POINTE SCIENTIFIC, INC.
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Calcium Reagent Set

Intended Use
For the quantitative determination of Calcium in serum. For *in vitro* diagnostic use only.

Method History
The various methods developed for the determination of calcium include colorimetric, fluorescent, gravimetric, ion selective, titrimetric, and atomic absorption. The atomic absorption method has been recommended as the reference method but it requires complicated and expensive equipment.[1] A rapid, convenient, simple and inexpensive method is one based on the interaction of calcium with a suitable chromogenic agent. *o*-Cresolphthalein complexone was first used as a complexing reagent for metals in 1954.[2,3] It was adapted to a spectrophotometric method for determining calcium in 1956.[4] Gitelman modified the procedure in 1967 to include 8-hydroxquinoline to remove magnesium interference and used diethylamine for a buffer pH of 12.[5] Baginski, et al modified the procedure further in 1973 to include DMSO to lower the dielectric constant of the working reagent and repress ionization of the *o*-cresolphthalein complexone.[6] The present procedure is based on modifications of the above methods.

Principle
Alkaline Medium
Calcium + *o*-Cresolphthalein Complexone ----------➢

Calcium – Cresolphthalein Complexone Complex (purple color)

Calcium reacts with CPC in an alkaline medium to form a purple color that absorbs at 600nm. The intensity of the color is proportional to the calcium concentration.

Reagents
1. Reagent 1: 2-Amino-2-Methyl-1-Propanol, pH 10.6±0.1
2. Reagent 2: *o*-CPC 0.3mM, surfactant.

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. Reagents R1 and R2 may be irritating to skin. Avoid contact.

Reagent Preparation
Reagents (R1 and R2) are ready to use.

Reagent Storage and Stability
1. All reagents should be stored at room temperature (15-30°C).
2. The R1 and R2 reagents remain stable until expiration date listed on reagent vial labels when stored tightly capped at room temperature.

Reagent Deterioration
The reagents should be clear; turbidity indicates deterioration and the reagents should not be used.

Specimen Collection and Storage
1. Fasting non-hemolyzed serum is the specimen of choice.
2. Anticoagulants other than heparin should not be used.[7]
3. Remove serum from clot as soon as possible since red cells can absorb calcium.[8]
4. Older serum specimens containing visible precipitate should not be used.[9,10]
5. Tubes with cork stoppers should not be used.[11]
6. Serum calcium is stable for twenty-four hours at room temperature, one week refrigerated (2-8°C) and up to five months frozen and protected from evaporation.[12]
7. Specimen collection should be carried out in accordance with NCCLS M29-T2. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

Interferences
1. Substances that contain calcium or complex calcium should not come in contact with the test specimen. Examples: EDTA, citrate, oxalate, fluoride.
2. Specimens from patients receiving bromosulfophthalein (BSP) or EDTA should not be used.[13]
3. For a list of substances affecting the accuracy of calcium values with this procedure see references 14 and 15.

Materials Provided
Calcium reagent set (R1 and R2)

Materials Required but not Provided
1. Controls.
2. Calibrator.
3. Beckman Coulter AU™ analyzer.
4. Application and Instrument manuals.

Procedure (Beckman Coulter AU™400 application)

SPECIFIC TEST PARAMETERS

TEST NUMBER: **#** TEST NAME: **Calcium** ∇ TYPE: **Serum** ∇ OPERATIONAL: **Yes** ∇
SAMPLE VOL.: **6** DIL. VOL.: **0** PRE-DILUTION RATE: **1**
REAGENTS: R1 VOLUME: **150** DIL. VOL.: **0** MIN. OD MAX. OD
R2 VOLUME: **60** DIL. VOL.: **0** L H
REAGENT OD LIMIT:
WAVELENGTH: PRI. **570** ∇ SEC. **660** ∇ FIRST L: **0.000** FIRST H: **2.500**
METHOD: **END**∇ LAST L: **0.000** LAST H: **2.500**
REACTION SLOPE: **+** ∇ DYNAMIC RANGE:
MEASURING POINT 1: FIRST: **0** LAST: **27** L: **#** H: **#**
MEASURING POINT 2: FIRST: **0** LAST: **10** CORRELATION FACTOR:
LINEARITY: % A: **1.000** B: **0.000**
NO LAG TIME: ∇ ON BOARD STABILITY PERIOD: **#**

SPECIFIC TEST PARAMETERS

VALUE FLAG: **#** ∇ LEVEL L: **#** LEVEL H: **#**

NORMAL RANGES:	SEX	AGE L YEAR	AGE L MONTH	AGE H YEAR	AGE H MONTH	L	H
○ 1.	# ∇	#	#	#	#	#	#
○ 2.	# ∇	#	#	#	#	#	#
○ 3.	# ∇	#	#	#	#	#	#
○ 4.	# ∇	#	#	#	#	#	#
○ 5.	# ∇	#	#	#	#	#	#
○ 6.	# ∇	#	#	#	#	#	#
7. NONE SELECTED						#	#
8. OUT OF RANGE		L		H		#	#

PANIC VALUE: **#** **#** UNIT: **mg/dl** DECIMAL PLACES: **1**

Calcium
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CALIBRATION SPECIFIC PARAMETERS

CAL TYPE: **AB** ∇ FORMULA: **Y=AX+B** ∇ COUNTS: **2** PROCESS: **CONC.** ∇

	CAL. NO.	OD	CONC.	FAC/OD-L	FAC/OD-H
POINT 1.	#		#	**-9999999**	**9999999**
POINT 2.					
POINT 3.					
POINT 4.					
POINT 5.					
POINT 6.					
POINT 7.					

1-POINT CAL. POINT: ○ WITH CONC-0

MB TYPE FACTOR: CALIBRATION STABILITY PERIOD: **#**

#: User-Defined

The above reagent parameters are intended to serve as a guide for use with Pointe Scientific, Inc. reagent. The parameters are based on data generated by Pointe Scientific, Inc. Please note: These parameters should be used in conjunction with your laboratory Quality Control Program for validation.

NOTE: For other instrument specific applications please contact Pointe Scientific, Inc. Technical Service Department at 1-800-445-9853

Procedure Notes
Samples with values above 20 mg/dl should be diluted 1:1 with saline, re-assayed and the result multiplied by two.

Calibration.
Follow instrument application instructions for calibration. Refer to instrument manual instructions for calibration procedures and frequency. It is recommended that each laboratory determine its own frequency of calibration.

Quality Control
The validity of the reaction should be monitored by use of control sera with known normal and abnormal calcium values. These controls should be run at least with every working shift in which calcium assays are performed. It is recommended that each laboratory establish its own frequency of control determination.

Calculation
$$\frac{\text{Absorbance of Unknown}}{\text{Absorbance of Standard}} \times \text{Conc. of Std.} = \text{Calcium (mg/dl)}$$

Example: If the Absorbance of Unknown = 0.47, Absorbance of Standard = 0.50, Concentration of Standard = 10 mg/dl, then:

$$\frac{0.47}{0.50} \times 10 = 9.4 \text{ mg/dl}$$

Note: To convert mg/dl to mEq/L, divide mg/dl by two.

Expected Values[16]
8.5 – 10.5 mg/dl
Children under 12 usually have higher normal values that fall with aging.
It is strongly recommended that each laboratory establish its own normal range.

Performance
1. Linearity: 20.0 mg/dl
2. Analytical Measuring Range: 0.1 – 20.0 mg/dl.
3. Comparison: A comparison study performed between the Beckman Coulter AU™400 and Roche Hitachi 717 using this method resulted in a correlation coefficient of r = 0.992 with a linear regression equation of y= 1.047x - 0.98. (n = 38, range 7.9 – 16.3 mg/dl)
4. Precision:
   Within - day precision study was performed using three levels of material.
   Between - day precision study was performed using two levels of control material assayed over a 20 day period with 2 runs per day and 2 replicates per run.

Within Day (N=20)			Day to Day		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
4.7	0.09	1.9	9.5	0.22	2.3
7.8	0.09	1.2	13.1	0.40	3.1
10.6	0.19	1.8			

Precision and Linearity studies were performed following modifications of CLSI Protocols EP5 and EP6[17] using a Beckman Coulter AU™400 analyzer

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Rev. 01/13 P803-OC902-01