Linearity and stability of the AVL and Nova magnesium and calcium ion-selective electrodes

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We studied the stability and linearity of the AVL and Nova Mg and Ca ion-selective electrodes and the relation between the ionized Ca and ionized Mg results reported by each analyzer. The response of the electrodes to different concentrations of Mg and Ca was determined for saline solutions, aqueous solutions, and serum samples. The electrodes from both manufacturers demonstrated acceptable stability for the time of the study. The response of the electrodes was linear within the range specified by each manufacturer, but relative nonlinearity and the values for the linear limits differed between the AVL and Nova analyzers. The ionized Mg results varied with the concentration of Ca. The relation between ionized Ca and ionized Mg results was nonlinear and differed between the AVL and Nova electrodes. Intermethod comparison between the electrodes showed poor agreement for ionized Mg results, especially at low and high concentrations of total Ca and total Mg.

INDEXING TERMS: electrolytes • intermethod comparison

The magnesium ion (Mg2+), like the calcium ion (Ca2+), circulates in blood bound to protein, complexed to inorganic and organic ligands, and as a free hydrated ion. These three fractions, routinely measured as total concentration, are in an equilibrium that can be altered by in vivo processes. As with other blood electrolytes, the physiological action of Mg is related only to the activity of free Mg2+ in plasma water. Because changes in the Mg2+ activity are not necessarily related to the changes in the substance concentration of total Mg (T>Mg), measurement of ion activity should be clinically more useful than measurement of the total ion concentration.1

In clinical practice, the ion concentration in plasma is estimated by determining the activity of the free ion potentiometrically with an ion-selective membrane electrode (ISE). At present, the concentrations of several blood electrolytes are measured routinely with ISEs [1] incorporated in commercial analyzers. To determine Mg2+ activity, Simon et al. [2, 3] developed several electrically neutral complexing agents (neutral ionophores) in a liquid carrier membrane. The major difficulty in the design of the Mg ISE for clinical application is the insufficient selectivity of the ionophore in the membrane for Mg2+ over Ca2+ and Na+. However, three commercial instrument manufacturers recently introduced ISEs for routine clinical measurement of free Mg2+ concentration in blood, plasma, and serum; the instruments involved are the AVL 988-4 (AVL, Graz, Austria), the Microlyte 6 (KONE, Espoo, Finland), and the Nova CRT (Nova, Waltham, MA). All three instruments simultaneously determine the concentration of free Mg2+ ["ionized magnesium" (iMg)] and free Ca2+ ["ionized calcium" (iCa)] and use the iCa result to correct for the selectivity of the Mg ISE for Ca2+. This computer-based correction is called chemometrics.

Results of analytical performance and clinical applications of Mg ISEs have been reported [4-6]. However, published information about the stability and linearity of the Mg and Ca ISEs, and about the effect of iCa on the iMg result, is preliminary [6-9] or not available. Given that the determination of iMg requires a simultaneous determination of iCa, the analytical performance of both Mg and Ca ISEs is crucial for the accuracy and precision of the iMg assay. We have investigated the stability and linearity of the AVL and Nova ISEs for Ca and Mg and determined the relation between the iMg and iCa results reported by each analyzer.

Materials and Methods

The Nova CRT analyzer uses ISEs to determine pH and the concentrations of Na, iCa, iMg, and K. The analyzer has modes for two sample types: whole blood and serum/plasma. The sensor for both Ca and Mg ISEs is a neutral carrier ionophore (of proprietary composition) contained in a poly(vinylchloride) membrane. The manufacturer’s analytical range for iMg is

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1 Nonstandard abbreviations: T>Mg, total magnesium; ISE, ion-selective electrode; iMg, ionized magnesium; iCa, ionized calcium; TCa, total calcium; AA, atomic absorption; and SLS, series of linearity solutions.

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0.1–2.5 mmol/L and for iCa is 0.1–5.0 mmol/L. The two-point calibration is made at assigned Mg^{2+} concentrations of 0.50 and 1.50 mmol/L and at assigned Ca^{2+} concentrations of 1.00 and 2.00 mmol/L. The manufacturer warrants the performance of the Mg ISE for 4 days (or 200 samples) and the Ca ISE for 7 days (or 300 samples) from date of installation. The analyzer automatically determines iCa with every iMg determination. If the iCa result is lower than the manufacturer’s analytical range, the iMg result is suppressed. An older model, the Nova 8 analyzer, also uses ISEs and determines pH and iCa with the same sensor as that in the Nova CRT.

The AVL 988–4 analyzer determines pH and the concentrations of iMg, iCa, and Na. The analyzer has six measurement (sample type) modes: blood, serum, standard (calibrator), urine, dialysis solution, and quality control. The sensor for the iCa electrode is a neutral carrier ionophore contained in a polyvinylchloride membrane. The ionophore for the iMg electrode is ETH 7025. The manufacturer’s analytical range for iMg is 0.1–3.0 mmol/L for iCa, it is 0.1–6.0 mmol/L. The two-point calibration is made at assigned Mg^{2+} concentrations of 0.30 and 0.90 mmol/L and at assigned Ca^{2+} concentrations of 0.90 and 1.25 mmol/L. The manufacturer warrants the lifetime of the Mg ISE for 2.5 months and of the Ca ISE for 4 months from shipment date. The analyzer determines iCa and Na with every iMg determination. For samples that have an iCa or Na result lower than the manufacturer’s specified analytical ranges, the iMg result is suppressed.

To determine total Ca concentration (TCa) and TMg, we used an atomic absorption (AA) spectrophotometer (Model 5100PC, Perkin-Elmer, Norwalk, CT).

REAGENTS

Aqueous linearity solutions. We prepared solutions of MgCl_2 and CaCl_2 in unbuffered saline (NaCl 145 mmol/L, pH 7.4) at four different concentrations of TCa (1.0, 1.5, 2.0, and 2.5 mmol/L), each paired with a low and a high concentration of TMg (0.00 and 4.00 mmol/L). Four series of linearity solutions (SLS) for Mg were prepared by mixing appropriate volumes of low- and high-TMg solutions (e.g., containing the high-TMg solution at 100%, 90%, 80%, 60%, 40%, 20%, 10%, and 0% by volume, each prepared in triplicate, for a total of 20 samples). Similarly, four SLS for Ca were prepared from a set of solutions with four different concentrations of TMg (0.30, 1.00, 1.50, and 2.00 mmol/L), each paired with a low and a high concentration of TCa (0.00 and 6.00 mmol/L). Similar Mg and Ca SLS were prepared with the aqueous calibrators from both manufacturers (AVL Standard A, no. BP1598; assay values Mg^{2+} 0.30 mmol/L, Ca^{2+} 0.90 mmol/L, pH 7.38; Linearity Solution/External Standard for Nova Analyzers Chem Set C Level 1, no. 13519; assay mean values Mg^{2+} 0.35 mmol/L, Ca^{2+} 0.72 mmol/L, TCa 1.00 mmol/L, pH 7.17). For Mg SLS in saline an additional five dilutions were made within the TMg range of 0.00–0.40 mmol/L.

Serum samples. We prepared three different serum samples with TCa concentrations of 2.32, 3.32, and 4.30 mmol/L and TMg concentrations of 0.65, 0.73, and 0.86 mmol/L by adding 1 part of saline CaCl_2 solution to 24 parts of serum. Each serum sample was also prepared to contain TMg at 3.68, 3.72, and 3.82 mmol/L by adding 1 part of saline CaCl_2/MgCl_2 solution to 24 parts of serum. Three dilution series (DS1, DS2, and DS3) were prepared, in triplicate, by mixing the appropriate low- and high-TMg samples as described above for the aqueous linearity solutions. All samples were collected in plain glass tubes with glycerin-coated stoppers (cat. no. 6397; Becton Dickinson, Rutherford, NJ). To minimize the change of serum pH, we prepared each dilution series from blood collected from apparently healthy volunteers just before analysis. The mean (and range) of the pH measured with each analyzer for the three dilution series was as follows: DS1_{AVL} = 7.56 (7.54–7.59); DS1_{Nova CRT} = 7.63 (7.60–7.67); DS2_{AVL} = 7.59 (7.56–7.60); DS2_{Nova CRT} = 7.65 (7.6–7.68); DS3_{AVL} = 7.57 (7.52–7.66); DS3_{Nova CRT} = 7.61 (7.57–7.65).

PROCEDURES

All samples were analyzed after maintenance (daily or weekly) and after daily calibration and quality-control procedures performed as recommended by each manufacturer. Each series of samples was analyzed at the same time in randomized order with the AVL analyzer and both Nova analyzers. During the analysis, a serum pool sample was assayed before the first and after every 12th aqueous linearity sample. For all measurements, the AVL was used in the serum mode and the Nova CRT was in the serum/plasma mode. We recorded the results for iCa, iMg, Na, and pH. For two Ca and Mg saline SLS, we also recorded the voltage responses of the four ISEs.

Stability studies. For this study we used the SLS prepared with saline and the aqueous calibrators. The first set of results (day 1) was obtained with newly installed Ca and Mg ISEs that were exposed to serum samples during the first 4 h of use. The same solutions were reanalyzed on the nth day (n = 3, 5, 8, and 10 for saline, n = 3, 5, 10, 13, 15, 17, and 19 for aqueous calibrators). The last set of data for aqueous calibrators (day 19) was obtained with only the AVL analyzer because the Nova Mg ISE failed to calibrate. Throughout the study, patients’ serum samples were assayed with both analyzers.

Linearity studies. We verified the linear range specified by each manufacturer for both Mg and Ca ISEs with the SLS that were prepared in saline and in the AVL and Nova aqueous calibrators. The linearity of the Ca ISEs was verified with the SLS for Ca (main ion) at four different constant concentrations of Mg (background ion) for each type of diluent. Similarly, the linearity of the Mg ISEs was verified with the SLS for Mg (main ion) at four different concentrations of Ca (background ion). All results were obtained with newly installed ISEs exposed to serum samples during the first 24 h of use. We assessed assay linearity by the procedure of Kroll and Emancipator [10, 11]. The limits of the linearity range were the lowest and the highest results obtained with each analyzer for each SLS. The order of the polynomial regression was that which best fit the data in the given range. We calculated the relative nonlinearity [11] to quantify the nonlinearity of the higher-order polynomials.
Relation and comparison of iCa and iMg results. Statistical analysis was done with StatView, version 4.01 (Abacus Concepts, Berkeley, CA).

Results

ISE STABILITY

The effect of aging on the voltage response of the Ca and Mg ISEs to increasing concentrations of TCa was determined for each day of the study by using linear regression (\( x = \text{log} \text{ TCa} \), \( y = \text{volts} \)). Fig. 1 shows the typical day-to-day variation in the response of Mg ISEs to unbuffered saline solutions that we observed with all solutions used in this study. For the AVL, the slopes and intercepts of the regression lines varied randomly. For the Nova, the regression line for a newly installed ISE (day 1) had lowest slope and greatest intercept observed for this ISE during the study; this was followed by a random pattern of variation as the electrode aged (day \( \geq 3 \)). The variation in the response of the three Ca ISEs was random. The results of the regression analysis for all ISEs are summarized in Table 1.

The effect of aging on the iCa and iMg results was determined by using linear regression to compare the results on each day of study (day \( n \)) with the results obtained with the newly

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\begin{align*}
\text{Table 1. Change in the voltage response with age of the ISEs.}\quad & \cr
\text{Sample} & \text{Min (Day)*} & \text{Max (Day)} & \text{Min (Day)} & \text{Max (Day)} & r \\
\text{Mg ISE} & & & & & \\
\text{AVL} & \text{Saline} & 21.69 (10) & 21.98 (8) & 13.48 (1) & 17.61 (8) & >0.997 \\
 & \text{AVL cal.} & 22.14 (11) & 23.06 (15) & 11.75 (17) & 13.03 (11) & >0.997 \\
 & \text{Nova cal.} & 20.95 (15) & 22.43 (18) & 13.98 (18) & 15.19 (11) & >0.998 \\
\text{Nova CRT} & \text{Saline} & 12.63 (1) & 13.81 (3) & 3.67 (10) & 9.40 (1) & >0.996 \\
 & \text{AVL cal.} & 15.58 (1) & 16.37 (4) & -2.69 (4) & 5.24 (1) & >0.994 \\
 & \text{Nova cal.}\quad & 23.62 (11) & 24.88 (4) & 1.47 (4) & 9.50 (1) & >0.974 \\
\text{Ca ISE} & & & & & \\
\text{AVL} & \text{Saline} & 27.83 (8&10) & 28.25 (1) & -7.48 (5) & -5.19 (8) & >0.998 \\
 & \text{AVL cal.} & 26.91 (11) & 27.70 (18) & -8.91 (17) & -8.32 (11) & >0.997 \\
 & \text{Nova cal.} & 26.95 (15) & 27.61 (18) & -8.15 (18) & -7.61 (11) & >0.997 \\
\text{Nova CRT} & \text{Saline} & 27.99 (8) & 29.24 (3) & 3.82 (1) & 5.94 (3) & >0.998 \\
 & \text{AVL cal.} & 27.44 (11) & 27.93 (11&18) & 4.01 (11) & 4.86 (18) & >0.997 \\
 & \text{Nova cal.}\quad & 26.62 (15) & 27.53 (18) & 4.68 (15) & 5.53 (18) & >0.996 \\
\text{Nova 8} & \text{Saline} & 28.81 (5) & 29.42 (3) & -3.14 (1) & -2.23 (10) & >0.999 \\
 & \text{AVL cal.} & 29.33 (11) & 30.85 (17) & -3.64 (18) & -4.10 (11) & >0.997 \\
 & \text{Nova cal.}\quad & 28.00 (18) & 29.55 (17) & -3.11 (11) & -2.50 (18) & >0.997 \\
\end{align*}
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*Results of the linear regression for \( x = \text{log} \text{ TCa}, y = \text{mV} \), where TCa in saline = 1.2–3.0 mmol/L, TCa in AVL calibrator (cal.) = 0.9–1.8 mmol/L, and TCa in Nova cal. = 1.0–2.0 mmol/L.

*Age of the ISE in days is listed in parentheses.

TCa = 1.0–1.6 mmol/L.
installed ISEs (day 1). The results of the AVL and Nova Ca ISEs for both manufacturer-supplied calibrators varied randomly during the 19 days of study (interassay SDs <0.3 mmol/L). With the newly installed Mg and Ca ISEs, the relation between the iCa (x) and iMg (y) results was y = 0.28 ± 0.03x for AVL and y = 0.20 ± 0.13x for Nova CRT analyses of the AVL aqueous calibrators, and y = 0.31 ± 0.08x for AVL and y = 0.23 ± 0.16x for Nova CRT analyses of the Nova aqueous calibrators. Fig. 2 shows the regression lines (x = iCa, y = ΔiMg) for the AVL Mg ISE with the AVL calibrator and for the Nova Mg ISE with the Nova calibrator. With the AVL Mg ISE the difference in the iMg results was greatest on day 3 (slope = −0.09, P_{slope} = 0 <0.0001, intercept = 0.078) and day 19 (slope = 0.051, P_{slope} = 0 <0.0001, intercept = −0.046). Between days 5 and 17, ΔiMg varied randomly: −0.018 ≤ slope ≤ 0.037, P_{slope} = 0 <0.04; −0.033 ≤ intercept ≤ 0.032, P_{intercept} = 0 <0.08.

The iMg results obtained with the Nova Mg ISE on days 3 and 5 were not significantly different from the results obtained with the newly installed ISE: slope −0.012, P_{slope} = 0 >0.08; intercept ≤0.016, P_{intercept} = 0 >0.07. Between days 10 and 17, the difference in the results gradually increased (−0.034 ≤ slope ≤−0.025, 0.052 ≤ intercept ≤ 0.082), and this change was statistically significant: P_{slope} = 0 <0.003, P_{intercept} = 0 <0.0001. The results of the AVL Mg ISE for the Nova calibrator and of the Nova Mg ISE for the AVL calibrator were similar and are not shown.

**ISE LINEARITY**

The linearity results obtained by polynomial regression analysis for the Nova 8 Ca ISE and the AVL and Nova CRT Ca and Mg ISEs are summarized in Table 2. For the AVL and Nova 8 Ca ISEs, the slopes and intercepts of the regression equations were not statistically different for saline and for AVL calibrator SLS with different constant concentrations of TMg (P >0.1) but were for the Nova calibrator SLS (P <0.01). For the Nova CRT Ca ISE, the effect of the TMg concentration was statistically

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**Table 2. Linearity of AVL and Nova CRT ISEs.**

<table>
<thead>
<tr>
<th></th>
<th>AVL</th>
<th>Nova CRT</th>
<th>Nova 8</th>
<th>AVL</th>
<th>Nova CRT</th>
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<td>Range, mmol/L</td>
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<td>0.31–5.38</td>
<td>0.03–6.03</td>
<td>0.10–2.50</td>
<td>0.19–2.48</td>
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<td>RNL, %</td>
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<td>≤5</td>
<td>NA</td>
<td>≤2</td>
<td>≤7</td>
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<td><strong>AVL calibrator SLS</strong></td>
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<tr>
<td>Range, mmol/L</td>
<td>0.90–5.80</td>
<td>0.83–5.37</td>
<td>0.86–5.65</td>
<td>0.33–2.93</td>
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<td>RNL, %</td>
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<td>Range, mmol/L</td>
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<td>0.77–5.37</td>
<td>0.76–5.60</td>
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<td>RNL, %</td>
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<td>NA</td>
<td>NA</td>
<td>≤2</td>
</tr>
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RNL = relative nonlinearity; NA = not applicable.
significant for all SLS ($P < 0.01$). A similar effect of TCa on the regression equations was found for the Nova CRT Mg ISE for all SLS ($P < 0.02$) and for the AVL Mg ISE for the saline SLS ($P < 0.005$).

The regression equation for the voltage response of the ISEs to the increasing concentration of the main ion in the saline SLS was first-order for Nova 8, second-order for the Nova CRT analyzer, and third-order for the AVL analyzer. The regression equation for the voltage responses of both Mg ISEs to the increasing concentration of TCa was second-order. In the physiological concentration range (TMg 0.2–1.2 mmol/L, TCa constant at 1.0 and 2.0 mmol/L; or TCa 0.6–2.4 mmol/L, TMg constant at 0.5 and 1.5 mmol/L), the regression equations for the voltage responses of the Mg and Ca ISEs were second-order.

Fig. 3 shows the linear regressions of the results obtained with solutions that had concentrations of both the main and the background ions in the physiological range.

**Relation Between iCa and iMg Results**

We used the results obtained with the SLS to investigate the adequacy of the chemometric correction for Ca interference with Mg ISEs and to investigate the effect of iMg concentration on the Ca ISEs. Fig. 4 shows the relationship between the iCa and iMg results obtained with AVL and Nova CRT ISEs for three saline SLS for Mg and three saline SLS for Ca. For the AVL, an increase in the iMg concentration in saline ($x$) did not affect the iCa result ($y$). The regression of the data for the SLS with constant TCa concentration was linear, and the difference

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**Fig. 3.** Voltage response of the Ca and Mg electrodes with the saline linearity solutions: AVL ISE (○); Nova CRT ISE (□).

(---) linear regression, ( - - ) second-order regression. (Top) Voltage response of the Mg ISEs to various concentrations of TMg at two constant concentrations of TCa: (filled symbols, TCa = 1.0 mmol/L) $y = 9.86 + 7.22x$ for AVL, $y = 12.34 + 15.93x$ for Nova CRT; (open symbols, TCa = 2.0 mmol/L) $y = 15.33 + 4.69x$ for AVL, $y = 14.87 + 12.54x$ for Nova CRT. (Bottom) Voltage response of the Ca ISEs to various concentrations of TCa at two constant concentrations of TMg: (filled symbols, TMg = 0.5 mmol/L) $y = -7.94 + 27.47x$ for AVL, $y = 7.35 + 27.23x$ for Nova CRT; (open symbols, TMg = 1.5 mmol/L) $y = -6.00 + 27.19x$ for AVL, $y = 7.53 + 26.44x$ for Nova CRT.

**Fig. 4.** Relation between iMg and iCa results obtained with AVL (○) and Nova CRT (●) ISEs for the saline SLS at (top) three constant concentrations of Ca (1.00, 1.50, and 2.00 mmol/L) and (bottom) three constant concentrations of Mg (0.30, 1.00, and 2.00 mmol/L).

(Top) At Ca = 1.00 mmol/L, $y = 1.06 - 0.008x$ for AVL, $y = 0.84 + 0.36x - 0.17x^2 + 0.03x^2$ for Nova CRT; at 1.50 mmol/L, $y = 1.53 - 0.006x$ for AVL, $y = 1.31 + 0.24x - 0.09x^2 + 0.01x^2$ for Nova CRT; at 2.00 mmol/L, $y = 2.03 - 0.006x$ for AVL, $y = 1.62 + 0.33x - 0.07x^2$ for Nova CRT. (Bottom) At Mg = 0.30 mmol/L, $y = 0.33 + 0.03x - 0.04x^2$ for AVL, $y = 0.02 + 0.16x - 0.005x^2$ for Nova CRT; at 1.00 mmol/L, $y = 1.01 - 0.02x + 0.02x^2$ for AVL, $y = 0.61 + 0.20x - 0.008x^2$ for Nova CRT; at 2.00 mmol/L, $y = 2.10 - 0.06x - 0.02x^2$ for AVL, $y = 1.35 + 0.24x - 0.01x^2$ for Nova CRT. Each point represents the mean of triplicate results.
in the slopes for each SLS was not statistically significant (P >0.1). However, for the Nova CRT, an increase in the iMg concentration effected an increase in the iCa result. The regression was a third-order polynomial, and the differences in the slopes for the SLS with the four constant TCa concentrations was statistically significant (P <0.01). The iMg value at the point of intersection of the regression curves increased with increasing concentrations of TCa.

The increase in the iCa value (x) affected both the AVL and the Nova CRT iMg results (y). The regression for both analyzers was a third-order polynomial, and the slopes for each SLS were significantly different (for AVL, P <0.005; for Nova CRT, P <0.0001). The iCa value at the point of intersection of the regression curves increased with increasing concentrations of TMg. The relation between the iCa and iMg for the SLS of aqueous calibrators and for dilution series of serum samples was similar and is not shown.

**COMPARISON OF ICA AND IMG RESULTS**

We compared the iCa results obtained for the saline calcium SLS among the three analyzers. The linear regressions (x = TCa, y = iCa) were as follows:

For TMg 0.30 mmol/L, y = 0.06 + 1.04x for AVL, y = 0.21 + 0.82x for Nova CRT, y = 0.01 + 1.00x for Nova 8.

For TMg 1.50 mmol/L, y = 0.07 + 1.05x for AVL, y = 0.30 + 0.84x for Nova CRT, y = 0.04 + 1.00x for Nova 8.

As indicated, the increase in TMg affected only the Nova CRT iCa results. The change in the slope was minimal, but the intercept increased. The agreement between the Nova 8 and AVL results was better than between the Nova 8 and Nova CRT results. The best agreement among the results of all three ISEs was at TCa = 1.2 mmol/L.

To compare the iMg ISEs, we used the results obtained for the saline magnesium SLS and for the serum dilution series. Fig. 5 shows the regression of the AVL and Nova CRT results for SLS with TCa of 1.00 and 1.50 mmol/L. For the TMg range of 0.00–1.60 mmol/L, the first-order regression gave the best fit for the AVL results and the second-order polynomial best fit the Nova CRT results. The AVL results were not affected by the TCa (slope near 1.0 and intercept near 0.0). For the Nova CRT, the intercept was positive and did not change, but the slope increased with an increase in TCa. Therefore, the TMg concentration at the point of intersection for the AVL and Nova regression lines increased when the TCa concentration increased.

For the serum dilution series, the concentration of the iCa was not constant but increased with the increasing TMg concentration. The baseline (at low TMg concentration) and high (at high TMg concentration) iCa ranges, and the results of linear regression for the serum dilution series with three different TCa concentrations, are summarized in Table 3. For the dilution series with TCa at 2.36 and 3.30 mmol/L, the increase in iCa from baseline for the Nova CRT was about twice that for the AVL. For the dilution series with TCa at 4.30 mmol/L, the increase in baseline iCa was comparable for both analyzers. However, each dilution series showed a difference between the AVL and Nova CRT slope and intercept. As with the saline SLS, the TMg at the point of intersection for the AVL and Nova CRT regression lines increased with increasing TCa.

**Discussion**

The response of the AVL and Nova Mg ISEs to changes in the TCa concentration varied with the age of the ISE. For the AVL electrode, the largest difference from the baseline (new electrode) for iMg results occurred on the third day of use, at which time the iMg results were underestimated when the measured iCa was >1.20 mmol/L. The same decrease in iMg results was observed with two other electrodes and was also seen during the linearity study (Fig. 4, lower panel) performed with 2-day-old AVL Ca and Mg ISEs. With further aging of the AVL electrode, the difference in iMg results varied randomly.
For the Nova Mg ISE, the change in the iMg results was least during the first five days of the study. Beyond the fifth day of use, this electrode overestimated the iMg concentration, particularly at low concentrations of iCa. However, the iMg results on the fifth day (or after 356 sample analyses) were still not significantly different from the baseline results (slope and intercept \(P > 0.07\)). Even though the difference in the iMg results obtained on day 10 was statistically significant, the interassay precision of the electrode for the first 10 days of use (CV \(\leq 3.3\%\)) was similar to the interassay precision (CV \(\leq 3.83\%\)) reported by Altura et al. [5]. Note that the change in the Nova iMg results was not related to the calibration slope, which varied between 12.1 (day 3) and 12.7 (days 1 and 13) and was within the limits recommended by the manufacturer (10.0-14.0) throughout the study. Generally, the difference between the response of the new and the aging electrode was smaller for the AVL than for the Nova when iCa \(\leq 1.2\) mmol/L.

The response of the AVL and Nova Ca ISEs varied during the time of the study, but the difference from baseline iCa results was \(\leq 0.04\) mmol/L and the voltage response was comparable on all days of the study. Because the changes in the iCa results were small, the variations in the iMg results with time of use could be the result of changes in the membrane selectivity of the Mg ISEs to Ca\(^{2+}\). However, the changes observed in the voltage responses of the aging Mg ISEs do not support such a conclusion. The response of the 3-day-old AVL electrode did not reflect the aberrant iMg results obtained with this relatively new electrode. Further, there was very little difference between the responses of the 3- and 5-day-old electrodes. The Nova CRT showed no obvious drift in the slope past the 5 days of use, and, in general, the voltage responses of this electrode were comparable up to the last day of acceptable calibration. On the basis of our results, we suggest that, despite the day-to-day variation in the voltage responses of both Mg ISEs to Ca ions, this variation can be corrected by calibration of the electrodes at appropriate Ca\(^{2+}\) and Mg\(^{2+}\) concentrations—which should minimize the effect of aging of the ISEs on the iMg results.

The linearity of both the AVL and Nova Ca and Mg ISEs was within the ranges specified by the manufacturers; however, the results obtained with the three analyzers for the same solutions were different. The first-order equation gave the best fit only with the Nova 8 iCa and voltage response results, and the linearity range extended to 6.0 mmol/L, which is beyond the limits recommended by the manufacturer (0.1-5.5 mmol/L).

For the AVL and Nova CRT analyzers, higher-order polynomials gave the best fit with both the iCa and voltage response results; however, we found major differences between the Nova 8 and the Nova CRT iCa results. For all solutions used in the study, the Nova CRT iCa results were higher at the low iCa concentrations and lower at the high iCa concentrations than the Nova 8 results. The overestimation of the iCa concentration by Nova CRT was greatest for the saline solution that did not contain Ca (i.e., no added CaCl\(_2\)). The Nova 8 results were consistently close to zero (0.03-0.04), but the Nova CRT results were never <0.31 (range 0.31-0.40). The lowest AVL iCa result was 0.14. In general, the agreement was better between the Nova 8 and AVL results than between the Nova 8 and Nova CRT results. The different performance characteristics of the two Nova Ca ISEs are consistent with the results for serum samples we reported previously [12].

The regression of the iMg results for the aqueous calibrators was both first-order and second-order, with a relative nonlinearity of \(\leq 3\%\). However, with these solutions, we could not verify the iMg linearity range for values <0.3 mmol/L. For the saline SLS, with TMg concentrations covering the entire linearity range of the ISEs, the best fit of iMg and voltage results was also obtained with higher-order polynomials. But the relative nonlinearity for Nova iMg results increased, mainly because of the extension of the range tested (i.e., TMg <0.3 mmol/L). All electrodes are calibrated at two concentrations of Ca\(^{2+}\) (at log Ca\(^{2+}\) of 0.00 and 0.30 mmol/L for Nova CRT and of -0.05 and 0.10 mmol/L for AVL) and Mg\(^{2+}\) (at log Mg\(^{2+}\) of -0.30 and 0.18 mmol/L for Nova CRT and of -0.52 and -0.05 mmol/L for AVL). Because the voltage responses of the ISEs deviate from the first-order equation, the two-point calibration of the electrodes is not adequate to produce linear results, especially when the calibration points do not cover the usable concentration ranges of the electrodes. Even though the relative nonlinearity of \(\leq 2\%\) for the AVL iMg results may be acceptable for clinical application, the relative nonlinearity of \(\leq 7\%\) for the Nova CRT may cause important overestimation of iMg results for samples with low TMg concentration.

The responses of the AVL and Nova CRT Ca ISE to variations in the TMg concentration differed. The response of the Nova CRT Ca ISE was affected by the iMg result for all SLS used in the study. The response of the AVL and Nova 8 Ca ISE was affected only by the increase in iMg in the Nova calibrator. For the saline SLS, however, we found that the voltage re-

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**Table 3. Effect of TCa on AVL and Nova CRT iMg results (mmol/L) for three serum dilution series.**

<table>
<thead>
<tr>
<th>AA TCa, mmol/L</th>
<th>iCa range, mmol/L</th>
<th>Slope</th>
<th>Intercept, mmol/L</th>
<th>AVL</th>
<th>iMg vs TMg</th>
<th>Nova CRT</th>
<th>iMg vs TMg</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.36</td>
<td>1.14-1.29</td>
<td>0.70</td>
<td>-0.041</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.30</td>
<td>1.67-1.88</td>
<td>0.65</td>
<td>-0.093</td>
<td></td>
<td></td>
<td>0.13-1.32</td>
<td>0.41</td>
</tr>
<tr>
<td>4.30</td>
<td>2.49-2.60</td>
<td>0.73</td>
<td>-0.242</td>
<td></td>
<td></td>
<td>1.54-1.91</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.23-3.23</td>
<td>0.57</td>
<td>0.124</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Results of linear regression of \(x = TMg, y = iMg\).
\(^b\) Results determined by atomic absorption spectrophotometry.
\(^c\) Results determined with Ca ISE for each serum dilution series.
responses of all three Ca ISEs were not affected by the increase in TMg concentration. In addition, as shown in Fig. 3 (lower panel), the slopes of linear regression (mV/log TCa) were similar at the two constant concentrations of TMg for both AVL and Nova CRT ISEs. Therefore, the observed iMg effect on the iCa result cannot be caused by analytical interference of Mg ions with the Ca ISE. We suggest that, with the Nova calibrator SLS, the addition of MgCl₂ affected the complexed Ca/Ca²⁺ equilibrium and resulted in relative changes in the Ca²⁺ concentration. We cannot explain the observed iMg effect on the Nova CRT iCa results with the saline and AVL calibrator SLS. However, compared with the AVL, the Nova CRT underestimated iCa at low iMg values and overestimated the iCa results as the iMg increased in all SLS used in this study.

The chemometric correction of the iMg result for the measured iCa concentration for both instruments does not seem to eliminate the interference by Ca ions on the iMg determinations. In this study, the responses of both the AVL and the Nova Mg ISEs were affected by the increase in the measured iCa concentration. For the AVL result of iCa >1.29 mmol/L, the iMg concentration was underestimated (see Fig. 2, lower panel). However, this underestimation did not correlate with a similar decrease in the voltage response of the Mg electrode; therefore, the apparent error must have been introduced during the mathematical computation of the iMg results. The Nova CRT iMg results increased with the increase in iCa results. In comparison with AVL, the Nova CRT iMg was underestimated at low iCa results and overestimated at high iCa results. The iCa result at the point of intersection of the AVL and Nova CRT regression curves increased with the increasing TMg concentration. The same pattern between the iCa and iMg results was also seen for serum samples, indicating that the observed effect was not specific for the matrix of aqueous solutions. Again, this trend cannot be seen in the voltage responses of the Mg ISE to increasing TCa concentrations. This pattern for the relation between iCa and iMg results is what would occur if the iCa results used for the chemometric correction of the iMg results were inaccurate: An overestimation of iCa at a low concentration would falsely decrease the iMg results, and an underestimation of iCa at the higher concentration would falsely increase the iMg result. We found this relative error in the Nova CRT iCa results when we compared the three different Ca ISEs. In comparison with the AVL and Nova 8, the Nova CRT underestimated the iCa results at the high TCa concentration and overestimated the iCa results at the low TCa concentration. We could not determine the accuracy of Ca ISEs because no reference material for ionized calcium is available. However, for the SLS prepared with unbuffered saline, the agreement between the expected TCa results and the iCa results was good for the Nova 8 and AVL analyzers, yielding slopes close to 1 and intercepts near 0. For the Nova CRT iCa results, the slope was <1, and the intercept was >0.

To summarize, we found poor agreement between the AVL and Nova CRT iMg results, mainly because of the difference in the effect of iCa on the iMg results. As shown in Fig. 5, the concentration of TMg at which the AVL and Nova CRT iMg results were identical increased with increasing TCa concentration. Thus, the crossover point of the TMg/TCa concentrations below (or above) which the AVL iMg results were higher (or lower) than the Nova CRT results varied. This variation in the difference between the two analyzers for iMg results may be clinically important, especially for samples with abnormal TCa and TMg concentrations. Although the intercepts of the regression equations for the saline SLS and the serum dilution series were close to or <0 for the AVL, they were positive for the Nova CRT—indicating that the iMg results at low TMg concentration may be overestimated. We believe that the performance of the Ca and Mg ISEs can be improved by increasing the number of calibration points at the appropriate Ca²⁺ and Mg²⁺ concentrations and by correcting for the deviation of the voltage response results from the first-order equation in the mathematical computation of the iMg results.

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References