



# Impact of Bicarbonate Interference on Routine Ion-Selective Electrode Chloride Measurements

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**Background:** Chloride measurement is crucial for calculating the anion gap. Bicarbonate can interfere with chloride measurements; however, there is no information on the specific types of electrodes affected or the changes in bicarbonate non-selectivity over time. We evaluated the interference of bicarbonate on chloride measurements using different electrodes and the stability of this interference over time.

**Methods:** The effect of bicarbonate on chloride measured with electrodes of various manufacturers was assessed. When non-selectivity toward bicarbonate was observed, the stability of this interference during the electrode's lifetime was explored. The impact of the bicarbonate concentration on the calibrator was also evaluated.

**Results:** Non-selectivity was observed for electrodes using quaternary ammonium salts (Beckman Coulter, Siemens, and Roche), with overestimated or underestimated chloride values observed at high or low bicarbonate concentrations, respectively. The degree of selectivity varied among electrodes. With the Roche electrode, interference became more pronounced over time, whereas the Siemens electrode appeared to gain selectivity during its lifetime. For the Roche system, adjusting the calibrator's bicarbonate concentration from 30 mmol/L to 20–24 mmol/L reduced the number of samples with unacceptable bias (>3%) from 77.3% to 12.6%. Lot-to-lot variations in the calibrator bicarbonate concentration increased the uncertainty of chloride measurements.

**Conclusions:** The extent of bicarbonate-induced error varied according to the type, manufacturer, and wear of the electrode; the bicarbonate concentration in the calibrators and the tested sample; and the chloride content. Laboratories should be aware of the impact of bicarbonate on routine chloride measurements to establish appropriate QC procedures.

**Key Words:** Chloride, Bicarbonate, Ion-selective electrode

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## INTRODUCTION

Chloride is the major extracellular anion in the human body, participating in water balance, osmotic equilibrium, and acid-base balance [1-4]. Chloride is typically measured using potentiometry. To measure a single ion with a high degree of specificity, ion-specific membranes are used to construct an ion-selective elec-

trode (ISE) [5].

Chloride concentration can be measured using a solid electrode such as Ag/AgCl or using chloride-selective ion exchangers embedded in a polyvinyl chloride (PVC) membrane together with a plasticizer [6]. Quaternary ammonium salts are frequently used as chloride-selective ion exchangers. Such ion exchangers are not very specific for chloride, since all ions with an equiva-

lent or lower ion hydration energy can bind to them; there are reports of bromide, iodine, thiocyanate (e.g., in smokers), salicylate, bicarbonate, and heparin interference [7]. Since bicarbonate is the second most abundant anion in the human body after chloride, it most significantly interferes with chloride measurements [1, 3, 4, 7, 8].

Schiemsky, *et al.* [1] described positive bicarbonate interference on the chloride electrode on a Cobas c501 instrument (Roche Diagnostics, Mannheim, Germany), along with under-recovery of chloride in the absence of bicarbonate. The latter is relevant when evaluating the anion gap in critically ill patients. For example, in metabolic acidosis, low bicarbonate concentrations will result in falsely decreased chloride values, leading to an erroneously increased anion gap and a possible misdiagnosis between normal and increased anion gap types of metabolic acidosis [8].

Bicarbonate interference on chloride measurements has been described [1-4, 7]. However, data comparing bicarbonate interference on electrodes by multiple manufacturers are lacking. In this study, the interference of bicarbonate on chloride measurement by different electrodes and the stability of this interference were evaluated over time. The influence of varying bicarbonate calibrator concentrations was also investigated for the Roche electrodes.

## MATERIALS AND METHODS

### Aqueous solutions

To evaluate the influence of bicarbonate on chloride measurements, six aqueous solutions with a fixed chloride concentration of 100 mmol/L and increasing bicarbonate concentrations (0, 20, 40, 60, 80, and 100 mmol/L) were prepared by dissolving appropriate amounts of NaCl (Merck, Darmstadt, Germany) and NaHCO<sub>3</sub> (BioWest, Nuaille, France) in Type I water generated with a Milli-Q system (Merck Millipore, Darmstadt, Germany). Supraclinical bicarbonate concentrations were used to amplify the effect occurring in the clinical range. Bicarbonate and chloride concentrations of each solution were measured in duplicate immediately after calibration of the ISE module. The relation between the bicarbonate concentrations and measured chloride results were analyzed using ordinary least-squares regression (OLR). An electrode was considered non-selective when the calculated slope was significantly different from zero ( $P < 0.05$ ) and the maximal deviation exceeded the total bias criterion of 3%. Additional solutions of 100 mmol/L HCO<sub>3</sub><sup>-</sup> (solution 1) and 100 mmol/L NaCl+100 mmol/L HCO<sub>3</sub><sup>-</sup> (solution 2) were used to

monitor the influence of bicarbonate over time on the electrodes with proven non-selectivity. Bicarbonate and chloride values were determined in duplicate for at least five consecutive days during the recommended lifetime of the electrode (Supplemental Data Table S1). The effect of time on the Roche electrodes was investigated by analyzing the data from QC materials (Roche Precinorm urine [PNU] and Precipath urine [PPU], PreciControl ClinChem [PCCC] Multi 1 and 2) and daily patient medians for the same time period (Supplemental Data Table S2).

### Patient samples

To exclude matrix-related effects, the influence of bicarbonate on chloride measurements in serum samples was investigated. A series of six serum samples with increasing bicarbonate concentrations (8.7–88 mmol/L) and a constant chloride concentration (97.5 mmol/L) were prepared. Stored serum samples with a known low bicarbonate concentration were pooled, divided into portions of 400 μL, and spiked with a constant volume (105 μL)

**Table 1.** Bicarbonate-induced error on chloride measurements at day 1 and day 63

Material* Calibrator: 30 mmol/L HCO <sub>3</sub> <sup>-</sup>	Target tested solution <sup>†</sup>		Mean Cl <sup>-</sup> bias (mmol/L)			
	Cl <sup>-</sup> (mmol/L)	HCO <sub>3</sub> <sup>-</sup> (mmol/L)	Day 1	Day 63	Δ <sup>‡</sup>	SDΔ
Patient median <sup>†</sup>	102.5	24	-0.2	-1.8	-1.6	0.7
Precinormal urine <sup>†</sup>	85.0	15	5.8	2.1	-3.7	2.1
Precipath urine <sup>†</sup>	109.1	30	1.7	1.4	-0.3	0.7
PreciControl Multi 1 <sup>†</sup>	85.4	0	4.8	-1.0	-5.8	2.3
PreciControl Multi 2 <sup>†</sup>	108.0	0	5.9	-1.5	-7.4	0.9
HCO <sub>3</sub> <sup>-</sup> 100	0.0	88	19.0	29.1	10.0	1.4
HCO <sub>3</sub> <sup>-</sup> 100 Cl <sup>-</sup> 100	100.0	88	9.8	18.3	8.5	2.9
HCO <sub>3</sub> <sup>-</sup> 25 Cl <sup>-</sup> 100	100.0	25	-2.6	-3.8	-1.2	0.6
Calibrator: 20–24 mmol/L HCO <sub>3</sub> <sup>-</sup>						
Patient median	102.5	24	0.1	-0.1	-0.2	0.9
Precinormal urine	85.0	15	44.2	42.6	-1.6	0.3
Precipath urine	109.1	30	55.0	55.7	0.7	0.5
PreciControl Multi 1	85.4	0	44.8	41.0	-3.8	1.0
PreciControl Multi 2	108.0	0	55.8	52.4	-3.4	0.8

\*Daily patient median values and quality control material (Precinorm urine, Precipath urine, PreciControl ClinChem Multi 1 and 2) were investigated using two different calibrator formulations: (A) 30 mmol/L HCO<sub>3</sub><sup>-</sup> and (B) 20–24 mmol/L HCO<sub>3</sub><sup>-</sup>. Additionally, 3 aqueous solutions (100 mmol/L HCO<sub>3</sub><sup>-</sup>, 100 mmol/L NaCl+100 mmol/L HCO<sub>3</sub><sup>-</sup> and 25 mmol/L NaCl+100 mmol/L HCO<sub>3</sub><sup>-</sup>) were measured over time using 30 mmol/L HCO<sub>3</sub><sup>-</sup> in the calibrator; <sup>†</sup>Values represent the mean and SDs from four electrodes of two modules; <sup>‡</sup>Δ demonstrates the difference of error at day 63 compared to day 1, which represents the instability over time.

of an increasing sodium bicarbonate concentration.

To determine the clinical impact of bicarbonate concentration on the chloride calibrators, a retrospective analysis of measured chloride and bicarbonate results was performed on 27,254 routine patient samples collected between January 1, 2020, and May 15, 2020, at AZ Groeninge Hospital. This study was approved by the Institutional Review Board of the University Hospital Ghent, Ghent, Belgium (approval no: B670201523174).

### Measurements

Bicarbonate interference was investigated on chemistry analyzers from five different manufacturers: Vitros 4600 from Ortho Clinical Diagnostics (OCD) (Rochester, NY, USA), Atellica CH 930 from Siemens (Erlangen, Germany), AU5800 from Beckman Coulter (Brea, CA, USA), Architect c16000 from Abbott (Park City, IL, USA), and Cobas c501 from Roche Diagnostics (Basel, Switzerland). The different electrodes are described in more detail in Table 1, Supplemental Data Tables S1, and S2.

### Allowable bias criterion

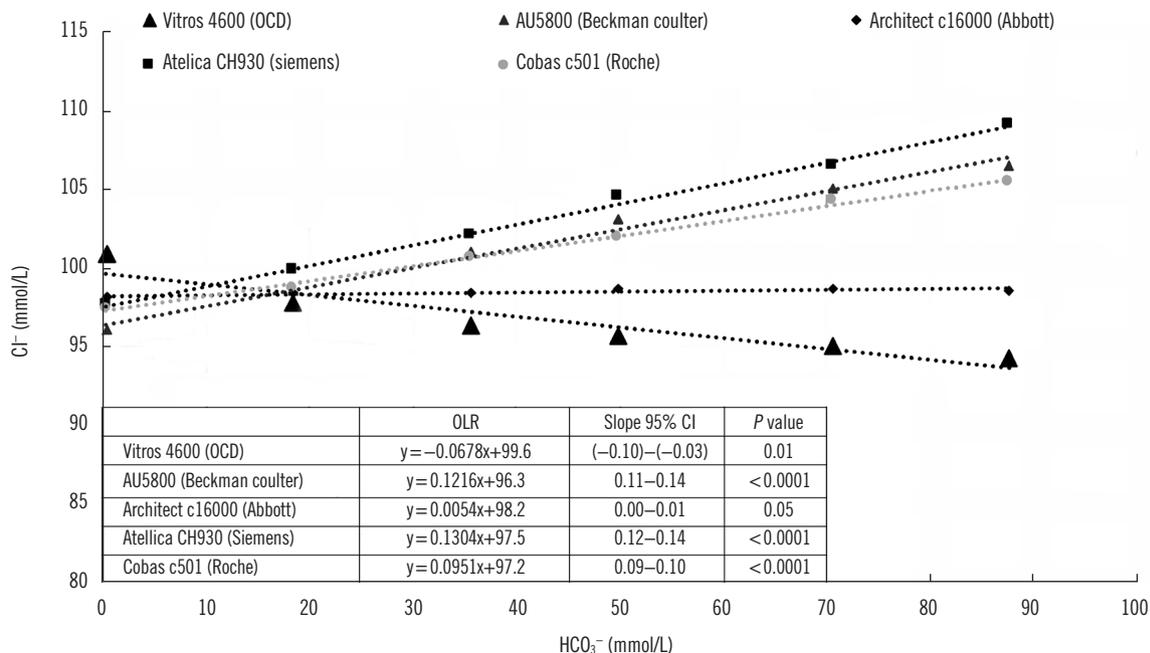
The European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) published data on the intra- and inter-individ-

ual biological variation of serum chloride [7]. Bias specifications derived from these data are very narrow ( $\leq 0.6\%$ ) and cannot be achieved during routine practice. As an alternative, we applied a bias criterion that was indirectly derived from the allowable total error limit of 5% defined by Sciensano, the national institute responsible for external quality control in Belgium [9]. Considering a long-term imprecision of approximately 1.2% on the chloride measurements in our laboratories, a maximum allowable bias of 3.0% was determined ( $= 5\% - 1.65 \times 1.2\%$ ). This bias criterion is much narrower than the limits used by the manufacturers in their kit inserts to evaluate interference, which are often not parameter-specific and are not related to clinical use of the parameter.

## RESULTS

### Effect of varying bicarbonate concentrations for different types of electrodes

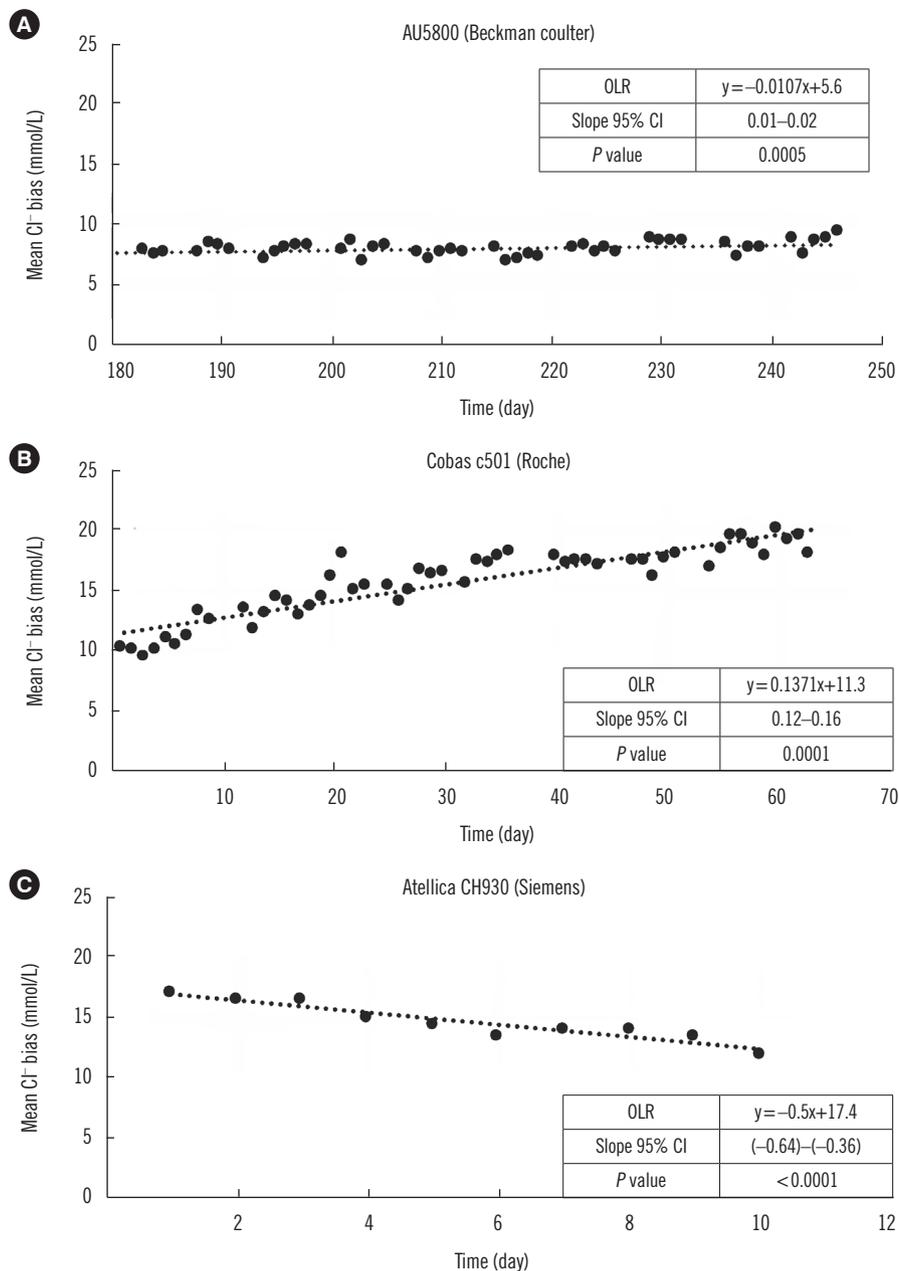
Plotting chloride measurements as a function of the bicarbonate concentration (Fig. 1) showed different trends depending on the type of electrode used. No bicarbonate interference was observed for the Architect c16000 electrode; chloride values remained



**Fig. 1.** Measured chloride values as a function of bicarbonate concentration (solution: 100 mmol/L NaCl+100 mmol/L NaHCO<sub>3</sub>) with electrodes from five different manufacturers: Ortho Clinical Diagnostics (OCD), Siemens, Beckman Coulter, Roche Diagnostics, and Abbott. Due to CO<sub>2</sub> evaporation during the dissolution of NaHCO<sub>3</sub>, the measured bicarbonate concentrations are approximately 10% lower than the concentrations calculated from the amount of bicarbonate added to the solution. For each instrument, the equation for ordinary least-squares regression (OLR), 95% confidence interval (CI) of the slope, and accompanying *P*-values are given. The slope is considered significant when *P*<0.05.

stable, independent of the bicarbonate concentration (mean chloride concentration: 98.5 mmol/L and  $P$ -value of the slope = 0.05). The chloride values determined with the Vitros 4600 electrode showed a slight but significant decrease as a function of the bicarbonate concentration ( $P$ -value of the slope = 0.01). The other analyzers showed an opposite trend, with an overestimation of chloride values at high bicarbonate concentrations and

an underestimation with low bicarbonate concentrations (Fig. 1). In the absence of bicarbonate, a negative bias of  $-2.3$  mmol/L (Atellica CH930) up to  $-4.0$  mmol/L (AU5800) was observed. In contrast, a positive bias of  $+5.4$  mmol/L (Cobas c501) up to  $+9.0$  mmol/L (Atellica CH930) was obtained with the highest bicarbonate concentrations. These three instruments use quaternary ammonium salts in the electrode membrane, highlighting



**Fig. 2.** Mean chloride bias ( $N=2$ ) as a function of time, expressed in days after installation of the electrode (solution: 100 mmol/L NaCl+100 mmol/L  $\text{NaHCO}_3$ ). Only electrodes based on quaternary ammonium salts were tested: (A) Beckman Coulter, (B) Roche, and (C) Siemens. For each instrument, the ordinary least-squares regression (OLR) equation, 95% confidence interval (CI) of the slope, and accompanying  $P$ -values are given. The slope is considered significant when  $P < 0.05$ .

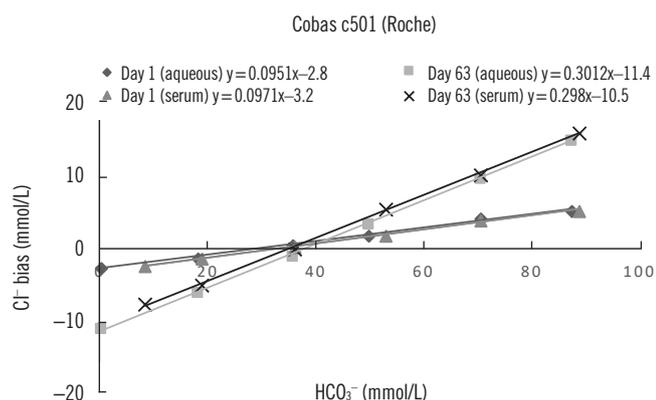
an issue with selectivity toward bicarbonate. Selectivity was quantified from the slope of the regression line. The lowest slope (0.0951) was obtained for the Cobas c501 electrode. The slopes for the AU5800 (0.1216) and Atellica CH930 (0.1304) electrodes were significantly higher, indicating less selectivity ( $P < 0.0001$ ).

### Stability of bicarbonate interference over time

If bicarbonate interference was detected in the first experiment, a second experiment was performed with a solution of 100 mmol/L NaCl and 100 mmol/L NaHCO<sub>3</sub> analyzed during the (complete) lifetime of the electrode. Fig. 2 shows the regression lines for the chloride values obtained with the electrodes based on quaternary ammonium salts, demonstrating different trends.

For the AU5800 electrode, almost no change over time could be discerned (slope=0.0107). However, results were only obtained for 65 days, starting at day 183 after installation of a new electrode. Consequently, it is not possible to arrive at any conclusions regarding the stability of interference during the first weeks after application of the electrode or at the end.

For the Atellica CH930 electrode, the measured values slightly, but significantly, decreased over time with a slope of  $-0.5$  ( $P < 0.0001$ ). On the Cobas c501 electrode, the initial overestimation increased even further as the electrode aged (from 11.5 mmol/L at day 1 up to +20 mmol/L at day 63). These data were obtained with a very high, supraclinical bicarbonate concentration ( $\pm 100$  mmol/L). A similar decrease of selectivity was observed in both aqueous solutions and serum samples spiked with increasing concentrations of bicarbonate (Fig. 3). Samples were analyzed just after installation of a new electrode (day 1) and at the end of its lifetime (day 63). Over this period, a substantial increase in the slopes was observed for both matrices.



**Fig. 3.** Chloride bias (mmol/L) as a function of bicarbonate concentration in serum (97.5 mmol/L chloride) and aqueous solutions (100 mmol/L chloride) measured on days 1 and 63 after installation of the Roche electrode.

Varying interference over time was also demonstrated using daily QCs (Fig. 4A–D: daily means from pre-run and post-run controls), on daily patient medians (data not shown), and on aqueous solutions analyzed on a daily basis (Fig. 4E and F) during the life cycle of two consecutive Roche electrodes. Depending on the bicarbonate concentration, decreasing chloride values (at lower bicarbonate concentration) or increasing chloride values (at higher bicarbonate concentration) were observed. Control materials without any bicarbonate (PreciControl Multi 1 and 2) exhibited the most pronounced decline (approximately  $-6$  mmol/L to  $-7.5$  mmol/L). Solutions with a very high bicarbonate concentration (solutions 1 and 2) showed a marked chloride value increase ( $+8.5$  mmol/L to  $+10$  mmol/L). When these time-induced differences were plotted against the bicarbonate concentration, a strong linear correlation was observed ( $y=0.1538x-5.8$ ,  $r=0.99$ ; Supplemental Data Fig. S1).

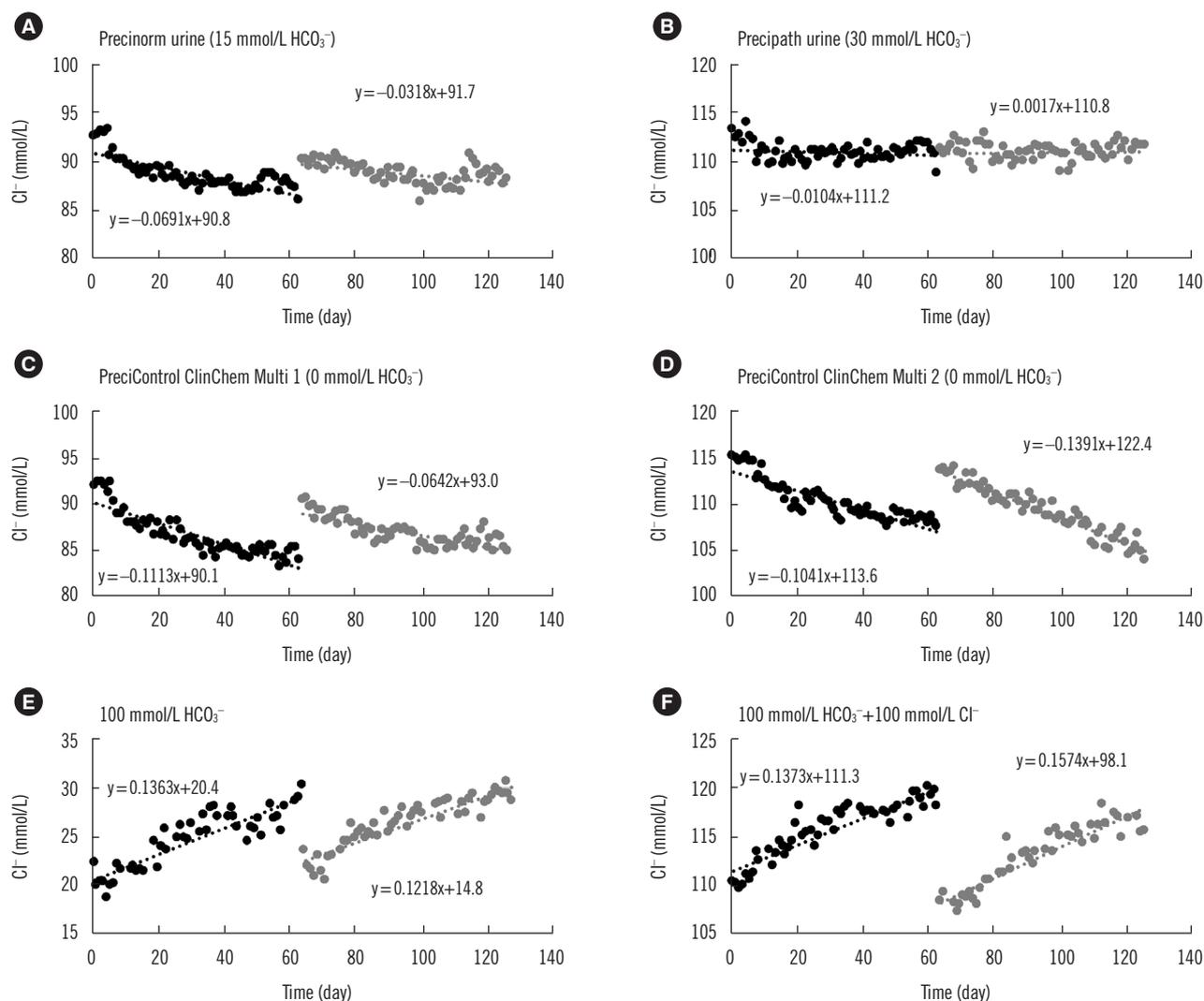
### Influence of the bicarbonate concentration of the calibrator

The above measurements on the Cobas c501 instrument were performed using calibrators with bicarbonate concentrations of approximately 30 mmol/L. Roche reduced the concentration of bicarbonate in their calibrators (20–25 mmol/L) to more closely mimic the physiological concentration. The effect of lowering the bicarbonate concentration in the calibrators was evaluated using a series of six serum samples with a constant chloride concentration (97.5 mmol/L) and increasing bicarbonate concentrations (8.7–88 mmol/L). When the bias on the measured chloride value was plotted against the bicarbonate concentration, the curve shifted to the left when using the new calibrator formulation (Fig. 5). Chloride overestimation in the high bicarbonate range was even more pronounced using the new calibrator ( $+18.8$  instead of  $+15.4$  mmol/L chloride at 88 mmol/L bicarbonate). The chloride underestimation was reduced in the normal and the lower bicarbonate concentration ranges, which are clinically more relevant ( $-6.6$  instead of  $-7.9$  mmol/L chloride at 8.7 mmol/L bicarbonate).

Table 1 shows the bias induced during the lifetime of the electrode for both calibrator formulations. As the bicarbonate concentration of the sample approached the calibrator concentration, the bias became smaller, and the deviation became more limited over time. In the normal range (represented by the patient median), the negative bias almost completely disappeared, and the time-dependent bias effect was limited. For samples with abnormal bicarbonate concentrations, significant deviations remained.

The bicarbonate range in which a reliable chloride measure-

Cobas c501 (Roche)



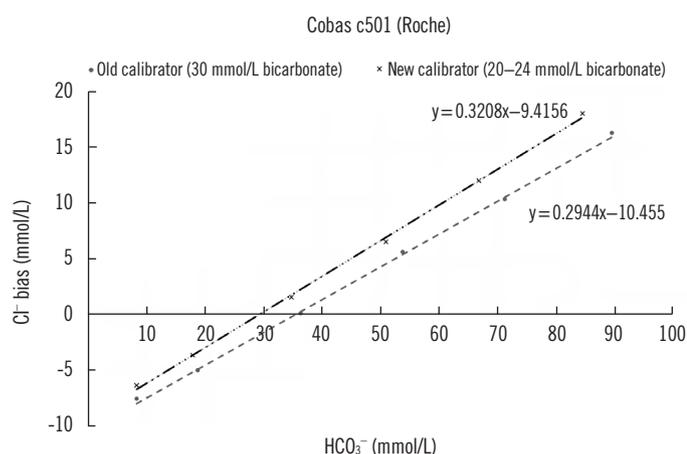
**Fig. 4.** Daily means from pre-run and post-run chloride controls over time for multiple QC materials and aqueous solutions: (A) Roche Precinorm urine, (B) Precipath urine, (C and D) PreciControl ClinChem Multi 1 and 2, (E) solution 1 (100 mmol/L HCO<sub>3</sub><sup>-</sup>), and (F) solution 2 (100 mmol/L NaCl+100 mmol/L HCO<sub>3</sub><sup>-</sup>). Data from two consecutive Roche electrodes are presented: the first electrode was used from day 1 to day 63 (dark gray), and the second was used from days 64 to 126 (light gray).

ment can be consistently obtained was calculated (Fig. 5). When applying the total bias criterion of 3%, measurements were deemed analytically correct within a range of 25.6–45.4 mmol/L using 30 mmol/L bicarbonate or within the range of 20.2–38.5 mmol/L when using calibrators containing 20–24 mmol/L bicarbonate. Based on the distribution of bicarbonate concentrations in routine patient samples from AZ Groeninge Hospital, only 22.7% of the reported results fell within the assured range when using the 30 mmol/L calibrators. For the calibrators with a lower bicarbonate concentration, the percentage of reliable results increased to 87.4%. This simulation illustrates how bicarbonate interference

can partially be controlled by choosing the appropriate bicarbonate concentration for the calibrator.

## DISCUSSION

We investigated bicarbonate interference on chloride electrodes of five different manufacturers. The Vitros 4600 electrode from OCD is the only analyzer that applies direct potentiometry, which implies that the chloride activity is measured without pre-dilution of the sample [5]. The activity coefficient depends on the ionic strength of the solution [10]. The negative trend observed in the



**Fig. 5.** Chloride bias (mmol/L) as a function of bicarbonate concentration in serum samples with a constant chloride concentration (97.5 mmol/L) and an increasing bicarbonate concentration (8.7–88 mmol/L) using old (30 mmol/L  $\text{HCO}_3^-$ ) and new (20–24 mmol/L  $\text{HCO}_3^-$ ) calibrator formulations. Measurements were performed on a Roche Cobas c501 instrument on day 63 after installation of the electrode.

chloride measurements shown in Fig. 1 can be explained by the decreasing chloride activity coefficient as a function of the increasing ionic strength in the solution due to bicarbonate. To exclude the effect of the ionic strength as well as to remove the influence of the decreasing activity coefficient on the measured chloride values, the chloride concentrations were manually recalculated using a constant chloride activity coefficient. After this correction, the negative trend disappeared (results not shown). We therefore conclude that there is no effect of bicarbonate on chloride measurements with the Vitros 4600 analyzer.

By contrast, the Siemens, Beckman Coulter, Abbott, and Roche Diagnostics analyzers use indirect potentiometry that involves a pre-dilution step with a fixed ionic strength solution [5, 11]. The Architect c16000 analyzer uses a solid-state AgCl electrode, which is not influenced by bicarbonate. The 95% confidence interval of the slope, obtained over the six solutions containing increasing bicarbonate concentrations, overlapped with zero, demonstrating a non-significant slope proving the selectivity.

Chloride values determined with the electrodes of Siemens, Beckman Coulter, and Roche Diagnostics were underestimated at low bicarbonate concentrations and were overestimated at high bicarbonate concentrations. We therefore conclude that electrodes using quaternary ammonium salts as the ion exchanger in their membrane are prone to bicarbonate interference. This ion exchanger is not specific for chloride, since bicarbonate having an equivalent ion hydration energy may also bind [7, 11, 12]. The extent of this interference can be calculated using the Nikol-

sky-Eisenman equation, an extension of the Nernst equation: the smaller the selectivity coefficient, the less interference by the interfering ion [13].

No proper selectivity study has been performed using the fixed interference or separate solution method. The selectivity coefficient has to be estimated using the slope of the curve obtained by measuring six solutions with a constant chloride concentration and an increasing bicarbonate concentration shortly after the electrode is installed. This study shows that the estimated selectivity coefficient of the chloride electrodes varies from manufacturer to manufacturer. Higher slopes indicate higher selectivity coefficients, representing greater susceptibility to interference. The largest bicarbonate interference was observed for the Siemens electrode and the smallest was observed for the Roche electrode ( $P < 0.0001$ ).

If the selectivity coefficient remains constant throughout the life of the electrode, the bicarbonate interference becomes predictable. This was the case for the Beckman Coulter electrode, since no trend was observed in the chloride values measured over time. However, the Beckman Coulter electrode has no pre-defined lifetime. According to the manufacturer, the ion-selective membrane contains approximately ten thousands macromolecule layers of quaternary ammonium salts to assure a long life. This molecular crystallization ensures a robust structure and prevents the absorption of endogenous components from the sample, which may contribute to lower interference by other ions.

For the Roche and Siemens electrodes, a variable and opposite selectivity coefficient over time was demonstrated. For the Atellica CH930 electrode, the measured values slightly decreased over time. To exclude the possibility that pre-analytical errors led to the evaporation of the bicarbonate in the test solution, the bicarbonate concentrations were inspected. The bicarbonate concentration decreased by 4 mmol/L over 10 days, which could only explain a decrease in chloride of 0.6 mmol/L (data not shown). A possible explanation might be linked to the structure of the membrane itself. More specialized studies are required to clarify this reason, which is beyond the present scope.

For the Roche electrode, newly installed electrodes appeared to allow a lower number of bicarbonate molecules to bind and therefore exhibited greater selectivity toward chloride. As the electrode aged, the selectivity decreased and the difference between the actual and measured chloride values increased. Co-extraction of other (positively charged) molecules from the sample might explain the higher chloride values obtained over time [14]. This will influence the membrane selectivity, allowing more

interfering ions to move into the membrane phase, which may contribute to the potential build-up. Since the bicarbonate interference is not stable over time, it is very difficult to predict the impact of bicarbonate interference on a patient's chloride result.

The chloride content in the sample also influences the impact of bicarbonate interference. This is demonstrated in Table 1 for the Roche electrode. A solution consisting solely of 100 mmol/L bicarbonate induced a bias ranging from 19 to 29.1 mmol/L (depending on the age of the electrode), whereas a solution containing bicarbonate and NaCl at the same concentration (100 mmol/L) resulted in a bias ranging from 9.8 to 18.3 mmol/L. More specialized studies are required to explain this effect.

Choosing an appropriate bicarbonate concentration in a calibrator is important to control the impact of the interference in routine practice. During calibration, the offset due to the interference of bicarbonate in the calibrators is set to zero. Samples containing less bicarbonate compared with that in the calibrators will be overcompensated, yielding falsely decreased chloride values, whereas samples with a higher bicarbonate concentration will be undercompensated, resulting in falsely elevated chloride values. Only samples within a limited bicarbonate range, close to the concentration in the calibrator, will be measured with minimal bias.

When monitoring time-dependent selectivity, the control materials should contain bicarbonate concentrations that differ from those present in the calibrators (Table 1). With the Precipath urine QC material, which contains approximately 30 mmol/L of bicarbonate, only a very limited time effect was observed ( $-0.3$  mmol/L during the electrode's lifetime). By contrast, Precipath ClinChem Multi controls, without any bicarbonate, exhibited a much more pronounced time effect ( $-5.8$  to  $-7.4$  mmol/L during the electrode's lifetime). Each laboratory should define the clinically relevant bicarbonate range to choose appropriate control materials. A control material with a bicarbonate concentration close to that of the calibrator can be used to monitor inherent analytical variation, without issues of interference. A second control material, at the border of the clinically relevant bicarbonate range, should be included to specifically monitor the selectivity of the electrode.

Recently, Roche reduced the concentration of bicarbonate in their calibrator. The bicarbonate concentration in the calibrator lots used in this study changed from 30 mmol/L for both calibrator concentrations to 20 mmol/L in the high ISE standard (120 mmol/L chloride) and 24 mmol/L in the low standard (80 mmol/L chloride). The lower bicarbonate concentrations caused the complete calibration curve to shift toward less negative elec-

tromotive force (EMF) values, and the slope of the calibration curve became less steep in the ISE high standard compared with that of the low standard. The chloride results became more accurate in the normal and lower bicarbonate ranges, which, from a clinical point of view (e.g., anion gap calculation in the case of metabolic acidosis), is more important than the higher bicarbonate range.

The bicarbonate concentration of a calibrator depends on the calibration lot owing to factors in the production process. The bicarbonate concentrations in the tested calibrator lot numbers of Roche changed from 24 to 25.1 mmol/L for the low standard and from 20 to 22.4 mmol/L for the high standard. For Beckman Coulter, the low standard changed from 21 to 23.8 mmol/L and the high standard changed from 20.7 to 23.4 mmol/L. The extent of over/underestimation of chloride values is defined by the difference in the bicarbonate concentration between the sample and the calibrator. This induces yet another source of variation on the chloride measurement, making it even more complex to predict the magnitude of the induced error and emphasizing the importance of carefully selected internal QC materials.

A limitation of this study is that data for the stability of the Beckman Coulter electrode were obtained over 65 days, starting at day 183 after installation of a new electrode, and the stability was not investigated until defect. This makes it impossible to infer the stability of the selectivity of the electrode over its entire lifetime. Furthermore, a supraclinical solution with a bicarbonate concentration up to 100 mmol/L was used to enlarge the effect occurring in the clinically relevant range.

In conclusion, bicarbonate interference is inherent to electrodes that use quaternary ammonium salts as the ion exchanger in the membrane. The selectivity coefficient varies among manufacturers. Relevant instability over time was observed for the Roche and Siemens electrodes. The bicarbonate-induced error on the measured chloride value depends on the difference in the bicarbonate concentration between the calibrator and test sample. The bicarbonate-induced error varies according to the type, manufacturer, and wear of the electrode; the bicarbonate concentration in the calibrators and the tested sample; and the chloride concentration. Clinical laboratories should be aware of the impact of bicarbonate interference on chloride measurements to assess the reliability of the obtained chloride results and to establish appropriate QC procedures to monitor the effect over time.

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## AUTHOR CONTRIBUTIONS

Oyaert M, Van Dalem A, Godefroid M, Cuykx M, and Monteyne T performed all the experiments, each on a different analyzer. Monteyne T drafted the manuscript. Callewaert N was the project supervisor. All authors participated in revising the paper, provided important intellectual contributions, accept their responsibility for the entire content of the manuscript, and gave final approval of the submitted version.

## CONFLICTS OF INTEREST

There is no conflict of interest.

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None declared.

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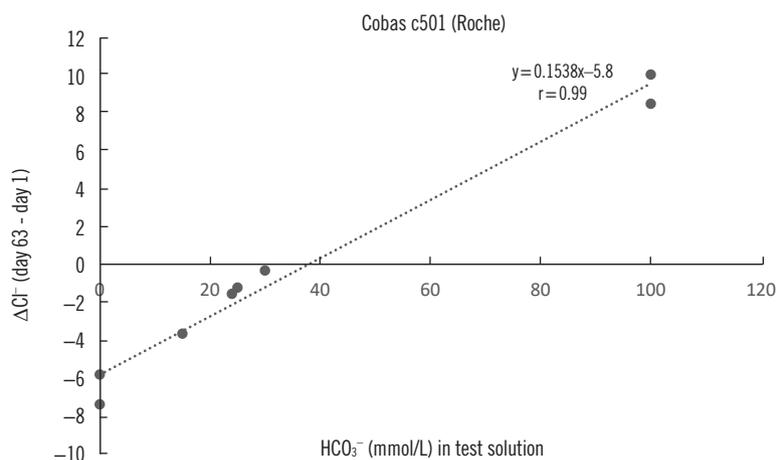
**Supplemental Data Table S1.** Overview of the equipment used to compare electrodes of multiple manufacturers

Analyzer	Company	Electrode type	Measurement principle	Recommended lifetime
Vitros 4600	Orthoclinical Diagnostics	Dry slide chemistry AgCl electrode	Direct potentiometry	Single use
Atellica CH930	Siemens	Polymer membrane electrode using quaternary ammonium salts	Indirect potentiometry	14 days or 5,000 tests
AU5800	Beckman Coulter	Polymer membrane electrode using quaternary ammonium salts	Indirect potentiometry	Change in case of defect
Architect c16000	Abbott	Solid-state AgCl electrode	Indirect potentiometry	90 days, 20,000 tests, or slope < 0.45
Cobas c501	Roche Diagnostics	Polymer membrane electrode using quaternary ammonium salts	Indirect potentiometry	2 months or 9,000 tests

**Supplemental Data Table S2.** Bicarbonate content in QC material/calibrators

Chemical analyzer	QC material	HCO <sub>3</sub> <sup>-</sup> in QC level 1 (mmol/L)	HCO <sub>3</sub> <sup>-</sup> in QC level 2 (mmol/L)	HCO <sub>3</sub> <sup>-</sup> in QC level 3 (mmol/L)	HCO <sub>3</sub> <sup>-</sup> in Cal 1 (mmol/L)	HCO <sub>3</sub> <sup>-</sup> in Cal 2 (mmol/L)	HCO <sub>3</sub> <sup>-</sup> in Cal 3 (mmol/L)	HCO <sub>3</sub> <sup>-</sup> in Cal 4 (mmol/L)
Vitros 4600	BioRad Multiqual unassayed QCs	15.7	/*	26.9	9.8	35.7	< 5.0	23.3
Atellica CH930	BioRad Multiqual unassayed QCs	15.0	21.0	/	4.0	Not detected	/	/
AU5800	BioRad Multiqual unassayed QCs	15.2	19.4	27.2	21.0	20.7	/	/
Architect c16000	BioRad Multiqual unassayed QCs	15.7	19.8	26.8	Not detected	Not detected	/	/
Cobas c501	Roche PCCC Multi	16.7	29.9	/	Old: 30.0 New: 24.0	Old: 30.8 New: 20.5	/	/

\*Not detected; only two standards were used for these assays.  
Abbreviation: Cal, calibrator.



**Supplemental Data Fig. S1.** Difference in measured chloride (mmol/L) between day 63 and day 1 with the Roche electrodes as a function of the bicarbonate concentration in the tested solutions (Precinorm urine, Precipath urine, PreciControl ClinChem Multi 1 and 2, aqueous solution 1 [100 mmol/L  $\text{HCO}_3^-$ ] and solution 2 [100 mmol/L  $\text{NaCl}$ +100 mmol/L  $\text{HCO}_3^-$ ]). The equation of ordinary least-squares regression and the Pearson correlation coefficient ( $r$ ) are given. See Table 1 for the chloride and bicarbonate concentrations of the tested solutions.