SYNCHRON® System(s)
Chemistry Information Sheet

CALC
Calcium
REF & 467915 467935
REF & A28945 A28937

For *In Vitro* Diagnostic Use

ANNUAL REVIEW

Reviewed by:	Date	Reviewed by:	Date

PRINCIPLE

INTENDED USE

ISE Electrolyte Buffer reagent and ISE Electrolyte Reference reagent, when used in conjunction with SYNCHRON LX® System(s), 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.

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.[1,2]

CHEMICAL REACTION SCHEME

The calcium ion selective electrode consists of a calcium ionophore membrane cast on a solid support. When sample buffer mixture contacts the electrode, changes in electrode potential occur as calcium ions react with the ionophore. These changes in potential are referenced to the sodium reference electrode. The "referenced potential" follows the Nernst equation and allows the calculation of calcium concentration:

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

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For more accurate measurement, the reference reagent containing calcium ions is introduced into the flow cell following the sample cycle, and the same reaction scheme takes place. The differential potential (voltage) between sample and reference reagent cycles is used for the calculation.

Under ideal conditions, the electrode imparts a selectivity of 1000:1 over sodium and potassium and is insensitive to hydrogen ions in solution buffered from pH 4 to 10.

SPECIMEN

TYPE OF SPECIMEN

Biological fluid samples should be collected in the same manner routinely used for any laboratory test.[3] 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.[4]
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.[4]
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.[5] Urine should be acidified with 10 mL of 6N HCl added to the container before collection begins.[6]

ADDITIONAL SPECIMEN STORAGE AND STABILITY CONDITIONS AS DESIGNATED BY THIS LABORATORY:

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.

CRITERIA FOR UNACCEPTABLE SPECIMENS

Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens.

CRITERIA FOR SAMPLE REJECTION AS DESIGNATED BY THIS LABORATORY:

PATIENT PREPARATION

SPECIAL INSTRUCTIONS FOR PATIENT PREPARATION AS DESIGNATED BY THIS LABORATORY:

SPECIMEN HANDLING

SPECIAL INSTRUCTIONS FOR SPECIMEN HANDLING AS DESIGNATED BY THIS LABORATORY:

REAGENTS

CONTENTS

Each kit contains the following items:
ISE ELECTROLYTE BUFFER REAGENT:
Two Electrolyte Buffer Reagent Bottles (2 x 2 L)
ISE ELECTROLYTE REFERENCE REAGENT:
Two Electrolyte Reference Reagent Bottles (2 x 2 L)

VOLUMES PER TEST

Sample Volume	40 µL
Reagent Volume	
ISE Electrolyte Buffer	1.27 mL
ISE Electrolyte Reference	3.23 mL
	(not part of sample dilution)

REACTIVE INGREDIENTS

REAGENT CONSTITUENTS

ISE ELECTROLYTE BUFFER REAGENT:	
Tris	230 mmol/L
ISE ELECTROLYTE REFERENCE REAGENT:	
Sodium	7 mmol/L
Potassium	0.2 mmol/L
Chloride	5 mmol/L
Carbon Dioxide	1.5 mmol/L
Calcium	0.1 mmol/L

Also non-reactive chemicals necessary for optimal system performance.

Avoid skin contact with reagent. Use water to wash reagent from skin.

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

SYNCHRON® Systems AQUA CAL 1 and 2
At least two levels of control material

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

1. 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.
2. 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.
3. 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.

ISE ELECTROLYTE BUFFER REAGENT AND ISE ELECTROLYTE REFERENCE REAGENT STORAGE LOCATION:

CALIBRATION

CALIBRATOR REQUIRED

SYNCHRON® Systems AQUA CAL 1 and 2

CALIBRATOR PREPARATION

No preparation is required.

CALIBRATOR STORAGE AND STABILITY

1. 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.
2. Repetitive refrigeration of the aqueous calibrators may facilitate crystal formation. Once removed from refrigerated storage, these calibrators should remain at room temperature.

CALIBRATOR STORAGE LOCATION:

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 24 hours or with each new bottle of reagent and also with certain parts replacement or maintenance procedures, as defined in the SYNCHRON LX *Maintenance Manual and Instrument Log*, or the UniCel DxC 600/800 System *Instructions for Use* (IFU) manual.
3. For detailed calibration instructions, refer to the SYNCHRON LX *Operations Manual*, or 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 SYNCHRON LX *Diagnostics and Troubleshooting Manual*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

TRACEABILITY

For Traceability information refer to the Calibrator instructions for use.

QUALITY CONTROL

At least two levels of control material should be analyzed daily. In addition, these controls should be run with each new calibration, with each new bottle of reagent, and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws.

The following controls should be prepared and used in accordance with the package inserts. Discrepant quality control results should be evaluated by your facility.

NOTICE
Do not use controls containing diethylamine HCl.

TABLE 1 QUALITY CONTROL MATERIAL

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

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 operations.

CALCULATIONS

The SYNCHRON® System(s) performs 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.

REPORTING RESULTS

Equivalency between the SYNCHRON LX and UniCel DxC 600/800 Systems has been established. Chemistry results between these systems are in agreement and data from representative systems may be shown.

REFERENCE INTERVALS

Each laboratory should establish its own reference intervals based upon its patient population. The following reference intervals were taken from literature and a study performed on SYNCHRON Systems.[6]

TABLE 2 REFERENCE INTERVALS

RANGE	SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
Literature	Serum or Plasma	8.6 – 10.0 mg/dL	2.15 – 2.50 mmol/L

RANGE	SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
	Urine (timed)	100 – 300 mg/24 hrs	2.50 – 7.50 mmol/24 hrs
SYNCHRON	Serum or Plasma	8.9 – 10.3 mg/dL	2.23 – 2.58 mmol/L

RANGE	SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
Laboratory			

Refer to References (7,8,9) for guidelines on establishing laboratory-specific reference intervals.

ADDITIONAL REPORTING INFORMATION AS DESIGNATED BY THIS LABORATORY:

PROCEDURAL NOTES

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:

TABLE 3 COMPATIBLE ANTICOAGULANTS

ANTICOAGULANT	LEVEL TESTED FOR IN VITRO INTERFERENCE	AVERAGE PLASMA-SERUM BIAS (mg/dL)
Ammonium Heparin	14 Units/mL	NSI[a]
Lithium Heparin	14 Units/mL	NSI
Sodium Heparin	14 Units/mL	NSI

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

1. The following substances were tested for interference with this methodology:

TABLE 4 INTERFERENCES

SUBSTANCE	SOURCE	LEVEL TESTED	OBSERVED EFFECT[b]
Bilirubin (unconjugated)	Bovine	30 mg/dL	NSI[c]
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

2. Serum or plasma from patients receiving EDTA therapy may yield depressed calcium values.
3. Flint glass containers contain calcium and should not be used to store samples.
4. Lipemic samples with visual turbidity >3+, or with a Lipemia Serum Index >10, should be ultracentrifuged and the analysis performed on the infranate.
5. Refer to References (10,11,12) for other interferences caused by drugs, disease and preanalytical variables.

PERFORMANCE CHARACTERISTICS

Analytic Range

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

TABLE 5 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 deionized water and reanalyzed.

REPORTABLE RANGE (as determined on site):

TABLE 6 REPORTABLE RANGE

SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS

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).

EQUIVALENCY

Equivalency was assessed by Deming regression analysis of patient samples to accepted clinical methods.

Serum or Plasma (in the range of 2.19 to 20.29 mg/dL):

Y (SYNCHRON LX Systems)	= 0.981X + 0.17
N	= 92
MEAN (SYNCHRON LX Systems)	= 9.50
MEAN (SYNCHRON CX Systems)	= 9.51
CORRELATION COEFFICIENT (r)	= 0.996

Urine (in the range of 1.34 to 31.00 mg/dL):

Y (SYNCHRON LX Systems)	= 0.982X + 0.60
N	= 97
MEAN (SYNCHRON LX Systems)	= 13.18
MEAN (SYNCHRON CX Systems)	= 12.80
CORRELATION COEFFICIENT (r)	= 0.998

Serum or Plasma (in the range of 2.0 to 19.3 mg/dL):

Y (UniCel DxC Systems)	= 1.007X - 0.03
N	= 184
MEAN (UniCel DxC Systems)	= 9.6
MEAN (SYNCHRON LX Systems)	= 9.6
Correlation Coefficient (r)	= 0.999

Urine (in the range of 2.1 to 30 mg/dL):

Y (UniCel DxC Systems)	= 0.983X + 0.09
N	= 103
MEAN (UniCel DxC Systems)	= 9.9
MEAN (SYNCHRON LX Systems)	= 10.0
Correlation Coefficient (r)	= 0.999

Refer to References (13) for guidelines on performing equivalency testing.

PRECISION

A properly operating SYNCHRON® System(s) should exhibit imprecision values less than or equal to the maximum performance limits in the table below. Maximum performance limits were derived by an examination of the imprecision of various methods, proficiency test summaries, and literature sources.

TABLE 7 MAXIMUM PERFORMANCE LIMITS

TYPE OF PRECISION	SAMPLE TYPE	1 SD		CHANGEOVER VALUE[f]		% CV
		mg/dL	mmol/L	mg/dL	mmol/L	
Within-run	Serum/Plasma	0.2	0.05	10.0	2.5	2.0
	Urine	0.3	0.08	10.0	2.5	3.0

TYPE OF PRECISION	SAMPLE TYPE	1 SD		CHANGEOVER VALUE[f]		% CV
		mg/dL	mmol/L	mg/dL	mmol/L	
Total	Serum/Plasma	0.3	0.08	10.0	2.5	3.0
	Urine	0.45	0.11	10.0	2.5	4.5

Comparative performance data for the SYNCHRON LX System evaluated using the NCCLS Approved Guideline EP5-A appears in the table below.[14] Each laboratory should characterize their own instrument performance for comparison purposes.

TABLE 8 NCCLS EP5-A PRECISION ESTIMATE METHOD

TYPE OF IMPRECISION	SAMPLE TYPE		No. Systems	No. Data Points[g]	Test Mean Value (mg/dL)	EP5-A Calculated Point Estimates	
						SD	%CV
Within-run	Serum	Control 1	1	80	7.69	0.16	2.0
	Serum	Control 2	1	80	13.45	0.11	0.9
	Urine	Control 1	1	80	8.59	0.18	2.1
	Urine	Control 2	1	80	11.05	0.12	1.1
Total	Serum	Control 1	1	80	7.69	0.17	2.2
	Serum	Control 2	1	80	13.45	0.13	1.0
	Urine	Control 1	1	80	8.59	0.20	2.3
	Urine	Control 2	1	80	11.05	0.16	1.5

NOTICE

These degrees of precision and equivalency were obtained in typical testing procedures on a SYNCHRON LX® System and are not intended to represent the performance specifications for this reagent.

ADDITIONAL INFORMATION

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

SHIPPING DAMAGE

If damaged product is received, notify your Beckman Coulter Clinical Support Center.

REFERENCES

1. Anker, P., Wieland, E., Ammand, D., Dohner, R. E., Asper, R., Simon, W., "Neutral Carrier Based Ion-Selective Electrode for the Determination of Total Calcium in Blood Serum", *Anal. Chem*, 53:1970, 4 (1981).

2. Perrin, D. D., Dempsey, B., *Buffers for pH and Metal Ion Control*, Chapman and Hall, London (1984).

3. Tietz, N. W., "Specimen Collection and Processing; Sources of Biological Variation", *Textbook of Clinical Chemistry*, 2nd Edition, W. B. Saunders, Philadelphia, PA (1994).

4. National Committee for Clinical Laboratory Standards, *Procedures for the Handling and Processing of Blood Specimens*, Approved Guideline, NCCLS publication H18-A, Villanova, PA (1990).

5. National Committee for Clinical Laboratory Standards, *Routine Urinalysis and Collection, Transportation and Preservation of Urine Specimens*, Tentative Guideline, NCCLS publication GP16-T, Villanova, PA (1992).

6. Tietz, N. W., *Clinical Guide to Laboratory Tests*, 3rd Edition, W. B. Saunders, Philadelphia, PA (1995).

7. National Committee for Clinical Laboratory Standards, *How to Define, Determine, and Utilize Reference Intervals in the Clinical Laboratory*, Approved Guideline, NCCLS publication C28-A, Villanova, PA (1995).

8. Tietz, N. W., ed., *Fundamentals of Clinical Chemistry*, 3rd Edition, W. B. Saunders, Philadelphia, PA (1987).

9. Henry, J. B., *Clinical Diagnosis and Management by Laboratory Methods*, 18th Edition, W. B. Saunders Company, Philadelphia, PA (1991).

10. Young, D. S., *Effects of Drugs on Clinical Laboratory Tests*, 4th Edition, AACC Press, Washington, D. C. (1995).

11. Friedman, R. B., Young, D. S.,*Effects of Disease on Clinical Laboratory Tests*, 3rd Edition, AACC Press, Washington, D.C. (1997).

12. Young, D. S., *Effects of Preanalytical Variables on Clinical Laboratory Tests*, 2nd Edition, AACC Press, Washington, D. C. (1997).

13. National Committee for Clinical Laboratory Standards, *Method Comparison and Bias Estimation Using Patient Samples*, Approved Guideline, NCCLS publication EP9-A, Villanova, PA (1995).

14. National Committee for Clinical Laboratory Standards, *Evaluation of Precision Performance of Clinical Chemistry Devices*, Approved Guideline, Vol. 19, No. 2, NCCLS publication EP5-A, Villanova, PA (1999).

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ENDNOTES

a NSI = No Significant Interference (within ±0.4 mg/dL or 4%).

b Plus (+) or minus (-) signs in this column signify positive or negative interference.

c NSI = No Significant Interference (within ±0.4 mg/dL or 4%).

d Intralipid is a registered trademark of KabiVitrum, Inc., Clayton, NC 27250.

e NA = Not applicable.

f When the mean of the test precision data is less than or equal to the changeover value, compare the test SD to the SD guideline given above to determine the acceptability of the precision testing. When the mean of the test precision data is greater than the changeover value, compare the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.

g The point estimate is based on the pooled data from one system, run for twenty days, two runs per day, two observations per run on an instrument operated and maintained according to the manufacturer's instructions.