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New Method for Calcium on the ADVIA Analyzer is Free from Interference of Gadolinium-Type Contrast Agents

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Recently, Siemens Diagnostics released a new calcium assay (CA_2) based on complex formation of calcium with Arsenazo III dye for use on the three automated, random access ADVIA Chemistry analyzers (1650, 2400, and 1200). We evaluated this method for analytical performance as well as potential interference from gadolinium-containing magnetic contrast agents. With Siemens Chemistry serum and urine controls, 2-levels each, the imprecision for the new method was ($n = 40$ each): within-run and total CV of <2.2 and $<3.8\%$, respectively, over all three platforms. The analytical range/linearity of the method (all three systems) was 1–16 mg/dl (serum or plasma) and 1–32 mg/dl (urine). The new method on all three platforms correlated well with a reference (Inductively Coupled Plasma Atomic Emission Spectroscopy) method ($n = 61$, range 4.03–10.30 mg/dl). The ADVIA 1650 CA_2 method also correlated well with the Roche Modular system® Calcium method. The new method showed $<10\%$ interference with unconjugated or conjugated bilirubin (50 mg/dl), hemoglobin (1,000 mg/dl), lipids (1,000 mg/dl), and two magnetic resonance contrast agents containing Gadolinium (OptiMARK® 1 mmol/l and Omniscan 1.5 mmol/l). On the contrary, the Roche Calcium method showed significant negative interference with gadolinium-containing contrast agents. We conclude that the ADVIA Ca_2 method can measure serum, plasma, or urine calcium concentrations accurately and is also free from interferences of gadolinium-containing agents. J. Clin. Lab. Anal. 23:399–403, 2009. © 2009 Wiley-Liss, Inc.

Key words: calcium; ADVIA; interference; gadolinium

INTRODUCTION

Serum, plasma, or urine calcium measurements are used in the diagnosis and treatment of parathyroid disease, various bone diseases, chronic renal disease, tetany, and malignancies. The two most common causes of hypercalcemia are hyperthyroidism and neoplastic disease (1). Malignancy-associated hypercalcemia is also common among hospitalized patients. Such complications develop in almost 10% of patients with advanced cancer representing the most frequent cause of death in several patients with cancer. Parathyroid hormone-related protein has a strong homology with parathyroid hormone and acts as a hormonal mediator causing hypercalcemia (2,3). Milk-Alkali syndrome consists of hypercalcemia, various degrees of renal failure, and metabolic alkalosis due to ingestion of large amounts of calcium. This syndrome was first identified after initiation of medical treatment of peptic ulcer with milk and alkali at the beginning of 20th Century. However, this problem has recently reemerged due to wide availability and use of calcium carbonate for the prevention of osteoporosis (4). Calcium is measured in serum or urine using various techniques including atomic absorption spectrometry, flame photometry as well as spectrophotometric methods based on the formation of complex between calcium and various dyes (5). An automated calcium method based on

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complex formation between calcium and *O*-cresolphthalein is a popular method adopted on various automated platforms for routine analysis of calcium in clinical laboratories (6,7). Although less common, Arsenazo III can also be used for serum calcium measurement using complex formation between calcium and this dye (8). However, many colorimetric calcium methods show negative interference with gadolinium-containing magnetic resonance contrast agents (9,10).

Gadolinium (Gd) is a silver white rare-earth metal (atomic weight: 157.2), which has a valency of 3+. Because of high paramagnetism and exceptionally long electronic relaxation time, contrast reagents containing gadolinium complexes are routinely used intravenously in magnetic resonance imaging (MRI) examinations. Gadolinium is clear and appears like water, but after injecting into the vain, gadolinium accumulates in the abnormal tissue and causes abnormal areas to become very bright on the MRI. This enhancement of MRI helps easier identification of the abnormal tissue such as tumor. To prevent gadolinium's interaction with calcium-dependent biological systems, ion-channels, and precipitation above pH 6, gadolinium is administered chelated by polyamino polycarboxylic ligands. These chelates have shown relatively little toxicity or allergic reaction in vivo, but interfere in colorimetric dye-binding assays of divalent metal ions, especially calcium as mentioned above. The interference of gadolinium-containing contrast agents in both *O*-cresolphthalein (9,11–15) and Arsenazo III (16) based calcium methods have been described. The mechanism of the interference is possibly from gadolinium 3+ ion binding to the dye, and thus causing a negative interference in the calcium assay. Since calcium measurements are critical components in the diagnosis of various diseases, such interference is undesirable in clinical calcium assays. Recently, Siemens released an improved calcium assay (Ca_2) for use on the three automated, random access ADVIA Chemistry analyzers: the ADVIA® 1650, ADVIA® 2400, and ADVIA® 1200 systems. We evaluated this new method for analytical performance as well as interference from gadolinium-contrasting agents, gadoversetamide (OptiMARK® from Mallinckrodt Medical), and gadodiamide (Omniscan). Here we report our findings.

MATERIALS AND METHODS

The ADVIA Chemistry Ca_2 method photometrically measures calcium concentrations in serum, plasma, or urine samples, by complex formation with the Arsenazo III dye. In this method, as run on the ADVIA 1650, ADVIA 2400, or ADVIA 1200 system, sample is added to the reagent (R1), which contains the dye in acetate buffer (pH 5.9). After five minutes of incubation at 37°C, the absorbance is measured at 658 nm (endpoint). All three methods use two-point calibration: blank (water) and a single calibrator (Siemens Chemistry Calibrator). Of the three ADVIA Chemistry systems used in our studies, the ADVIA 1650 and ADVIA 2400 use sample pre-dilution: 5× for serum and 10× for urine. The ADVIA 1200 system, on the other hand, uses undiluted neat sample (serum) and 2× prediluted sample (urine). The dilution is done automatically by the system using the system diluent (saline). From the diluted sample multiple assays can be run. All three ADVIA Chemistry systems use the same reagent packs, calibrators, and controls. A single calibrator value and control ranges are used across all three platforms. The method has a minimum of 30 days on-system and calibration stability (on all three systems).

The reference method for calcium was done by using the Inductively Coupled Plasma Atomic Emission Spectroscopy and calibrators traceable to NIST (from National Bureau of Standards) Standard Reference Materials. The Roche calcium method was run on the Roche MODULAR® system using manufacturer recommended materials and protocol.

To study interference of bilirubin, hemoglobin, and lipids in the ADVIA Ca_2 assay, aliquots of serum or urine specimens containing known amounts of calcium were further supplemented with bilirubin (up to 50 mg/dl), hemoglobin (up to 1,000 mg/dl), and triglycerides (up to 1,000 mg/dl).

For interference study using gadolinium-containing contrast agents; Omniscan® (gadolinium diamide) was obtained from Amersham Health (Buckinghamshire, UK) and OptiMARK® (gadolinium versetamide) was obtained from Covidien, Inc. (formerly, Mallinckrodt Medical, Hamilton, Bermuda). Both were obtained as aqueous (stock) solutions of known gadolinium concentrations. To investigate the interference of gadolinium contrast agents with this new method and Roche method, we prepared two different serum pools. The total calcium concentration of each pool was measured using both methods. Then, aliquots of each pool were supplemented with the stock gadolinium solutions up to 1,500 mg/dl of Gd; other aliquots of the serum pools were spiked with same volumes of saline to form the control (no Gd) samples. These pair of solutions for each Gd-agent were mixed in different ratios to obtain the various amounts of either gadodiamide or gadoversetamide. Calcium concentrations were measured in each sample again using both methods. Each measurement was performed in triplicate and the mean value was used for comparison of both methods regarding the interference from gadolinium contrast agents.

RESULTS

Precision

Precision studies of the ADVIA Chemistry Ca_2 method were done using a set of bi-level commercial serum (Siemens Assayed Chemistry 1 and 2) and urine (ADVIA Chemistry Urine: Normal and Abnormal) Controls available from the Siemens Diagnostics. Each sample was assayed two times per run, two runs per day, for 10 days (total number of replicates for each sample being 40). Imprecision estimates were computed according to NCCLS document EP5-A, "Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline" (Table 1).

TABLE 1. Imprecision of ADVIA Chemistry Calcium (Ca_2) Method Using Serum and Urine Controls ($n = 40$ Each)

System	Control	MEAN (mg/dl)	Within run SD	Within run CV	Total SD	Total CV
1650	Chemistry 1	5.82	0.08	1.4	0.12	2.1
	Chemistry 2	9.81	0.12	1.3	0.13	1.3
	Urine normal	5.95	0.07	1.2	0.18	3.0
	Urine abnormal	11.80	0.11	0.9	0.18	1.6
2400	Chemistry 1	5.95	0.12	2.0	0.13	2.2
	Chemistry 2	9.89	0.19	1.9	0.19	2.0
	Urine normal	6.15	0.14	2.2	0.23	3.8
	Urine abnormal	11.79	0.13	1.1	0.26	2.2
1200	Chemistry 1	5.97	0.11	1.8	0.12	2.1
	Chemistry 2	9.93	0.09	0.9	0.24	2.4
	Urine normal	6.01	0.13	2.1	0.23	3.8
	Urine abnormal	11.89	0.25	2.1	0.29	2.4

Linearity

The linearity of the ADVIA Chemistry Ca_2 method was determined for serum and urine by nine-level dilution of a high serum or urine pool with saline. The observed ADVIA Chemistry (ADVIA 1650, ADVIA 2400, and ADVIA 1200) results with all three methods were compared with expected results (Table 2). The data support the claimed analytical ranges of the methods: Serum, 0–16 mg/dl; and Urine, 0–32 mg/dl. The ranges of the method can further be extended by auto-rerun conditions, where samples with calcium concentration >15 mg/dl for serum (or 30 mg/dl for urine) will be automatically rerun after an extra $2\times$ dilution for serum and $5\times$ dilution for urine samples. The upper range of the method can thus be extended on all three systems up to at least 32 mg/dl (serum) and 150 mg/dl (urine).

Method Comparison

In a first study, 61 serum samples were analyzed by the ADVIA Chemistry Ca_2 method (run on the ADVIA 1650, ADVIA 2400, and ADVIA 1200 systems) and by the reference calcium method (Inductively Coupled Plasma Atomic Emission Spectroscopy). The correlation data are presented in Table 3 and Figure 1. In a second study, 50 serum samples were compared on the ADVIA 1650 Calcium_2 and Roche MODULAR Calcium methods (Table 3 and Fig. 2). Good agreement was observed in each method comparison.

TABLE 2. Linearity of ADVIA Chemistry Calcium (Ca_2) Method on ADVIA 1650, ADVIA 2400 and ADVIA 1200 Platforms for both Serum and Urine Calcium Measurement

Method	Analyzer	Range tested (mg/dl)	Regression equation: y (observed) vs. x (expected) Slope	Intercept	r
Serum	1650	0–16.92	0.990	−0.10	0.999
	2400	0–16.92	1.013	0.59	0.999
	1200	0–16.92	0.978	−0.04	0.999
Urine	1650	0–32.88	1.006	0.11	0.999
	2400	0–32.88	0.995	−0.17	0.999
	1200	0–32.88	1.013	0.07	0.999

TABLE 3. Correlation of ADVIA Chemistry Calcium (Ca_2) Method Using ADVIA 1650, ADVIA 2400 and ADVIA 1200 Platform with Inductively Coupled Plasma Atomic Emission Spectroscopy (Reference Calcium) and ROCHE Calcium Method on the MODULAR Analyzer

Method (x)	Method (y)	Regression equation	Sy/x	r	n
Reference calcium[a]	ADVIA 1650 Ca_2	$y = 1.02x - 0.13$	0.20	0.995	61
Reference calcium[a]	ADVIA 2400 Ca_2	$y = 1.02x - 0.15$	0.22	0.994	61
Reference calcium[a]	ADVIA 1200 Ca_2	$y = 0.99x + 0.06$	0.26	0.990	61
Roche MODULAR	ADVIA 1650 Ca_2	$y = 0.95x + 0.17$	0.40	0.980	50

[a]Reference Calcium Method: Inductively Coupled Plasma Atomic Emission Spectroscopy.

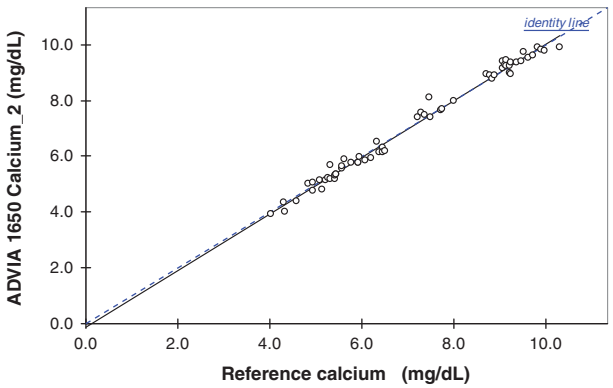

Fig. 1. Regression equation showing correlation between ADVIA 1650 Ca_2 method with the reference method (Inductively Coupled Plasma Atomic Emission Spectroscopy).

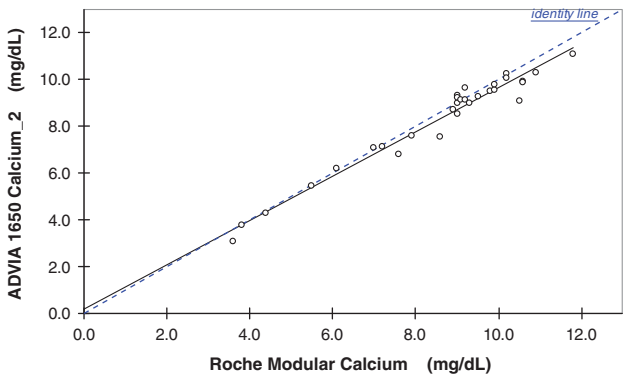

Fig. 2. Regression equation showing correlation between ADVIA 1550 Ca_2 method with the Roche calcium method on the modular analyzer.

Interference

The new method showed <10% interference with unconjugated or conjugated bilirubin (50 mg/dl), hemoglobin (1,000 mg/dl), and lipids (1,000 mg/dl). The lipid study was performed using Intralipid (20% soybean oil containing mainly triglycerides with predominately unsaturated fatty acids) and 1.2% egg yolk phospholipid.

Interference Study with Contrast Agents

When aliquots of serum pools were supplemented with either gadodiamide or gadoversetamide, we observed significant negative interference using the Roche calcium method, but the new calcium method on the ADVIA analyzer showed no significant interference with either contrast agents. For example, when an aliquot of serum pool 1 containing 6.1 mg/dl of calcium (Roche method) was supplemented with gadodiamide to achieve a final concentration of the contrast agent of only 0.5 mmol/l, the observed mean calcium value was reduced to 3.8 mg/dl, a change that is both clinically and

TABLE 4. Effect of Gadolinium Containing Contrast Agents on Serum Calcium Measurement by ADVIA and Roche Calcium Method

			Observed calcium (mg/dl)[a]						
	Serum pool	Gadolinium concentration (mmol/l)	ADVIA 1650	Recovery (%)	ADVIA 2400	Recovery (%)	ADVIA 1200	Recovery (%)	Roche	Recovery (%)
Omniscan (Gadodiamide)	1	0	5.91	100.0	6.22	100.0	6.06	100.0	6.1	100.0
		0.5	6.04	102.1	6.37	102.4	6.27	103.4	3.8	62.3
		1	6.28	106.3	6.55	105.3	6.43	106.0	2.0	32.8
		1.5	6.37	107.7	6.69	107.6	6.52	107.5	0.8	13.1
	2	0	11.64	100.0	11.99	100.0	11.89	100.0	11.8	100.0
		0.5	12.16	104.5	12.09	100.8	12.03	101.2	7.1	60.2
		1	11.96	102.8	12.35	103.0	12.24	102.9	4.6	39.0
		1.5	12.61	108.3	12.46	103.9	12.37	104.1	1.5	12.7
OptiMARK (Gadoversetamide)	1	0	5.90	100.0	6.17	100.0	6.04	100.0	6.1	100.0
		0.5	6.17	104.5	6.41	103.9	6.27	103.9	3.8	62.3
		1	6.36	107.7	6.64	107.6	6.50	107.7	2.0	32.8
		1.5	6.52	110.5	6.87	111.4	6.68	110.7	1.1	18.0
	2	0	12.16	100.0	11.86	100.0	11.87	100.0	12.1	100.0
		0.5	12.45	102.4	12.17	102.6	12.10	101.9	8.8	72.7
		1	12.86	105.8	12.39	104.5	12.40	104.5	6.4	52.9
		1.5	12.59	103.6	12.61	106.4	12.54	105.7	5.2	43.0

[a]Each measurement performed in triplicate, the value presented here represents the mean value.

statistically significant. On the contrary, using the new calcium method on the ADVIA 1200 analyzer, the calcium value changed from 6.1dl to 6.3 mg/dl. This change is nonsignificant both statistically and clinically. At the highest gadolinium concentration tested (1.5 mmol/L), the maximum interference with the new Ca_2 method (on any ADVIA Chemistry system) vs. the Roche Calcium method was 8.3 vs. −87.3% (gadodiamide) and 11.4 vs. −82.0% (gadoversetamide), respectively (Table 4).

DISCUSSION

There are three dye-binding assays commonly used in determining serum or plasma calcium concentrations using automated analyzers. These dyes include *O*-cresolphthalein, methylthymol blue, and Arsenazo III. This new Ca_2 method for application on the ADVIA platform based on the binding of calcium with Arsenazo III forming a colored complex that can be measured photometrically correlates well with the reference Inductively Coupled Plasma Atomic Emission Spectroscopy as well as with widely used colorimetric calcium method based on dye binding with *O*-cresolphthalein (Roche Calcium Method).

It has been known that only gadodiamide and gadoversetamide out of five currently available contrast agents are less stable and interfere with colorimetric calcium assay (12). Therefore, we used these two agents only for our interference study. In addition, these agents are more commonly used because of their short half-life (less than 2 hr). The concentrations of both agents used in our study is expected in vivo concentrations at various time points after administration of these contrast agents also similar to concentrations used by other investigators to report interference of these agents with colorimetric calcium tests. Both the manufacturers of gadolinium contrast agents (OMNISCAN) and serum calcium measurement Kits (Roche Diagnostics, Indianapolis, IN) have issued customer bulletins alerting falsely low calcium results after exposure to gadolinium-containing contrast agents. The possible mechanism of interference is dissociation of gadolinium 3+ ions from the gadodiamide followed by binding of this ion with *O*-cresolphthalein, thus underestimating true calcium value.

Fortunately, the new calcium method on the ADVIA analyzers is free from such interferences probably because Arsenazo III has more affinity for calcium than gadolinium 3+ ion. We conclude that the new calcium method on ADVIA analyzers can be used routinely for serum calcium measurements including patients receiving gadolinium-based contrast agents such as gadodiamide and gadoversetamide.

REFERENCES

1. Pecherstorfer M, Brenner K, Zojer N. Current management stratigies for hypercalcemia. Tret Endocrinol 2003;2:273–292.
2. Lumachi F, Brunello A, Roma A, Basso U. Cancer induced hypercalcemia. Anticancer Res 2009;29:1551–1555.
3. Lumachi F, Brunello A, Roma A, Basso U. Medical management of malignancy-associted hypercalcemia. Curr Med Chem 2008;15:415–421.
4. Medarov BI. Milk-alkali syndrome. Mayo Clinica Proc 2009;84:261–267.
5. Robertson WG, Marshall RW. CRC Crit. Rev Clin Lab Sci 1979;11:271–304.
6. Zak B, Epstien E, Baginski ES. Review of calcium methodologies. Ann Clin Lab Sci 1975;5:195–215.
7. Hartmann AE, Lewis LR. Evaluation of the ASTRA-O-cresolpthalein complexone calcium method. Am J Clin Pathol 1984;82:182–187.
8. Janssen JW, Helbing AR. Arsenazo III: an improvement of the routine calcium determination in serum. Eur J Clin Chem Clin Biochem 1991;29:197–201.
9. Proctor KA, Rao LV, Roberts WL. Gadolinium magnetic resonance contrast agents produce analytical interference in multiple serum assays. Am J Clin Pathol 2004;121:282–292.
10. Emerson J, Kost G. Spurious hypocalcemia after Omniscan or OptiMark enhanced magnetic resonance imaging: an algorithm for minimizing a false positive laboratory value. Arch Pathol Lab Med 2004;128:1151–1156.
11. Kang HP, Scott MG, Joe BN, et al. Model for predicting the impact of gadolinium on plasma calcium measured by teh O-cresolphthalein method. Clin Chem 2004;50:741–746.
12. Gandhi M, Narra VR, Brown JJ, et al. Clinical and economic impact of falsely decreased calcium values caused by gadoversetamide interference. Am J Roentgenol 2008;190:W213–W217.
13. Prince MR, Erel HE, Lent RW, et al. Gadodiamide administration causes spurious hypocalcemia. Radiology 2003;227:639–646.
14. Lin J, Idee JM, Port ME, et al. Interference of magnetic resonance contrast agents with serum calcium measurement technique using colorimetric reagents. J Pharm Biomed Anal 1999;21:931–943.
15. Lowe A, Balzer T, Hirt U. Interference of gadolinium containing contrast enhancing agents with colorimetric calcium laboratory testing. Invest Radiol 2005;40:521–525.
16. Normann PT, Froysa A, Svaland M. Interference of gadodiamide injection (OMNISCAN) on the colorimetric determination on serum calcium. Scand J Clin Lab Invest 1995;55:421–426.