Evaluation of an electrolyte analyzer for measurement of ionized calcium and magnesium concentrations in blood, plasma, and serum of dogs

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Objective—To evaluate an electrolyte analyzer for measurement of ionized calcium (Ca i) and magnesium (Mg i) concentrations in blood, plasma, and serum; investigate the effect of various factors on measured values; and establish reference ranges for Ca i and Mg i in dogs.

Animals—30 healthy adult dogs of various breeds.

Procedure—Precision in a measurement series, day-to-day precision, and linearity were used to evaluate the analyzer. The effects of exposure of serum samples to air, type of specimen (blood, plasma, or serum), and storage temperature on sample stability were assessed. Reference ranges were established with anaerobically handled serum.

Results—The coefficient of variation for precision in a measurement series was ≤ 1.5% for both electrolytes at various concentrations. The Ca i and Mg i concentrations were significantly lower in aerobically handled serum samples, compared with anaerobically handled samples. The Ca i and Mg i concentrations differed significantly among blood, plasma, and serum samples. In anaerobically handled serum, Ca i was stable for 24 hours at 22ºC, 48 hours at 4ºC, and 11 weeks at −20ºC; Mg i was stable for 8 hours at 22ºC, 48 hours at 4ºC, and < 1 week at −20ºC. In anaerobically handled serum, Ca i was stable for 24 hours at 22ºC, 48 hours at 4ºC, and 11 weeks at −20ºC; Mg i was stable for 8 hours at 22ºC, < 24 hours at 4ºC, and < 1 week at −20ºC. In anaerobically handled serum, Ca i was stable for 24 hours at 22ºC, 48 hours at 4ºC, and 11 weeks at −20ºC; Mg i was stable for 8 hours at 22ºC, < 24 hours at 4ºC, and < 1 week at −20ºC. In anaerobically handled serum, Ca i and Mg i concentrations differed significantly among blood, plasma, and serum samples. In anaerobically handled serum, Ca i was stable for 24 hours at 22ºC, 48 hours at 4ºC, and 11 weeks at −20ºC; Mg i was stable for 8 hours at 22ºC, < 24 hours at 4ºC, and < 1 week at −20ºC.

Conclusions and Clinical Relevance—The electrolyte analyzer was suitable for determination of Ca i and Mg i concentrations in dogs. Accurate results were obtained in anaerobically handled serum samples analyzed within 8 hours and kept at 22ºC.

Calcium and magnesium are 2 essential electrolytes that are important in various intracellular and extracellular functions as well as bone development.14 In serum, these cations may be ionized, protein-bound, or complexed with substances such as phosphate, citrate, or lactate. However, only the ionized fraction is biologically active, and it is maintained in a narrow concentration range that is controlled by several feedback mechanisms.14 For financial and technical reasons, most laboratories measure only the total concentration of calcium and magnesium. Although in many instances this provides important information about electrolyte abnormalities, the total concentration correlates poorly with the concentration of the ionized form in conditions such as hypoproteinemia, hyperproteinemia, acid-base imbalance, and renal insufficiency.5,6 Thus, it would be advantageous to be able to directly measure the concentrations of ionized calcium (Ca i) and ionized magnesium (Mg i). Only a few analyzers with ion-selective electrodes (ISEs) for measurement of Ca i and Mg i are available.8,9 A number of studies reported8,11 that these recently developed analyzers produce reliable results, whereas other studies16,17 on the comparison of different analyzers revealed that marked analyzer-specific variations exist. For this reason, it is necessary to use analyzer-specific reference ranges. Furthermore, to assure accurate results, specific techniques for sample collection and handling must be followed.8,9,10,12

One such analyzer can measure the concentrations of Ca i and Mg i in blood, plasma, and serum.1 One number of studies16,18,19 report that this analyzer is well suited for use in human medicine. To the authors’ knowledge, it has not been evaluated in veterinary medicine. Therefore, the goals of the study reported here were to evaluate the analyzer for its usefulness to determine Ca i and Mg i concentrations in dogs, determine analyzer-specific reference ranges for Ca i and Mg i in healthy dogs, and investigate the effects of various external factors on the measurements obtained.

Materials and Methods

Analyzer—The analyzer is equipped with ISEs and is designed for simultaneous measurements of Hct, Na+, K+, pH, Mg i, and Ca i in blood, plasma, and serum. The analyzer provides calculated results for Ca i (measurement range, 0.1 to 5.0 mmol/L) and Mg i (measurement range, 0.1 to 2.5 mmol/L), normalized to a pH of 7.4. The equation used for this calculation is:

\[
\log [\text{electrolyte}] = \log [\text{electrolyte}]_X - 0.24 (7.4 - X)
\]

where X is the measured pH of the sample. In addition, the electrical signal from the Mg i-selective electrode is adjusted by the signal from the Ca i electrode by an algorithm that uses the selectivity constant K MgCa. The analyzer performs a 2-point calibration with 2 Ca i and Mg i aqueous solutions. A sample volume of 180 µL is required, and the measurement cycle is 55 seconds.20

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 Dogs—Thirty dogs owned by employees of the Faculty of Veterinary Medicine, University of Zurich, were used for this study. These dogs were considered healthy on the basis of history and results of physical examination, CBC, and serum biochemical analyses. The dogs were of 17 breeds and ranged in age from 1 to 13 years (mean, 5.6 years). There were 7 sexually intact females, 8 spayed females, 9 sexually intact males, and 6 castrated males. Of these 30 dogs, 20 dogs were divided into subgroups to study the influence of sample handling and storage conditions. Six dogs were used to investigate the effect of exposure of samples to air; 7 were used for comparison of different types of blood specimens and stability at 4°C, and 7 were used to study sample stability at 4°C and –20°C. All 30 dogs served as controls to establish reference ranges for Ca and Mg concentrations.

Precision, linearity, and analytical accuracy—For determination of precision in a measurement series, 2 standard samples from the manufacturer with a low concentration (Ca, 0.59 to 0.83 mmol/L; Mg, 0.26 to 0.42 mmol/L) and a high concentration (Ca, 1.50 to 1.90 mmol/L; Mg, 1.32 to 1.68 mmol/L) of Ca and Mg, and a canine serum sample with a midrange concentration (Ca, 1.12 mmol/L; Mg, 0.46 mmol/L) were measured 10 times within 10 minutes. For day-to-day precision, the manufacturer’s 2 standard samples with a low and a high concentration of Ca and Mg were measured every 24 hours for 10 consecutive days. Samples were stored anaerobically at 4°C.

For determination of linearity, highly concentrated aqueous solutions of calcium (4.84 mmol/L) and magnesium (2.49 mmol/L) were prepared from crystalline calcium chloride (CaCl₂ × 2H₂O) and crystalline magnesium chloride (MgCl₂ × 6H₂O), respectively. These stock solutions were diluted with isotonic saline (0.9% NaCl) solution to make 50%, 25%, 12.5%, and 6.25% solutions. For each solution, the concentration of Ca and Mg was measured with the analyzer and concentrations were calculated with actual electrolyte concentrations.

Effect of exposure to air—To determine the effect of exposure to air on measurements, blood was collected from the jugular vein of 6 dogs. For each dog, the blood was divided among 3 serum tubes such that 100%, 50%, and 25% of the tube was filled. In the 100%-filled tubes, any visible air bubbles, and closed tightly. After 10 to 15 minutes, the tubes were centrifuged at 7,500 g for 2 to 5 minutes and Ca and Mg were measured every 24 hours for 10 consecutive days. Samples were stored anaerobically at 4°C.

Within-run (n = 10)  | Between-run (n = 10*).
--- | ---
Ca, low† | 0.9 (0.65 ± 0.01)
Ca, high† | 0.6 (1.77 ± 0.01)
Serum | 0.9 (1.12 ± 0.01)
Mg, low† | 0.8 (0.39 ± 0.003)
Mg, high† | 0.5 (1.55 ± 0.007)
Serum | 1.5 (0.45 ± 0.007)

Stability of samples stored anaerobically at different temperatures—Serum was placed as 0.5-ml aliquots into completely filled, autoclave tubes. From 7 dogs, serum was stored at room temperature (22°C). Serum from 7 other dogs was stored either at refrigerator temperature (4°C) or at freezer temperature (–20°C). For all 3 temperature groups, the first measurement was performed immediately after centrifugation of the blood. For samples stored at 22°C, additional measurements were made 1, 2, 3, 4, 8, 24, and 48 hours later. For samples stored at 4°C, additional measurements were made every 24 hours for 5 days. For samples stored at –20°C, additional measurements were made 1, 3, 11, and 26 weeks later.

Reference values—Blood samples were collected from the jugular vein of all 30 dogs and placed into Eppendorf serum tubes. The tubes were filled completely, excluding any visible air bubbles, and closed tightly. After 10 to 15 minutes, the tubes were centrifuged at 7,500 g for 2 to 5 minutes and concentrations of Ca and Mg were immediately determined.

Statistical analyses—Data were compiled and analyzed by use of software programs. Data were analyzed via the QC-test for normality to confirm a Gaussian distribution. Precision was calculated from coefficients of variation. Linearity was assessed by use of regression analysis and correlation coefficients. An ANOVA was used for comparison of multiple means, and the Bonferroni-Dunn post hoc test was used for comparison of 2 individual means. Sample stability was analyzed by use of ANOVA for repeated measures. The Bonferroni-Dunn post hoc test was used for comparison of values after storage with those at the start of storage. Reference ranges were defined as the range from the 5th to the 95th percentiles. Unless otherwise stated, all values are reported as mean ± SD. Differences were considered significant at P ≤ 0.05.

Results

Precision, linearity, and analytical accuracy—The variation coefficients for precision in a measurement series for the different concentrations of Ca and Mg, ranged from 0.5% to 1.5%, and those for day-to-day precision ranged from 1.1% to 2.3% (Table 1).

For both Ca and Mg, there was good agreement between the calculated and measured concentrations in the different dilutions for the entire measurement range of the analyzer. The correlations and equations

Table 1—Determination of precision of an electrolyte analyzer for measurement of ionized calcium (Ca) and magnesium (Mg) concentrations in dogs

<table>
<thead>
<tr>
<th>Sample</th>
<th>Within-run (n = 10)</th>
<th>Between-run (n = 10*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CV (%)</td>
<td>Mean ± SD (mmol/L)</td>
</tr>
<tr>
<td>Ca, low†</td>
<td>0.9 (0.65 ± 0.01)</td>
<td></td>
</tr>
<tr>
<td>Ca, high†</td>
<td>0.6 (1.77 ± 0.01)</td>
<td>1.1 (1.73 ± 0.02)</td>
</tr>
<tr>
<td>Serum</td>
<td>0.9 (1.12 ± 0.01)</td>
<td></td>
</tr>
<tr>
<td>Mg, low†</td>
<td>0.8 (0.39 ± 0.003)</td>
<td>2.3 (0.38 ± 0.01)</td>
</tr>
<tr>
<td>Mg, high†</td>
<td>0.5 (1.55 ± 0.007)</td>
<td>1.9 (1.42 ± 0.03)</td>
</tr>
<tr>
<td>Serum</td>
<td>1.5 (0.45 ± 0.007)</td>
<td></td>
</tr>
</tbody>
</table>

*Performed on 10 consecutive days. Standard samples from the manufacturer. CV = Coefficient of variation.
The concentration of Ca\textsubscript{i} was significantly higher, compared with the concentration before storage (Table 2).

**Table 2—Effect of different storage times and temperatures on stability of Ca\textsubscript{i} and Mg\textsubscript{i} (mean ± SD [mmol/L] in anaerobically handled serum samples from 7 healthy dogs)**

<table>
<thead>
<tr>
<th>Time (h) at 22°C</th>
<th>Ca\textsubscript{i}</th>
<th>Mg\textsubscript{i}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.314 ± 0.055</td>
<td>0.464 ± 0.063</td>
</tr>
<tr>
<td>1</td>
<td>1.311 ± 0.051</td>
<td>0.461 ± 0.058</td>
</tr>
<tr>
<td>2</td>
<td>1.313 ± 0.061</td>
<td>0.461 ± 0.060</td>
</tr>
<tr>
<td>4</td>
<td>1.327 ± 0.057</td>
<td>0.470 ± 0.061</td>
</tr>
<tr>
<td>8</td>
<td>1.301 ± 0.050</td>
<td>0.463 ± 0.061</td>
</tr>
<tr>
<td>24</td>
<td>1.300 ± 0.057</td>
<td>0.491 ± 0.067*</td>
</tr>
<tr>
<td>48</td>
<td>1.330 ± 0.086*</td>
<td>0.474 ± 0.072</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time (h) at 4°C</th>
<th>Ca\textsubscript{i}</th>
<th>Mg\textsubscript{i}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.333 ± 0.030</td>
<td>0.481 ± 0.030</td>
</tr>
<tr>
<td>24</td>
<td>1.351 ± 0.039</td>
<td>0.499 ± 0.030*</td>
</tr>
<tr>
<td>48</td>
<td>1.341 ± 0.031</td>
<td>0.521 ± 0.032*</td>
</tr>
<tr>
<td>72</td>
<td>1.361 ± 0.032*</td>
<td>0.529 ± 0.033*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time (wk) at –20°C</th>
<th>Ca\textsubscript{i}</th>
<th>Mg\textsubscript{i}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.333 ± 0.030</td>
<td>0.481 ± 0.030</td>
</tr>
<tr>
<td>1</td>
<td>1.337 ± 0.049</td>
<td>0.526 ± 0.280*</td>
</tr>
<tr>
<td>3</td>
<td>1.313 ± 0.047</td>
<td>0.491 ± 047*</td>
</tr>
<tr>
<td>11</td>
<td>1.317 ± 0.067</td>
<td>0.513 ± 0.057*</td>
</tr>
<tr>
<td>26</td>
<td>1.172 ± 0.066*</td>
<td>0.469 ± 0.024</td>
</tr>
</tbody>
</table>

*Significantly (P ≤ 0.05) different from value at time 0.

Stability of samples stored anaerobically at different temperatures—The concentration of Ca\textsubscript{i} was stable for 24 hours at 22°C, 48 hours at 4°C, and 11 weeks at –20°C. The concentration of Mg\textsubscript{i} was stable for 8 hours at 22°C. After 24 hours at 4°C and 1 week at –20°C, the concentration of Mg\textsubscript{i} was significantly higher, compared with the concentration before storage (Table 2).

**Reference values**—The reference range developed by use of anaerobically handled serum was 1.20 to 1.35 mmol/L for Ca\textsubscript{i} and 0.42 to 0.58 mmol/L for Mg\textsubscript{i}.

**Discussion**

This study revealed that the analyzer was suitable for measurement of Ca\textsubscript{i} and Mg\textsubscript{i} in serum of dogs. Analysis of the precision and linearity of the analyzer yielded good results. As well, our results supported those of other studies,\textsuperscript{8,18-20} which revealed that specific handling, processing, and storage of samples are crucial for accurate measurement of ionized electrolyte concentrations.

Allowing serum to mix with air results in an increase in the serum pH, leading to a significant decrease in the concentration of Ca\textsubscript{i}.\textsuperscript{20} In our study, a significant decrease in the Ca\textsubscript{i} and Mg\textsubscript{i} concentrations occurred approximately 20 minutes after blood collection in tubes filled to 50% capacity, compared with tubes that were filled completely. Hence, our findings also underline the importance of strictly anaerobic sample handling and storage.

Concentrations of Ca\textsubscript{i} and Mg\textsubscript{i} can be measured in blood, plasma, or serum. However, anticoagulants used for the collection of blood and plasma may form complexes with electrolytes and lower their concentrations.\textsuperscript{21} It is also possible that ions contained in anticoagulants interfere with the electrodes and cause erroneously high values.\textsuperscript{21} In our study, the concentrations

for the linear regressions for Ca\textsubscript{i} were $y = -0.02 + 0.21x$ ($R = 0.99$) and for Mg\textsubscript{i} were $y = 0.03 + 0.39x$ ($R = 0.99$).

Direct measurements of Ca\textsubscript{i} and Mg\textsubscript{i}, compared with the actual weighed-in electrolyte concentrations, were linear in the measured range for both electrolytes. The correlations and equations for the linear regressions for Ca\textsubscript{i} were $y = 0.05 + 0.94x$ ($R = 1.00$) and for Mg\textsubscript{i} were $y = 0.03 + 0.88x$ ($R = 0.99$; Fig 1 and 2).

**Effect of exposure to air**—The concentrations of Ca\textsubscript{i} in tubes filled to 50% capacity (1.29 ± 0.05 mmol/L) and 25% capacity (1.26 ± 0.04 mmol/L) were significantly ($P = 0.01$) lower than the concentration in tubes that were completely filled (1.33 ± 0.06 mmol/L). This was also true for the concentrations of Mg\textsubscript{i}, which were 0.51 ± 0.06 mmol/L in completely filled tubes and 0.50 ± 0.06 and 0.48 ± 0.06 mmol/L in tubes filled to 50% and 25% capacity, respectively ($P < 0.01$).

**Comparison of Ca\textsubscript{i} and Mg\textsubscript{i} in blood, plasma, and serum**—The concentration of Ca\textsubscript{i} was significantly lower in plasma (1.21 ± 0.18 mmol/L), compared with blood (1.26 ± 0.18 mmol/L) and serum (1.25 ± 0.18 mmol/L). The concentration of Mg\textsubscript{i} was significantly

lower in serum (0.48 ± 0.07 mmol/L), compared with blood (0.54 ± 0.10 mmol/L) and plasma (0.53 ± 0.07 mmol/L).

Concentrations of Cai and Mgi can be measured in aqueous solutions obtained with an electrolyte analyzer.

Comparisons of Cai and Mgi in blood, plasma, and serum may form complexes with electrolytes and lower their concentrations. It is also possible that ions contained in anticoagulants interfere with the electrodes and cause erroneously high values. In our study, the concentrations

Figure 1—Linear correlation between direct measurements of ionized calcium (Ca\textsubscript{i}) and actual (weighed in) concentrations in aqueous solutions obtained with an electrolyte analyzer.

Figure 2—Linear correlation between direct measurements of ionized magnesium (Mg\textsubscript{i}) and actual (weighed in) concentrations in aqueous solutions obtained with an electrolyte analyzer.
of Ca, was significantly lower in plasma than in serum, which was possibly attributable to heparin binding with calcium ions. Although blood samples also contained heparin, Ca concentration was higher in those samples, compared with plasma samples that contained heparin. Our only explanation for this difference is the time frame of exposure to heparin because the blood samples were analyzed within seconds of collection, whereas heparinized plasma samples were analyzed 20 minutes thereafter.

In contrast, Mg concentration was significantly lower in serum. The reason for the higher Mg concentration in plasma and blood than in serum is also believed to be related to the anticoagulant. Lithium and zinc ions, both part of the anticoagulant, can interfere with the magnesium electrodes and increase the Mg concentration value. However, depending on the anticoagulant used, false low or increased values for Mg may be obtained.

Hence, serum is the sample best suited for clinical use because there is no interference by an anticoagulant and stability of ionized electrolytes appears to be better in serum than in blood. However, in addition to strict anaerobic handling, serum should be harvested as quickly as possible (<1 hour) to prevent glycolysis and lactate accumulation, which decreases the sample pH.

Results of our study indicate that for in-house analysis, serum does not need to be refrigerated because the concentrations of Ca and Mg remain stable at 22°C for 8 hours. Refrigeration (<4°C) is advised for determination of Ca concentration in serum samples that cannot be analyzed within 24 hours. If analysis will not be performed within 48 hours, the serum should be frozen (<20°C). In contrast, the concentration of Mg was significantly decreased after 24 hours when stored at 4°C and after 1 week when stored at −20°C. Reliable results for Mg can only be expected within 8 hours of collection when samples are kept at 22°C. For a more accurate determination of sample stability of Mg, stored at 4°C or −20°C, measurements in shorter time intervals would be necessary.

Reference ranges for Ca and Mg concentration should be established with regard to species, age, and sample type as well as to method of analysis. Reference ranges for Ca concentration have not previously been established for the electrolyte analyzer we evaluated here. Those obtained in this study were markedly lower (mean, 1.28 mmol/L) than those determined with another analyzer (mean, 1.37 mmol/L). Such analyzer-dependent differences have been described and are attributable to differences in analyzer components.

The reference range established for the serum Mg concentration was in agreement with that of Mann et al., who used the same analyzer. In the future, differences in technological features of different electrolyte analyzers may be eliminated, thus eliminating the need for analyzer-specific reference ranges.

The electrolyte analyzer used in our study provides, in addition to the actual measurements, values that are corrected for pH 7.4. The pH correction was not used in our study because it has not been validated for dogs. Furthermore, in vivo pH changes, which may influence the ionized electrolyte concentration of a patient, are ignored by this correction. The actual measurements from an anaerobically handled sample are considered to be the most accurate.

References

15. Elin RJ, Hristova EN, Cecco SA, et al. Comparison of preci-


