Discordance between Measured and Calculated Total Carbon Dioxide

J. P. J. Ungerer,¹ M. J. Ungerer,² and W. J. H. Vermaak¹

Recent studies on the agreement and correlation between measured and calculated total CO₂ (TCO₂) have yielded conflicting results. Pre-analytical variation could have been partially responsible. While keeping such variables at an absolute minimum, we found excellent correlation (r = 0.96) in 88 samples, with only a small variation in agreement between measured and calculated TCO₂ values (SD = 1.1 mmol/L), which could be a function of variation in apparent pK (pK'). A subsequent evaluation of 913 consecutive samples, routinely analyzed, yielded similar results. These results suggest that some of the discrepancies reported in the literature could be ascribable to differences in sample types and sample handling. Rigid control of pre-analytical procedures is therefore a prerequisite in studies on this topic. The two methods were found to agree over a wide range of values, such that either of them could be used to evaluate clinical acid–base status accurately.

Additional Keyphrases: blood gases · acid–base status · sample handling · variation, source of

In the assessment of acid–base status, total carbon dioxide can be measured directly (measTCO₂) or calculated (calcTCO₂) from measured pH and pCO₂ by way of the Henderson–Hasselbalch equation:

\[ \text{pH} = \text{pK}' + \log \frac{[\text{HCO}_3^-]}{[\text{CO}_2]} \]

The calcTCO₂ is equal to \([\text{HCO}_3^- + (S \cdot \text{pCO}_2)]\). pK' represents the apparent first dissociation constant of carbonic acid and S the solubility coefficient for CO₂ gas in plasma.

Despite numerous papers on the subject of measTCO₂ and calcTCO₂, controversy still exists with respect to the accuracy of each method and the degree with which they correlate (1–12). The constants used in the Henderson–Hasselbalch equation, especially the pK', are not constant in blood samples but vary because of the effect of changes in ionic strength, pH, temperature, and the concentrations of (e.g.) proteins, electrolytes, and urea. However, no consensus exists as to the degree of variability, especially in relation to its use in clinical medicine (13–20). Any variation in pK' would subsequently lead to inaccurate calcTCO₂. Some authors argue that the inconsistence of pK' diminishes the accuracy of calcTCO₂ to the extent that the use of measTCO₂ is warranted (1–6); others disagree (6–12). Contributing to this controversy is the fact that results found in studies comparing measTCO₂ and calcTCO₂ differ (1–12).

By scrutinizing the various publications on this topic, we came to the conclusion that much of the disparity of results among the studies could be partly explained by factors that are not directly method related. Studies differed in many respects, such as analytical methods used, the type of samples analyzed, sample treatment, and whether or not a correction was made to eliminate possible systemic bias between the two methods (1–12). For example, a few authors (1, 7) comparing arterial calcTCO₂ with venous measTCO₂ have, in some instances, not mentioned how the venous samples were handled before analysis. In contrast, others used both methods on identical samples (2, 9, 13).

In this study we assessed the agreement between measTCO₂ and calcTCO₂ methods, while minimizing the pre-analytical variables that could affect the results. We assessed the effect of specimen handling and compared blood–gas analyses in plasma and whole-blood specimens. MeasTCO₂ and calcTCO₂ were also obtained for >900 consecutive samples, to assess the agreement in diverse clinical situations in the routine setting.

Materials and Methods

pH and pCO₂ were measured, and the TCO₂ calculated from these values, with an ABL 3 blood-gas analyzer (Radiometer, Copenhagen, Denmark). The measTCO₂ was determined with an Astra 8 analyzer (Beckman, Fullerton, CA). In this direct measurement method, the sample is acidified and the released CO₂ is measured with an ion-selective electrode. Precision was calculated from internal quality-control measurements, by use of Qualiteccheck (an aqueous-based control material from Radiometer) with the ABL 3 analyzer and Decision control serum (Beckman) with the Astra 8 analyzer.

We selected 88 specimens from those received at our emergency laboratory, to include a wide range of acid–base values. Heparinized arterial blood samples were collected anaerobically. An aliquot was removed and blood-gas analysis was done immediately upon receipt of the sample. The remainder of the sample was transferred to capped tubes and centrifuged (5 min at room temperature). Blood-gas analysis and measTCO₂ were performed simultaneously on the plasma. All analyses were completed within 30 min of the sample's receipt.

The effect of exposure to air was investigated in 31 of these samples. After determining the measTCO₂, we divided the remaining plasma from each sample into two portions. One portion was drawn into a plastic syringe and sealed, the other was left in uncapped tubes. After 4 h at room temperature (–23°C), each sample portion was analyzed for measTCO₂.

To evaluate the agreement between measTCO₂ and calcTCO₂ in the routine clinical setting, we compared results from 913 consecutive samples sent to our emergency laboratory for blood-gas analysis. The samples, anaerobically treated arterial blood, were analyzed for blood gases. Immediately afterwards the blood was centrifuged in uncapped tubes and TCO₂ was measured in the serum, usually within 30 min. The samples originated mostly from various intensive-care units and the accident department of our hospital.

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Results
The blood-gas analysis and measTCO₂ results obtained from the 88 samples are summarized in Table 1. The pH values ranged from 6.94 to 7.61 and the pCO₂ from 14.9 to 72.7 mmHg. Table 2 presents the results of the linear-regression analysis between the various measurements on the different specimens.

The agreement between measTCO₂ and calcTCO₂ was assessed according to the methods proposed by Bland and Altman (21) and is illustrated with bias plots (Figure 1). The analytical precision studies, performed with quality-control samples, yielded an SD of 0.6 mmol/L for calcTCO₂ and 0.7 mmol/L for measTCO₂. The expected and observed limits of agreement (mean difference ± 2 SD) are shown. The expected limits, ± 1.82 mmol/L, are based on the combined imprecision for the two methods, calculated according to the following formula:

Expected SD = \sqrt{(SD measTCO₂)^2 + (SD calcTCO₂)^2}

Figure 2 illustrates the effect on the measTCO₂ in the 31 samples exposed to air over a 4-h period. The average decrease in measTCO₂ was 2.95 mmol/L (SD = 1.40, maximum decrease = 5.8 mmol/L). No change in measTCO₂ was observed for the sample portions not exposed to air.

The pH and pCO₂ values in the group of 913 consecutive samples varied from 6.83 to 7.78 and 11 to 113 mmHg, respectively. To illustrate the diversity of samples, we noted 21 samples with pH < 7.2. Linear-regression analysis of the results gave the following equation: calcTCO₂ = 1.0774(measTCO₂) + 2.7844 (r = 0.942, SEE = 1.7571). Before plotting the variation in differences (Figure 3), we corrected the measTCO₂ according to the linear-regression formula, so as to remove any systematic bias between the two methods. A similar procedure was followed by Masters et al. (9) The SD of the differences between measTCO₂ and calcTCO₂ was 1.76 mmol/L. Without the correction for bias this SD was 1.79 mmol/L. To assess its variability, we also calculated the pK' in each individual case, according to the equation pK' = pH - log[(measTCO₂ - pCO₂ - 0.0306)/ (pCO₂ - 0.0306)]. The frequency distribution of pK' is illustrated in Figure 4. The mean pK' was 6.96 (SD = 0.041, CV = 0.67%).

Discussion
According to the regression analysis (Table 1), blood and plasma calcTCO₂ values were well correlated (r = 0.99). The plasma calcTCO₂ correlated better with measTCO₂ than did blood calcTCO₂, but in both instances the correlation was near perfect. The mean calcTCO₂ was also 0.71 mmol/L lower in plasma than in whole blood, even though we used anaerobic conditions.

The effect of exposure to air was clearly demonstrated, with the measTCO₂ decreasing by as much as 5.8 mmol/L

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**Table 1. Acid–Base Results for 88 Specimens**

<table>
<thead>
<tr>
<th></th>
<th>Blood</th>
<th></th>
<th>Plasm</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>measTCO₂</td>
<td>CalcTCO₂</td>
<td>measTCO₂</td>
<td>CalcTCO₂</td>
</tr>
<tr>
<td>pH</td>
<td>mmol/L</td>
<td>mmol/L</td>
<td>mmol/L</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Mean</td>
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<td>35.64</td>
<td>22.55</td>
<td>7.47</td>
</tr>
<tr>
<td>SD</td>
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<td>14.49</td>
<td>7.83</td>
<td>0.13</td>
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</table>

**Table 2. Linear-Regression Analysis TCO₂ Results**

<table>
<thead>
<tr>
<th></th>
<th>Intercept</th>
<th>SEE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>0.992</td>
<td>1.206</td>
</tr>
<tr>
<td>Blood</td>
<td>1.037</td>
<td>0.978</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.944</td>
<td>0.538</td>
</tr>
</tbody>
</table>

**Figure 1.** Bland and Altman (21) plot of mean observed between calcTCO₂ and measTCO₂ vs the difference between the methods: (top) blood, (bottom) plasma. Observed limits of agreement are based on ±2 SD of the predicted combined analytical imprecision of the methods (1 SD = 0.92 mmol/L).

(Figure 2). In contrast, plasma samples could be stored anaerobically for long periods without adversely affecting acid–base results. Meticulous care pertaining to sample handling and time lapse before measurement is critical for accurate results. We did not evaluate the effect of storage time on acid–base measurements in whole blood.

Both blood and plasma calcTCO₂ show good agreement with measTCO₂ (Figure 1), with the SD of the difference being 1.64 and 1.43 mmol/L, respectively. The positive mean difference indicates a calibration disequilibrium between measTCO₂ and calcTCO₂. MeasTCO₂ could be a more nearly accurate substitute for calcTCO₂ if results were corrected for this difference.

We used the one-tailed statistical test of Levin (22) to test whether the various standard deviations for mean differences (Figure 1) were significantly different from each
other. The SD of the difference between plasma calcTCO₂ and measTCO₂ was less than between blood calcTCO₂ and measTCO₂ (1.43 vs 1.64 mmol/L), but not significantly so ($F = 1.3153, P > 0.05$). The expected SD of the difference between calcTCO₂ and measTCO₂ attributable to combined analytical variation was 0.91 mmol/L. This was significantly less than 1.43 mmol/L, the SD of the difference between plasma calcTCO₂ and measTCO₂ ($F = 2.4694, P = <0.001$). Because the plasma calcTCO₂ and measTCO₂ were determined simultaneously on the same sample, this greater than expected variation was probably caused by method-related factors, e.g., changes in $pK'$, which would affect calcTCO₂. This "method-related" variation can be quantified by the formula

$$\text{Method-related SD} = \sqrt{(\text{observed SD})^2 - (\text{expected SD})^2}$$

where observed SD represents the observed SD of the difference between plasma calcTCO₂ and measTCO₂ when the pre-analytical variables were being minimized. The calculated value for the method-related SD was 1.1 mmol/L. Thus the difference between measTCO₂ and calcTCO₂ attributable to differences inherent to the methods should be <2.2 mmol/L in 95% of samples analyzed.

If we apply the same formula to the results obtained by O’Leary and Langton (1), the method-related SD would be 3.2 mmol/L, not 1.1 mmol/L. The reason for a higher method-related SD may be related to variables not related to the methods per se. Sample treatment may have played an important role. Furthermore, another contributing factor could be that venous measTCO₂ was compared with arterial calcTCO₂. Arterial and venous acid–base analytes can differ markedly in certain clinical conditions (23–25), the difference in calculated bicarbonate being about 2 mmol/L on average (26). Venous samples could also contain fluids given intravenously, which would also affect measTCO₂. The difference between measTCO₂ and calcTCO₂ should not therefore be ascribed solely to $pK'$ changes, nor should the measTCO₂ be used to calculate $pK'$, if conditions are not standardized and identical plasma samples are not used.

In the group of 88 patients, which included patients with serious acid–base disorders, no obvious outliers were found that would have serious implications on patient treatment. In certain studies, in which the difference between measTCO₂ and calcTCO₂ was unacceptably large, the authors based their views on large discrepancies found in individual cases (2, 3). This prompted us to investigate a large number of consecutive acid–base determinations performed by our laboratory. The subjects included patients with a wide variety of disorders, some with severe acid–base and electrolyte disturbances.

The correlation for this group of 913 samples was excellent ($r = 0.942$). The "method-related" SD, or SD not explained by analytical variation, of the differences between measTCO₂ and calcTCO₂, calculated as above, was 1.51 mmol/L. The results from the studies of the group of 88 indicate that this variance would be even smaller if the calcTCO₂ had been determined in plasma instead of whole blood and if the samples had been centrifuged anaerobically. In calculating the $pK'$ from measTCO₂, pH, and $pCO₂$, the SD of the $pK'$ values was only 0.041. This variation includes the combined analytical variation of the three measurements, and one can assume that the true variation in $pK'$ is even less. However, we did encounter a few obvious "outliers," i.e., samples with large differences between calcTCO₂ and measTCO₂ ($a$–$f$ in Figure 3). These outliers resulted in abnormal values for calculated $pK'$, correspondingly marked $a$–$f$ in Figure 4. Serial samples were analyzed in the three patients in whom outliers $a$, $d$, 

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**Fig. 2:** MeasTCO₂ in 33 samples before and after 4 h of exposure to air at room temperature. 

**Fig. 3:** Bland and Altman (21) plot of mean observed plasma calcTCO₂ and measTCO₂ vs the difference between the methods, in 913 consecutive samples. 

- $a$–$f$: "outliers," as referred to in the text. Observed SD of the difference = 1.76 mmol/L; expected SD of the difference = 0.92 mmol/L. 

**Fig. 4:** Frequency distribution of $pK'$, as calculated from the 913 consecutive samples analyzed. 

The "outliers" marked $a$–$f$ correspond to those in Fig. 3. (see text)
and e occurred; the results are summarized in Table 3. The mean TCO₂ in samples a, d, and e does not appear to fit the pattern observed in the respective serial determinations; it therefore seems likely that the discrepant mean TCO₂ values were caused by a random laboratory error. The other three outliers were from an isolated analysis done only once on a patient, and cannot be explained by the available data. pK₂ differences may have been the cause but in light of the large number of specimens examined, as well as the three obvious errors of mean TCO₂, these "outliers" were more likely the result of random laboratory or analytical errors in one or more measurements. This is also the view of Gennari in explaining widely discrepant results (12).

In evaluating the results of these comparison studies with mean TCO₂ and calc TCO₂, it is also worth noting the work of Lustedt et al. (27), who studied the correlation between various contemporary methods for determination of mean TCO₂. They examined the correlation between the various mean TCO₂ methods by linear-regression analysis and obtained the following results: r and SEE ranged from 0.975 to 0.969 and from 0.932 to 1.962, respectively. These results actually vary more than ours (Table 2). Furthermore, a few "outliers" among the 60 patients they examined are evident in their linear-regression figures. For example, one method of mean TCO₂ gave a value of ~9 mmol/L corresponding to a value of 3 mmol/L by another method. Thus, differences between mean TCO₂ and calc TCO₂ cannot summarily be ascribed to variation in pK₂, because mean TCO₂ may also be the culprit.

In conclusion, differences between calc TCO₂ and mean TCO₂ may be caused by pre-analytical variation, analytical imprecision, or fluctuations in the constants (e.g., pK₂) used in the Henderson–Hasselbalch equation. It is, therefore, imperative to minimize the pre-analytical variables and to account for analytical imprecision in all studies in which calc TCO₂ and mean TCO₂ are compared, or in which the constancy of pK₂ is assessed. By adhering to these prerequisites, we found the variation in pK₂ so small and the agreement between calculated and measured TCO₂ good enough that clinical misinterpretation was unlikely in the vast majority of cases. Both of these methods seem to be valid and can be used interchangeably with reasonable confidence.

References