# Discrepancy between Measured Serum Total Carbon Dioxide Content and Bicarbonate Concentration Calculated from Arterial Blood Gases

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### Abstract

Large differences between the concentrations of serum total carbon dioxide (TCO<sub>2</sub>) and blood

gas bicarbonate (HCO<sub>3</sub><sup>-</sup>) were observed in two consecutive simultaneously drawn sets of samples of serum and arterial blood gases in a patient who presented with severe carbon dioxide retention and profound acidemia. These differences could not be explained by the effect of the high partial pressure of carbon dioxide on TCO<sub>2</sub>, by variations in the dissociation constant of the carbonic acid/bicarbonate system or by faults caused by the algorithms of the blood gas apparatus that calculate  $HCO_3^-$ . A recalculation using the Henderson-Hasselbach equation revealed arterial blood gas  $HCO_3^-$  values close to the corresponding serum  $TCO_2$ values and clarified the diagnosis of the acid-base disorder, which had been placed in doubt by the large differences between the reported  $TCO_2$  and  $HCO_3^-$  values. Human error in the calculation of  $HCO_3^-$  was identified as the source of these differences. Recalculation of blood gas  $HCO_3^-$  should be the first step in identifying the source of large differences between serum  $TCO_2$  and blood gas  $HCO_3^-$ .

**Categories:** Internal Medicine, Nephrology, Emergency Medicine **Keywords:** total co2 concentration, bicarbonate concentration, carbonic acid/bicarbonate pk', acidbase status, acidemia, hypercapnia, respiratory acidosis, metabolic acidosis

### Introduction

The measured total carbon dioxide  $(TCO_2)$  in serum has three main components, bicarbonate anion  $(HCO_3^{-})$ , dissolved carbon dioxide  $(dCO_2)$ , and carbonic acid  $(H_2CO_3)$ , which is the hydrated form of carbon dioxide. The concentration of  $dCO_2$  is calculated from the partial pressure of  $CO_2$  (pCO<sub>2</sub>). Not taking into account the concentration of  $H_2CO_3$  in this calculation is the source of a negligible error. In body fluids, one  $H_2CO_3$  molecule is at equilibrium with 340 molecules of  $dCO_2$  under normal temperature and physiologic conditions [1]. At a pH of

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7.40, the concentration of  $HCO_3^-$  is 20-fold higher than the concentration of  $dCO_2$  and the concentration of  $H_2CO_3$  is 1.2/340 or 0.004 mmol/L when the  $TCO_2$  concentration is 25.2 mmol/L and the  $HCO_3^-$  level is 24 mmol/L. The ratio  $HCO_3^-/dCO_2$  decreases progressively as the pH decreases but remains routinely high. Consequently, the concentrations of  $TCO_2$  in a serum sample and of  $HCO_3^-$  in a simultaneously drawn blood gas sample should differ only slightly, with  $TCO_2$  typically exceeding  $HCO_3^-$  by less than 3 mmol/L.

In a sample of blood gases, the concentration of  $dCO_2$  is expressed by multiplying the  $pCO_2$  by a proportionality coefficient *S* converting units of partial pressure to units of molar concentration. The value of  $HCO_3^-$  in blood gases is routinely obtained by entering the measured values of pH and  $pCO_2$  in an algorithm representing the Henderson-Hasselbach equation, the general expression of which is as follows:

$$pH = pK' + \log(HCO_3^{-}/[SxpCO_2])$$
 {1}

where pK' is the negative logarithm of the first dissociation constant of H  $_2$ CO $_3$ . The general expression of HCO $_3^-$  concentration is obtained by rearranging equation 1 as follows:

$$HCO_3^- = SxpCO_2 x 10^{(pH - pK)}$$
<sup>(2)</sup>

The coefficient *S* is not constant. The temperature and composition of the solution tested are factors affecting this coefficient. At body temperature, the coefficient *S* obtains the value 0.0301 mmol/L per mm Hg in blood [1]. The pK' of  $H_2CO_3$  in aqueous solutions is around 3.5 [2]. The calculation of this pK' in biological fluids assumes that  $H_2CO_3$  consists of the whole dCO<sub>2</sub>. Therefore, the apparent value of pK' is at or very close to 6.1 under normal conditions [1].

Large differences between simultaneously obtained concentrations of serum TCO<sub>2</sub> and arterial blood  $HCO_3^-$  are encountered in certain instances. These differences complicate the evaluation of the acid-base status of patients and may lead to diagnostic and therapeutic errors. One potential source of the discrepancy is rooted in the erroneous assumption that the pK' of  $H_2CO_3$  is constant at 6.1. It was suggested that this pK' is the cause of erroneous calculation of  $HCO_3^-$  in blood gases [3].

Differences in the concentrations of closely obtained TCO  $_2$  and HCO $_3^-$  may have other origins in addition to a wrong value of pK' applied in the calculation of HCO $_3^-$ . A systematic search for the cause of these differences is merited. We report a patient presenting with a large difference between serum TCO $_2$  and arterial blood gas HCO $_3^-$  in two consecutive sets of serum and blood gas samples. By our calculations, the pK' was not the cause of this difference. A different reason for the difference was detected and led to the correct diagnosis of the acid-base disturbance, which was in doubt because of the TCO $_2$ /HCO $_3^-$  discrepancy.

# **Case Presentation**

Patient

Permission to report this case was obtained from the Raymond G Murphy VA Medical Center Institutional Human Research Committee. This paper was approved as a case report with waiving of the informed consent with the proviso that all identifying information was removed from the text.

A 61-year-old man with acute confusion and shallow and infrequent respirations was transferred to this hospital from a nursing home. He carried the diagnosis of alcoholic cirrhosis with ascites. One year prior to this admission, he had a small bowel resection with ileostomy. Six months later, he had surgical repair of an incarcerated inguinal hernia.

In the days prior to admission, he had consumed an unknown number of oxycodone tablets. On admission, his temperature was  $36.9^{\circ}$  C, blood pressure - 132/68 mm Hg, and pulse rate - 89 per minute. Urine toxicology revealed large concentrations of opioids. Acute respiratory failure was diagnosed. He underwent tracheal intubation and was ventilated. Table *1* shows arterial blood acid-base parameters and serum TCO<sub>2</sub> in the first two sets of simultaneously drawn samples of arterial blood gases and serum. Arterial blood gas values were determined in a satellite "point-of-care" instrument almost immediately after collection of the samples. The concentration of serum TCO<sub>2</sub> exceeded that of arterial blood gas HCO<sub>3</sub><sup>-</sup> by 10.7 mmol/L in the first set of blood samples and by 7.6 mmol/L in the second set. In the first and second serum samples, respectively, sodium concentrations were 139 and 138 mmol/L, chloride concentrations were 102 and 103 mmol/L, and anion gaps were 8 and 9 mEq/L. Lactate level was 1.1 mmol/L in the first blood sample.

Study	Arterial pH	Arterial pCO <sub>2</sub> mm Hg	Arterial HCO <sub>3</sub> <sup>-</sup> mmol/	Serum TCO <sub>2</sub> mmol/L
1st	6.91	149	18.3	29.0
2nd	7.11	74	18.4	26.0

# TABLE 1: Reported simultaneously obtained serum and arterial blood gas acid-base parameters

The patient's respiratory acidosis improved rapidly with ventilation. He was extubated the next day. His mental status improved slowly and returned to baseline in four days. The concentration of serum TCO<sub>2</sub> was between 24 and 27 mmol/L in samples obtained after the first two samples during this hospitalization.

### Investigations of the source of the discrepancy between TCO<sub>2</sub>

### and HCO3<sup>-</sup>

Identification of the source of the difference between serum TCO <sub>2</sub> and arterial blood HCO<sub>3</sub><sup>-</sup> was attempted in three successive steps:

The Potential Effect of Elevated  $pCO_2$ 

The concentration of  $TCO_2$  is considered at first approximation to be equal to the sum of the

concentrations of  $dCO_2$  and  $HCO_3^-$ . Because it is a linear function of  $pCO_2$ ,  $dCO_2$  accounts for progressively larger differences between serum  $TCO_2$  and  $HCO_3^-$  at progressive levels of hypercapnia. We tested whether  $dCO_2$ , calculated as  $0.0301XpCO_2$ , accounted for the differences between serum  $TCO_2$  and blood gas  $HCO_3^-$  in our patient. Figure 1 shows this comparison for the first set of measurements. The figure shows the sum of blood gas  $HCO_3^-$ ,  $dCO_2$ , and  $H_2CO_3$  in an idealized normal subject with an  $HCO_3^-$  of 24 mmol/L and a  $dCO_2$  of 1.2 mmol/L and in the first set of arterial blood gases in the patient of this report, plus the measured  $TCO_2$  in the first blood sample of this patient.



### FIGURE 1: Components of total carbon dioxide content

A: Idealized normal subject. B: Arterial blood gas  $dCO_2$ , plus  $H_2CO_3$ , plus  $HCO_3^-$  in the first set of blood tests in the patient presented in this report. C: Serum  $TCO_2$  in the first set of blood tests in the patient of this report. The concentration of arterial  $dCO_2$  was substantially higher in B than in A. Despite adjustment for the high  $dCO_2$ , the measured concentration of  $TCO_2$  in serum (C) remained substantially higher than its computed concentration in the blood gasses (B).

Figure 2 shows the comparison between the sum of blood gas  $HCO_3^-$ ,  $dCO_2$ , and  $H_2CO_3$ , plus the measured serum  $TCO_2$  in the second set of measurements and in the idealized normal subject.



### FIGURE 2: Components of total Carbon dioxide content

A. Idealized normal subjects. B. Arterial blood gas  $HCO_3^-$ , plus  $dCO_2$ , plus  $H_2CO_3$  in the second set of blood tests in the patient of this report. C. Measured serum  $TCO_2$  in the second set of blood tests in the patient of this report. The measured serum  $TCO_2$  (C) remained substantially higher than the calculated blood gas  $TCO_2$  (B).

Figures 1 and 2 have two noticeable findings. The first finding is that the concentration of  $H_2CO_3$  was truly negligible. The concentration of  $H_2CO_3$  should be shown in red. Red colour columns did not appear in the figures even at a pCO<sub>2</sub> of 149 mm Hg. The main finding of Figures 1 and 2 is that accounting for  $dCO_2$  reduced the difference of the concentrations between serum  $TCO_2$  and arterial blood  $HCO_3^-$  but did not eliminate it. Substantial parts of this difference remained unaccounted for.

### Potential Effect of Changing pK'

The pK' of  $H_2CO_3$  in various solutions is not a fixed value. Variables affecting this pK' include the temperature, acidity, and ionic strength of the solution tested [3-7]. The first two variables have been studied extensively [3-6]. The pK' value increases with increasing acidity and decreasing temperature. Increasing the pK' value in equation 2 lowers the value of  $HCO_3^$ calculated from it for the same value of  $pCO_2$ . In the chapter by Madias and Cohen [1], Table 1-3 shows apparent pK' values in human plasma at temperatures between 10 and 40° C and pH values between 7.0 and 7.6. In this table, the lowest plasma pK', at a pH of 7.60 and a temperature of 40° C, was 6.077 while the highest pK', at a pH of 7.00 and a temperature of 10 °C, was 6.246. Figure 3 shows the values of  $HCO_3^-$  calculated from equation 2 for a  $pCO_2$  of 149 mm Hg and pK' values of 6.1, 6.077, and 6.246. The figure shows substantial differences

between these values. The lowest value of  $HCO_3^-$  was calculated for a pK' of 6.246 and the highest value for a pK' of 6.077.



# FIGURE 3: Bicarbonate values calculated at a pH of 6.91 from a pCO2 of 149 mm Hg by various pK' values

High pK' values, observed at low temperatures and low pH values result in low values of calculated  $HCO_3^-$ .

The purpose of Figure 3 was to show the direction of the changes in the HCO  $_3^-$  concentration that result exclusively from changes in the pK'. Consequently, the pH was kept at 6.91 and the temperature was kept at 37° C in all three calculations. Correction for the effect of temperature on the pCO<sub>2</sub> and use of the correct pK' for each temperature and pH in equation 2 would change the differences shown in this figure and could potentially increase them. Therefore, a variation in the pK' can potentially account for large variations in HCO<sub>3</sub><sup>-</sup> and large differences between serum TCO<sub>2</sub> and blood gas HCO<sub>3</sub><sup>-</sup>.

We tested the effect of changing pK' on the differences between serum  $TCO_2$  and arterial blood gas  $HCO_3^-$  in the patient presented in this report in two sets of calculations. In the first set, we repeated the calculations of  $HCO_3^-$  using a pK' of 6.1 by the following equation:

$$HCO_{3}^{-} = 0.0301 \text{xpCO}_{2} \text{x10}^{(\text{pH} - 6.1)}$$
 {3}

In the second set of calculations, we calculated the approximate pK' value at a pH of 6.91 by

performing a linear regression on pH and pK' values at a temperature of  $37^{\circ}$  C in Table 1-3 of the chapter by Madias and Cohen [1]. We obtained the following regression: pK' = 6.410 - 0.042xpH, r = -0.994. The pK' values calculated by this regression are 6.120 for a pH of 6.91 and 6.114 for a pH of 7.11. Figure 4 shows HCO<sub>3</sub><sup>-</sup> values reported and calculated from equations 3 and 2 for pK' values of 6.1 and 6.120 in the first set of measurements. The calculated values were substantially higher than the reported HCO<sub>3</sub><sup>-</sup> value and very close to each other and the measured serum TCO<sub>2</sub> of 29 mmol/L.



### FIGURE 4: Bicarbonate concentrations in the first arterial blood sample reported and calculated by the use of three different pK' values

The three calculated  $HCO_3^-$  values were substantially higher than the reported value and very close to each other and to the measured serum  $TCO_2$ .

Figure 5 shows  $HCO_3^-$  values reported and calculated from equations 3 and 2 for pK' values of 6.1 and 6.114 in the second set of measurements. Again, the calculated values were substantially higher than the reported  $HCO_3^-$  value and very close to each other and the measured serum  $TCO_2$  of 26 mmol/L.



# FIGURE 5: Bicarbonate concentrations in the second set of arterial blood gasses reported and calculated by three different pK'.

In the first and second set of blood gasses alike, the calculated  $HCO_3^-$  values were substantially higher than the reported value and very close to each other and to the corresponding measured serum  $TCO_2$ .

The pK' value that is required to obtain an  $HCO_3^-$  of 18.3 mmol/L, calculated from the Henderson-Hasselbach equation for a  $pCO_2$  of 149 mm Hg and a pH of 6.91 is 6.299, a value substantially higher than any of the values provided in the table published by Madias and Cohen [1]. From equation 1, the pH value required for the calculation of an  $HCO_3^-$  of 18.3 mmol/L at a  $pCO_2$  of 149 mm Hg is 6.71 when the pK is 6.1. We concluded that it is unlikely that the observed difference between serum  $TCO_2$  and arterial blood  $HCO_3^-$  was due to variation in the value of pK'.

### Potential Effect of Instrument Calibration Error or Human Error

From the previous calculations, we concluded that the reported blood gas HCO  $_3$  values were in error. The similarity of the calculated blood gas HCO $_3$  values in the first and second sets of measurements suggested a possibility that the error may be in the algorithms of the blood gas apparatus. We investigated whether the error in the calculations of HCO $_3$  was due to issues related to the blood gas apparatus or to errors of the operators of the blood gas apparatus by repeating the calculations using the logarithms for the pK' and HCO $_3$  calculations shown in the

manual of the apparatus. At a temperature of 37° C, the pK' values calculated by these algorithms are 6.118 for a pH of 6.91 and 6.114 for a pH of 7.11. Figures 4 and 5 show the calculated blood gas  $HCO_3^-$  values in the first and second set of measurements by the use of these pK' values. These values are also very close to the other calculated values and the values of the reported serum  $TCO_2$ . We concluded that the error in the reported  $HCO_3^-$  values cannot be attributed to the algorithms of the blood gas apparatus.

### **Discussion**

Acid-base disorders indicate health challenges that must be addressed because they result from profound respiratory or metabolic derangements and can be life-threatening. The first step in the management of an acid-base disorder is a correct diagnosis based on accurate measurement or calculation of the acid-base determinants in the blood and on the proper interpretation of these determinants [8]. The diagnosis is based on the observed combination of the measured value of blood pH and the measured or computed values of the pivotal carbonic acid/bicarbonate buffer system [9]. The patient presented in this report illustrates the difficulty encountered when measured and computed acid-base parameters lead to different diagnostic acid-base categories. A pathway leading to deciphering this difficulty is detailed.

In the first two sets of serum and arterial blood gas measurements of our patient, substantial differences were found between the reported concentrations of serum  $TCO_2$  and arterial blood

 $HCO_3^-$  (Table 1). These differences created differing impressions of the underlying acid-base disorder. The arterial blood gases suggested a picture of mixed respiratory and metabolic acidosis while the combination of arterial pH and pCO<sub>2</sub>, plus serum TCO<sub>2</sub>, indicated acute respiratory acidosis alone [10]. Previous medical history assists in the diagnosis of acid-base disorders [9]. In our patient, previous extensive gut surgery could have been the cause of metabolic acidosis, but his mental status on admission did not allow questioning about recent bowel movements. In any event, the critical issue in this and all other cases with similar findings is an identification of the correct one among the two conflicting acid-base parameters. Variations in the pK' of the carbonic acid/bicarbonate system have been extensively studied as a source of TCO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> differences.

Variation of pK' with temperature and acidity of the biological fluid tested was documented in both humans [3-6] and animals [11-12]. It was reported that variation in the pK' values results in substantial variation of the  $HCO_3^-$  values calculated by the Henderson-Hasselbach equation

[13]. Observed differences between serum  $TCO_2$  and  $HCO_3^-$  values calculated by using the Henderson-Hasselbach equation with the pK' fixed at 6.1 were attributed to errors in the value of  $HCO_3^-$  secondary to varying pK' [14-20]. This approach suggests that the correct acid-base value for diagnosing the acid-base disorder and assessing its magnitude is the serum  $TCO_2$ , the measurement of which is not affected by changes in pK'.

The concept that the bicarbonate/carbonic acid pK' is the main cause of discrepancies between serum  $TCO_2$  and blood gas  $HCO_3^-$  has been challenged [21]. One criticism of this concept is that the scale of the pK' change that has been calculated for changes in blood acidity and temperature is not large enough to explain large differences between  $TCO_2$  and  $HCO_3^-$  [22]. As noted, higher values of pK' resulting from high acidity or low temperature of the solution tested lead to lower values of  $HCO_3^-$  calculated by the Henderson-Hasselbach equation. Studies in critically ill patients found only a small variation of the pK' around 6.1 [23-26]. These studies

suggest that varying pK' is not the cause of large differences between  $TCO_2$  and  $HCO_3^-$ .

Another potential source of differences between serum TCO<sub>2</sub> and blood gas HCO<sub>3</sub><sup>-</sup> is an analytical overestimation of TCO<sub>2</sub> [27]. TCO<sub>2</sub> is measured by converting at a temperature of 37° C essentially all CO<sub>2</sub> in the tested sample to HCO<sub>3</sub><sup>-</sup> by a complex enzymatic method producing NAD<sup>+</sup> from a known substrate of NADH under the influence of HCO<sub>3</sub><sup>-</sup>. The remaining NADH is measured by reflectance spectrophotometry [28]. A large list of tested medications and organic compounds showed no interference with this assay [29]. However, overestimation of TCO<sub>2</sub> was reported in some cases and was attributed to interference by organic acids or carbamino compounds [30-31]. Based on this interpretation of the source of differences between serum TCO<sub>2</sub> and blood gas HCO<sub>3</sub><sup>-</sup>, Halperin and coauthors proposed that these differences are not caused by variations in the pK' and that the level of HCO<sub>3</sub><sup>-</sup>, rather than that of TCO<sub>2</sub>, is the correct measurement [32]. In a recent case report, serum TCO<sub>2</sub> was consistently low in a patient with normal blood pH and HCO<sub>3</sub><sup>-</sup> [33]. The presence in the patient's serum of paraproteins causing turbidity and interfering negatively with the enzymatic measurement of TCO<sub>2</sub> was identified as the source of the erroneously low TCO<sub>2</sub> values in this report.

We evaluated the pK' as the potential source of the difference between serum TCO  $_2$  and blood

gas  $HCO_3^-$  in the patient presented in this report. The arterial blood pH in the first set of measurements, at 6.91, was lower than the lowest pH value in Table *1*-3 of the Madias and Cohen chapter [1]. We calculated the pK' value for a pH of 6.91 by performing a linear regression of pH on the pK' values reported in the same table at a temperature of 37 °C. An apparent linearity of the relationship pK'/pH within "physiologic" pH values has been reported [34]. Changing ionic strength is the source of a curvilinear relationship pK'/pH [7]. However, the ionic strength of the blood of our patient was within normal limits. The slope of the pK'/pH regression that we performed was, at -0.042, within the range of slopes (-0.05 to -0.04) reported in the literature [17]. The pK' values that we calculated were almost identical to the values calculated by the algorithms of the blood gas apparatus.  $HCO_3^-$  values calculated using a pK' of 6.1 and pK' values calculated from our pH/pK' regression analysis of the Madias and Cohen Table *1*-3 and from the algorithms of the blood gas apparatus were very close to each other and to the corresponding serum TCO<sub>2</sub> values clarifying the diagnosis of the acid-base disorder and

identifying human error as the source of the observed  $TCO_2/HCO_3^-$  differences.

Based on the closeness of the HCO  $_3$  values calculated by using a pK' of 6.1 and pK' values obtained by other methods, we propose that the first step in identifying the source of large TCO<sub>2</sub>/HCO<sub>3</sub> differences should be a recalculation of the blood gas HCO  $_3$  value using equation 3 with a pK' of 6.1. This calculation is simple and will provide an easy answer to the question of error. If large differences TCO<sub>2</sub>/HCO<sub>3</sub> persist after the first recalculation of the blood gas HCO  $_2$  as 0.0301xPCO<sub>2</sub>. Persistence of large TCO<sub>2</sub>/HCO<sub>3</sub> differences should be to calculate the dCO  $_2$  as 0.0301xPCO<sub>2</sub>. Persistence of large TCO<sub>2</sub>/HCO<sub>3</sub> differences should lead to a new calculation of the HCO<sub>3</sub> using the algorithms of the blood gas apparatus because they may still reveal substantial differences in extreme acid-base disturbances. Persisting differences, unexplained by changes in pK' or pCO<sub>2</sub>, require a systematic investigation of the timing of blood gas and serum samples in patients with unstable acid-base status and investigation of the handling of

the blood samples, with emphasis on the blood gas sample (temperature, time period between drawing and measuring the sample, etc). If sampling issues are discovered, a new simultaneous set of blood gases and serum samples should be obtained. If differences persist in this new set, conditions affecting the measurement of  $TCO_2$  should be investigated [30-31, 33].

## Conclusions

Neither a variation in the apparent carbonic acid pK', nor a high PCO  $_2$  value appears to provide adequate explanations for very large differences between measured serum TCO $_2$  and calculated

blood gas  $HCO_3^-$ . Although analytical errors in the measurement of  $TCO_2$  may be present, the possibility of error in the input of values into the blood gas apparatus or in reporting acid-base parameters should be evaluated first. The first, and simple, step in identifying the source of large differences between serum  $TCO_2$  and blood gas  $HCO_3^-$  is an independent verification of

the calculation of  $HCO_3^-$  using a pK' of 6.1. Equation 3 should be used as the algorithm for this calculation.

# **Additional Information**

### **Disclosures**

**Human subjects:** Raymond G Murphy VA Medical Center IRB issued approval. Approved as a case report with waiving of informed consent with the proviso that all identifying information is removed from the text.

### References

- 1. Madias NE, Cohen JJ: Acid-base chemistry and buffering. Acid-Base. Cohen JJ, Kassirer JP (ed): Little, Brown and Company, Boston; 1982. 3-24.
- Loerting T, Bernard J: Aqueous carbonic acid (H2CO3). ChemPhysChem. 2010, 11:2305-9. 10.1002/cphc.201000220
- 3. Bradley AF, Severinghaus JW, Srupfel M: Variations of serum carbonic acid pK with pH and temperature. J Appl Physiol. 1956, 9:187-200.
- 4. Alexander SC, Gelfand R, Lambertsen CJ: The pK' of carbonic acid in cerebrovascular fluid . J Biol Chem. 1961, 236:592-596.
- Maas AH, van Heijst AN, Visser BF: The determination of the true equilibrium constant (pK 1g) and the practical equilibrium coefficient (pK 1g) for the first ionization of carbonic acid in solutions of sodium bicarbonate, cerebrospinal fluid, plasma and serum at 25 and 38. Clin Chim Acta. 1971, 33:325-43. 10.1016/0009-8981(71)90490-6
- Wills MR, Laite PA: Apparent change in the pK1 of carboinic acid in plasma in response to acute metabolic acidosis in normal subjects. Clin Chim Acta. 1971, 35:514-16. 10.1016/0009-8981(71)90233-6
- 7. Jungas RL: Best literature values for the pK of carbonic and phosphoric acid under physiological conditions. Anal Biochem. 2006, 349:1-15. 10.1016/j.ab.2005.08.020
- Adrogue HJ, Gennari FJ, Galla JH, Madias NE: Assessing acid-base disorders. Kidney Int. 2009, 76:1239-47. 10.1038/ki.2009.359
- 9. Harrington JT, Cohen JJ, Kassirer JP: Introduction to the clinical acid-base disturbances. Acid-Base. JJ Cohen, JP Kassirer (ed): Little, Brown and Company, Boston; 1982. 113-120.
- Brackett NC Jr, Cohen JJ, Schwartz WB: Carbon dioxide titration curve of normal man. Effect of increasing degrees of hypercapnia on acid-base equilibrium. N Engl J Med. 1965, 272:6-12. 10.1065/NEJM196501072720102
- Linden RJ, Norman J: The imprecision arising from the application of the Henderson-Hasselbalch relationship to the blood of anesthetized dogs. J Physiol. 1971, 215:491-507. 10.1113/jphysiol.1971.sp009481
- 12. Boutilier RG, Iwama GK, Heming TA, Randall DJ: The apparent pK of carbonic acid in rainbow

trout blood plasma between 5 and 15 degrees C. Respir Physiol. 1985, 61:237-54. 10.1016/0034-5687(85)90129-X

- 13. Agabiti E, Todisco T, Grassi V, Sorbini CA: Calculation of plasma bicarbonates using Henderson-Hasselbalch equation. Influence of pK changes as a function of pH (article in Italian). Minerva Nefrol. 1973, 20:23-27.
- 14. Sinclair MJ, Hart RA, Pope HM, Campbell EJM : The use of the Henderson-Hasselbach equation in routine clinical practice. Clin Chim Acta. 1968, 19:63-69. 10.1016/0009-8981(68)90189-7
- Tietz NW: Comparison of calculated and experimental pCO2 values. Ann Clin Lab Sci. 1973, 3:341-44.
- Natelson S, Nobel D: Effect of the variation of pK' of the Henderson-Hasselbach equation on values obtained for total CO2 calculated from pCO2 and pH values. Clin Chem. 1977, 23:767-69.
- 17. Hood I, Campbell EJM: Is pK OK?. N Engl J Med. 1982, 306:864-66. 10.1056/NEJM198204083061410
- 18. Rosan RC, Erlander D, Ellis J: Unpredictable error in calculated bicarbonate homeostasis during pediatric intensive care: the delusion of fixed pK'. Clin Chem. 1983, 29:69-73.
- Santoro A, Ferrari G, Spongano M, Cavali F, Zucchelli P: Effects of pK variability on bicarbonate balance in dialysis patients. Artif Organs. 1987, 11:491-95. 10.1111/j.1525-1594.1987.tb02715.x
- 20. Rana AP, Rana SP, Rana JP, Kaur T: Base excess significantly corrected for the variations in apparent dissociation constant for human blood gas testing. Indian J Physiol Pharmacol. 2006, 50:303-8.
- 21. Austin WH: Further comments on the vatiation of pK': computation of [HCO3-] independent of pK' and solubility coefficient. Clin Chem. 1978, 24:1081-82.
- 22. Gennari FJ: Is pK OK? (Letter). N Engl J Med. 1982, 307:683.
- 23. Austin WH, Ferrante V, Anderson C: Evaluation of whole blood pK' in the acutely ill patient . J Lab Clin Med. 1968, 72:129-35.
- 24. De Raedt M, Vandenbergh E, Van de Woestijne KP: Direct and indirect determination of partial pressure of CO2 in the arterial blood of patients with respiratory insufficiency. Clin Sci. 1968, 35:347-52.
- 25. Ryan DH, Holt J: Is pK OK?. N Engl J Med. 1982, 307:683-84.
- 26. Kruse JA, Hukku P, Carlson RW: Constancy of blood carbonic acid pK' in patients during cardiopulmonary resuscitation. Chest. 1988, 93:1221-24. 10.1378/chest.93.6.1221
- Mohler JG, Mohler PA, Pallivathucal RG: Failure of the serum CO2 determined by automation to estimate the plasma bicarbonate. Scand J Clin Lab Invest Suppl. 1987, 188:61-7. 10.1080/00365518709168174
- 28. Ortho-Clinical Diagnostics: Instructions for use ECO2. Corning 965 Carbon Dioxide Analyzer Manual. 1977, 1-10. Accessed: October 30, 2015: www.cmmc.org/cmmclab/IFU/CO2\_030.PDF.
- Krower JS, Tholen DW, Garber CC, Goldshmidt HMJ, Kroll MH, Linnet K, Meier K, Robinowitz M, Kennedy JW (NCCLS Document EPG9-A2): Method Comparison and Bias Estimation Using Patient Samples: Approved Guideline, 2nd edition. National Committee for Clinical Laboratory Standards (ed): CLSI, Wayne, PA; 2002.
- 30. O' Leary TD, Langton SR: Calculated bicarbonate or carbon dioxide?. Clin Chem. 1989, 35:1697-1700.
- Pichette C, Chen CB, Goldstein M, Stinebaugh B, Halperin M: Influence of solutes in plasma on the total CO2 content determination: implications for clinical disorders. Clin Biochem. 1983, 16:91-3. 10.1016/S0009-9120(83)90552-0
- Halperin M, Goldstein MB, Pichette C, Jungas RL, Stinebaugh BJ: Evaluation of the bicarbonate buffer system. Does the pK' really vary in the blood?. Am J Nephrol. 1983, 3:245-247. 10.1159/000166723
- Goldwasser P, Manjappa NG, Luhrs CA, Barth RH: Pseudohypocarbonatremia caused by an endogenous assay interferent: a new entity. Am J Kidney Dis. 2011, 58:617-20. 10.1053/j.ajkd.2011.06.003
- 34. Hastings AN, Sendroy J Jr: The effect of variation in ionic strength on the apparent first and second dissociation constant of the carbonic acid. J Biol Chem. 1925, 65:445-55.