Letter to the Editor

Jenny E. Kootstra-Ros*, Eline A.E. van der Hagen, Marith van Schrojenstein Lantman, Marc Thelen and Miranda van Berkel, on behalf of the SKML General Clinical Chemistry Group

(In)direct chloride ISE measurements, room for improvement

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To the Editor,

The calculation of the 'anion gap' (AG) is common practice in the diagnostic workup of patients with a metabolic acidosis. The AG is mostly calculated by subtracting the anions chloride (Cl^-) and bicarbonate (HCO_3^-) from the cation sodium and aids in differentiating between two types of metabolic acidosis: normal AG acidosis, where a decreased HCO_3^- concentration is compensated by increased Cl^-, and high AG acidosis, where the concentration of other anions than HCO_3^- or Cl^- is increased [1]. For disease management, calculation of the AG requires accurate measurement of each of its components.

For the determination of Cl^- multiple state-of-the-art techniques are available. The current reference method to determine Cl^- is the inductively coupled plasma-isotope dilution mass spectrometry (ICP-IDMS) method [2, 3]. In daily routine practice, Cl^- is measured with an ion selective electrode (ISE) using a perm-selective membrane to ensure selective transport of Cl^-. This is either a direct ISE (predominantly in whole blood) or indirect ISE (in plasma/serum).

In the past decades the accuracy of these indirect Cl^- electrodes has been questioned. Results from the Dutch External Quality Assurance (SKML) programs have shown a systematic negative bias of particularly Roche ISE platforms compared to the ICP-IDMS method [4]. Other reports confirm a non-selectivity of Roche Cl^- ISE membranes towards HCO_3^- [5–7]. A HCO_3^- concentration-dependent positive bias was observed for Cl^- measured on the C6000 ISE module [6]. Fortunately, a subsequent call for optimisation of the Roche ISE electrode has led to the launch of an improved Roche ISE calibrator procedure in 2020, where the amount of HCO_3^- in the calibrator was lowered to more physiological concentrations (Roche customer communication, April 2020).

To re-assess the performance of the Roche Cl^- electrode compared to other manufacturers we evaluated the accuracy of Cl^- measurement using EQA data of the first halves of 2019 and 2021 of the SKML schemes for Blood Gas Parameters and Clinical Chemistry in Blood. Samples with the lowest and highest Cl^- were included, with an additional 'spy sample' derived from pooled unprocessed serum. The Cl^- in blood gas analysis shows variation in bias between different platforms and concentrations of Cl^- and is most concordant between Siemens and Radiometer (Figure 1B

Jenny Kootstra-Ros and Eline van der Hagen contributed equally to this work.

Members of the SKML General Clinical Chemistry Group: R.J.A.C. de Beer, Laboratory of Medical Diagnostics, Rivierenland Hospital, Tiel, The Netherlands; D. L. Bakkeren, Department of Clinical Chemistry, Máxima Medical Center, Veldhoven, The Netherlands; M. van Berkel, Department of Laboratory Medicine, Radboud University Hospital, Nijmegen, The Netherlands; E.A.E. van der Hagen, MCA Laboratory, Queen Beatrix Hospital, Winterswijk, The Netherlands; A.A. van der Horst, Laboratory of Clinical Chemistry and Hematology, Jeroen Bosch Hospital, Den Bosch, The Netherlands; J.E. Kootstra-Ros, Department of Laboratory Medicine, University Medical Center Groningen, Groningen, The Netherlands; C. Ramakers, Department of Clinical Chemistry, Erasmus MC, Rotterdam, The Netherlands; M. van Schrojenstein Lantman, Department of Laboratory Medicine, Radboud University Hospital, Nijmegen, The Netherlands and SKML, Nijmegen, The Netherlands; S. Waaijers, Department of Laboratory Medicine, Radboudumc, Nijmegen, The Netherlands.

***Corresponding author: Jenny E. Kootstra-Ros,** Department of Laboratory Medicine, University Medical Center Groningen, Groningen, The Netherlands, Phone: +31 50 3610530, E-mail: j.e.kootstra@umcg.nl. https://orcid.org/0000-0002-1394-513X
Eline A.E. van der Hagen, MCA Laboratory, Queen Beatrix Hospital, Winterswijk, The Netherlands
Marith van Schrojenstein Lantman and Marc Thelen, Department of Laboratory Medicine, Radboud University Hospital, Nijmegen, The Netherlands; and SKML, Nijmegen, The Netherlands. https://orcid.org/0000-0002-5454-990X (M. van Schrojenstein Lantman)
Miranda van Berkel, Department of Laboratory Medicine, Radboud University Hospital, Nijmegen, The Netherlands

and D). For the Clinical Chemistry scheme, most EQA samples measured on Abbott Architect and Siemens Advia/Dimension show a bias within the tolerance limits of 1.1% (based on the combined uncertainty of the target value of 1.0% and maximally allowed desirable bias of 0.4%) as measured by the reference method ICP-IDMS which is consistently over time (Figure 1A and C). Beckman AU performs suboptimally both in 2021 and in 2019, but the accuracy as assessed by calculating the deviation from the target value, of Cl^- measurement on the DXC platform has improved in 2021 (Figure 1A and C). The Cl^- concentrations in EQA samples from 2019 measured by the Roche Cobas ISE platforms display the highest bias compared to the reference value (Figure 1A, left panel mean bias of –5.6% and –4.5% for the C6000 and C8000 platform respectively). Notably, for the 'spy sample' this negative bias is improved in 2021 for all Roche modules, although biases are still observed for the regular EQA samples with low and high Cl^- concentration (Figure 1C, mean bias of –5.0% for the C6000 and –3.6% for both the C8000 and pro platforms). One important difference between EQA samples and the 'spy sample' is the HCO_3^- concentration, which is 'normal' in the latter, and decreased in regular EQA samples due to handling processes. Remarkably, almost 50% of all users of Roche systems apply correction factors to their Cl^- concentration (38 of 112 in 2019 and 52 of 112 users

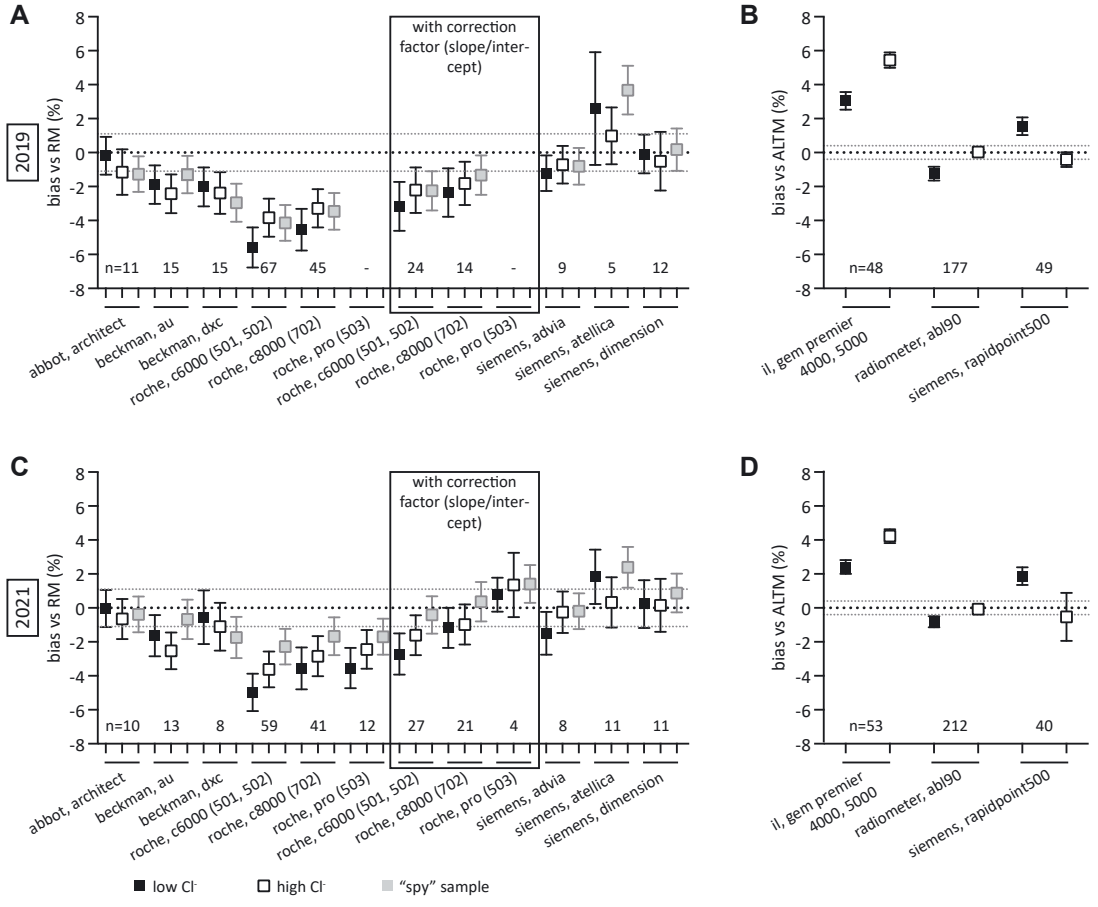

Figure 1: EQA results (SKML) for Cl^- in 2019 and 2021 per instrument type.
EQA results were obtained from the SKML EQA program of years 2019 Q1/Q2 and 2021 Q1/Q2. For each sample in the 'Clinical Chemistry in blood' the participants' results were stratified to analyser type. For blood gas parameters each concentration was measured in both rounds, for Clinical Chemistry each concentration was measured once, except for the 'spy sample' which was measured both in Q1 and Q2. Results deviating >3SD from the mean were excluded. Mean bias (%) vs. Reference Method (RM) target values for low (90.3 mmol/L, closed box), high (128.3 mmol/L, open box) and 'spy sample' (108.0 mmol/L, grey box), for 2019 (A) and for low (89.3, closed box), high (126.0, open box) and 'spy sample' (106.1 mmol/L, grey box), for 2021 (C). Mean bias (%) vs. ALTM for BGA for low (79.1 mmol/L, closed box) and high (118.2 mmol/L, open box) concentrations, for 2019 (B) and for low (77.8 mmol/L, closed box) and high (120.8 mmol/L, open box) concentrations, for 2021 (D). All data are presented as mean bias ± SEM, depicted n-numbers are the number of individual results. Dotted lines represent the combined uncertainty (1.1%) of the target value (1.0%) and maximally allowed desirable bias (0.4%) for A and C and combined uncertainty (0.4%) as derived from interlaboratory SEM (0.2%) and maximally allowed bias (0.4%) for B and D. Data were processed using R Studio and Graphpad Prism 6. Maximally allowed desirable bias is based on a CVi of 1.1% and CVg of 1.3% [8, 11].

in 2021; slope on average 1.03 (range 1.00–1.05) and intercept on average 2.2 in 2019 and 2.0 in 2021 (range 0.0–3.0). Although correction improves accuracy of Cl^- concentration in the 'spy sample', the HCO_3^--associated negative bias for Cl^- in the regular EQA samples persists (Figure 1 A, C inset). Taken together, measurement of Cl^- by direct and indirect methods lacks harmonization and exceeds the desirable bias based on biological variation [8]. Moreover, optimisation in Roche Cl^- calibration procedures only shows improvement when HCO_3^- levels are within normal ranges.

To evaluate the accuracy of Cl^- measurements in patient samples, an interlaboratory comparison was performed using pooled plasma samples with either a low (11 mmol/L), normal (22 mmol/L) or high (32 mmol/L) HCO_3^- concentration. Samples were measured on four different routine chemistry platforms (RC) and blood gas analysers (BGA) and compared to the Cl^- concentrations determined with the ICP-IDMS reference method (Instand, Düsseldorf, Germany). All RC and BGA analysers tested show a HCO_3^--dependent bias for Cl^- from the reference method ranging from –3.8% for the low HCO_3^- concentrations to +4.8% for samples with high HCO_3^--concentrations (Figure 2). The underestimation of Cl^- is most prominent in low HCO_3^- samples on the Cobas 8000 platform (mean bias –3.8%). On the other hand, BGA analysis overestimates Cl^- compared to the reference method in high HCO_3^- samples (mean bias +4.8% for RAPIDPoint and ABL, Figure 2). However, the relevance of this overestimation is limited for the calculation of the AG in these high HCO_3^- samples, since Cl^- concentrations are of particular importance in metabolic acidosis, characterised by a low HCO_3^- concentration.

We evaluated the potential effects of the observed biases in Figure 2 on the clinical decision-making associated with the diagnosis of a metabolic acidosis by in-silico modulation of patient results with high AG acidosis. Therefore, we obtained BGA Cl^-, sodium, and HCO_3^- patient results (RAPIDPoint) from the laboratory information system of a tertiary centre, including samples with a pH of <7.35 and HCO_3^- <20 mmol/L. We (re-)calculated the AG in three ways: (1) using the 'true' Cl^- value (reference-Cl^--AG), (2) using the BGA-Cl^- value of a RAPIDPoint analyser (BGA-Cl^--AG), and (3) using the Cl^- value as obtained by an RC Cobas 8000 (RC-Cl^--AG). For correction, the bias of the most appropriate bicarbonate pool per patient was used (e.g. patient bicarbonate levels of 16 mmol/L and lower were corrected with the bias found in the 11 mmol/L pool, whereas samples above this threshold were corrected with the 22 mmol/L pools) as shown in Figure 2 (–3.8% and –3.4% for RC and +1.5% and + 1.9% for BGA). Subsequently, the proportion of patients above the cut-off value of 12 mmol/L were assessed. The dataset consisted of 2274 patient samples, of which 56% would have a high AG acidosis when using the reference-Cl^--AG. With the BGA-Cl^--AG method 43% of the patients would have an AG above the cut-off, which is within the uncertainty range of

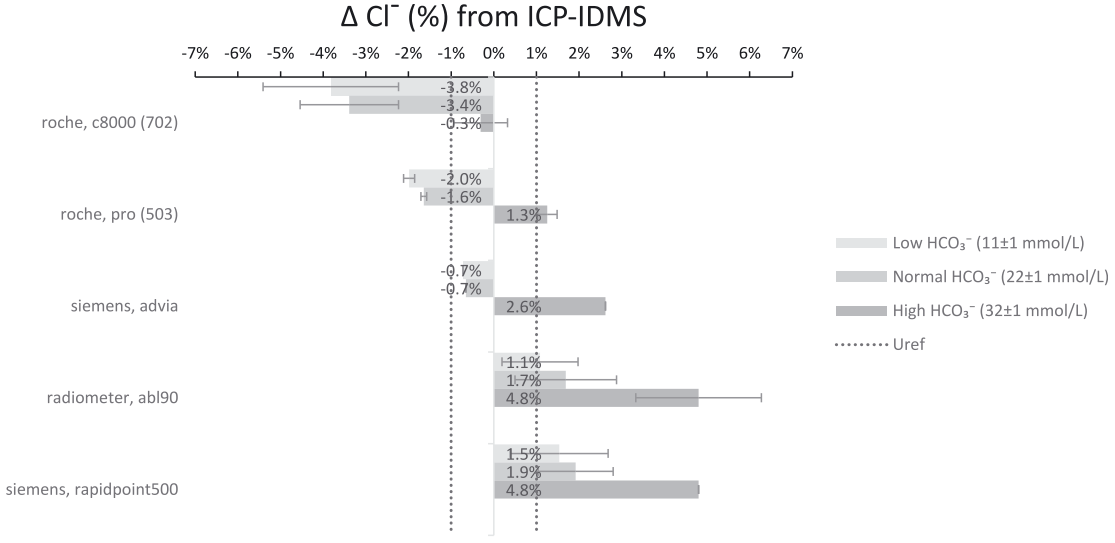

Figure 2: Cl^- deviation from reference laboratory (%) for samples with high, normal and low HCO_3^- levels.
LiHep plasma of anonymised residual patient samples with a low, normal and high HCO_3^- concentration were individually frozen, pooled into the three sample levels, and frozen until analysis. Samples were measured on four different RC platforms: Cobas 8000, Cobas Pro (both Roche, Mannheim, Germany), Advia XPT1 (Siemens, Erlangen, Germany), and BGA RAPIDPoint 500-I (Siemens) and ABL 90Flex(Plus) (Radiometer, Kopenhagen, Denmark). The reference measurement was performed with the ICP-IDMS reference method (Instand, Düsseldorf, Germany). Uref: expanded uncertainty of the reference method (k=2).

the reference-Cl^--AG considering the measurement uncertainty of 1.7 mmol/L [9, 10]. Notably, when AG was calculated with the RC-Cl^--AG as much as 87% of the results would be above the cut-off value of 12 mmol/L. These results indicate a similar referral rate for BGA and reference-based assessment of Cl^- for the determination of the AG, but the RC-Cl^--AG overestimates the AG due to a falsely decreased Cl^- value.

Correction of the RC-Cl^--AG with the average reported slope and intercept as mentioned above resulted in 46% of results above the cut-off value. These results indicate that the average used correction factor is sufficient in achieving positive rates similar to reference- and BGA-derived Cl^- values. However, the general use of a correction factor could negatively affect patient populations with a high HCO_3^- concentration, due to the HCO_3^--dependent nature of the interference.

Overall, our study shows that despite efforts to improve accuracy of Cl^- measurement by indirect ISE on Roche platforms, a HCO_3^--dependent negative bias still exists. Furthermore, our results indicate that BGA also suffer from a HCO_3^--interference albeit only present at the higher HCO_3^- concentrations.

With this study, we aim to create awareness of the clinically relevant bias – both negative and positive – that is observed in state-of-the-art ISE Cl^- measurements. Standardisation of Cl^- measurement is technically feasible since the traceability chain to a higher order measurement system with reference material is available. Nevertheless, methods subject to concentration-dependent interference due to non-selectivity cannot be harmonized or corrected. This study shows that about 50% of all Roche platform users apply a factor to compensate for the negative bias. However, proper correction would require sample-specific, interferent concentration-dependent correction factors.

We advise not to use the different methods interchangeably, since the interpretation of the AG will introduce diagnostic error. Also, we dispute the introduction of a factor to correct for this, since the magnitude of interference is not constant. Hence, we strongly urge the manufacturers to improve ISE Cl^- measurements and diminish non-selectivity to HCO_3^-, as technical feasibility has been proven by others.

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