAL

PROCEDURE: Calcium

PURPOSE:
The CA method used on the Dimension EXL200 Integrated chemistry system is an *in vitro* diagnostic test intended for the quantitative determination of calcium in **serum**, **plasma** and **urine**.

PRINCIPLE:
In blood, approximately 50% of the plasma calcium is free, 40% is protein bound and 10% is complexed. About 80% of protein –bound calcium is associated with albumin, with the remaining 20% associated with globins.[1] Calcium measurements are used in the diagnosis and treatment of parathyroid disease, a variety of bone diseases, chronic renal diseases and tetany (intermittent muscular contractions or spasms).
The calcium method is a modification of the calcium o-cresolphthalein complexone (OCPC) reaction originally reported by Schwartzenbach, et al.[1] Stern and Lewis[2] later adapted this reaction to a colorimetric calcium assay. Connerty and Briggs[3] demonstrated the use of 8-quinolinol to reduce magnesium interference, and Sarkar and Chauhan[4] 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.

$$CA^{++} + OCPC \xrightarrow[\text{pH 9.7}]{} \text{Ca-OCPC complex (absorbs at 577 nm)}$$

$$Mg^{++} + \text{8-quinolinol} \longrightarrow \text{Magnesium quinolinate (nonabsorbing at 577 nm)}$$

INSTRUMENT, REAGENTS & SUPPLIES:

Wells[a]	Form	Ingredient	Concentration[b]
1-6	Liquid	Glycine buffer	0.22 mmol/L

7-8	Liquid	OCPC/	0.39 mmol/L
		8-Quinolinol	6.60 mmol/L

a. Wells are numbered consecutively from the wide end of the cartridge.
b. Nominal final value per test at manufacture.

Precautions:

Used cuvettes contain human body fluids; handle with appropriate care to avoid skin contact and ingestion.

Safety Data Sheets can be found at www.siemens.com/healthcare

For *in vitro* diagnostic use

Reagent Preparation: All reagents are liquid and ready-to-use.

STORAGE & STABILITY:
Store at 2 – 8° C.
Expiration: Refer to carton for expiration date of individual unopened reagent cartridges. Sealed wells on the instrument are stable for 30 days. Once wells 1–6 have been entered by the instrument, they are stable for 24 hours. Once wells 7–8 have been opened by the instrument, they are stable for 10 days.

SPECIMEN REQUIREMENTS:
Normal procedures for collecting and storing serum and plasma may be used for samples to be analyzed by this method. Serum should be separated from red cells and analyzed promptly.

Specimen:

- patient preparation: none required
- specimen type: serum or heparinized plasma
- handling: analyze immediately or refrigerate at 2-8 degrees C

CONTROLS:
At least once daily run solutions at two levels of a quality control material with known concentrations.

For further details, refer to your Dimension® system manual. The result obtained should fall within limits defined by the day-to-day variability of the system as measured in the user's laboratory. If the results fall outside the laboratory's acceptable limits, follow the procedure in the quality control policy.

PROCEDURE:

Materials Provided

The CA Flex® reagent cartridge, Cat. No. DF23A

Materials Required But Not Provided

CHEM I Calibrator, Cat. No. DC18B or D18C

Quality Control Materials

Test Steps

Sampling,[c] reagent delivery, mixing, processing and printing of results are automatically performed by the Dimension® system. For details of this processing, refer to your Dimension® Operators Guide.

c. The sample container (if not a primary tube) must contain sufficient quantity to accommodate the sample volume plus the dead volume; precise container filling is not required.

Test Conditions

- Sample Size: 5 µL
- Reagent 1 Volume: 145 µL
- Reagent 2 Volume: 33 µL
- Diluent Volume: 258 µL
- Test Temperature: 37° C
- Wavelength: 577 and 540 nm
- Type of Measurement: bichromatic endpoint

Backup:
Refer to Brown Clinic Backup Policy

Calibration

Assay Range:	5.0–15.0 mg/dL [1.25–3.75 mmol/L][d]
Calibration Material:	CHEM I Calibrator, Cat. No. DC18B or D18C
Calibration Scheme:	Three levels in triplicate
Units	mg/dL [mmol/L]
	(mg/dL x 0.25) = [mmol/L]
Typical Calibration Levels	7, 10, 14 mg/dL [1.8, 2.5, 3.5 mmol/L]
Calibration Frequency:	Every 3 months for any one lot
A new calibration is required	-For each lot of Flex® reagent cartridges
	-After major maintenance or service, if indicated by quality control results
	-As indicated in laboratory quality control procedures
	-When required by government regulations
Assigned Coefficients:	C_0 1.000
	C_1 0.090

d. Système International d'Unitès [SI units] are in brackets

INTERPRETATION:
The instrument automatically calculates and prints the concentration of calcium in mg/dL [mmol/L] using the calculation scheme illustrated in your Dimension® system manual.

For purposes of diagnosis and treatment, results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

LIMITATIONS:
Analytical measurement Range (AMR): 5.0-15.0mg/dL [1.25-3.75 mmol/L]

This is the range of analyte values that can be measured directly from the specimen without any dilution or pretreatment that is not part of the usual analytical process and is equivalent to the assay range.

Samples with results in excess of 15.0 mg/dL [3.75 mmol/dL] should be repeated on dilution.

Manual Dilution: Serum/Plasma: Make appropriate dilutions with Reagent grade water to obtain result within the assay range. Enter dilution factor.
Urine: Dilute 1 part urine: 1 part Reagent grade water. Enter dilution factor of 2.
Serum/Plasma/Urine: Reassay. Resulting readout is corrected for dilution.

Auto Dilution (A/D): Refer to your Dimension® Operators Guide. (for serum/plasma/urine.)

The instrument reporting system contains error messages to warn the operator of specific malfunctions. Any report slip containing such error messages should be held for follow-up. Refer to your Dimension® Operator's Guide.

Expected Values[11]
Serum: 8.5-10.1 mg/dL [2.12-2.52 mmol/L]

Analytical Specificity

For Known Interfering Substances section refer to package insert.

For Known Non-Interfering Substance refer to package insert.

For Additional Technical Information refer to package insert.

Reference: CA Flex® reagent cartridge insert sheet PN 717123.002 Issue Date 2016-2-26

Origination Date: 9-10-07

Date of Implementation: 11-10-10

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Written By: ____Lori Murray MT(ASCP)/ Samantha Legg_____ Date: __11-30-15__

Approved By: _____Aaron Shives, MD_____ Date: ___10/21/2017_____
Laboratory Director

REVIEW - REVISION SUMMARY DOCUMENTATION

Date	By	Revision Summary
07/10	Lori Murray	New format
8-8-12	Lori Murray	changed instrumentation to EXL200
5/6/15	Natalie Brinkman	Package insert updated 5/6/15
11-24-15	Samantha Legg	updated calibrator material per package insert 2016-07-30
2/21/17	Lori Murray	backup method modified
7/11/17	LMurray	reference range
10/17/17	Heather Hall	updated information to package insert dated 2/26/16, modified backup method