Original

Time course of blood gas analysis and application to inter-instrument difference verification using patient blood sample as a control

Fuminori Ishitobi[*1], †*Shozo Yano, MD, PhD*[*1,2], *Kazuhiro Akeho*[*3], *Hiroyuki Umeda*[*3], *Tsukimi Shoji*[*1], *Atsushi Nagai*[*4]

†Correspondence: Department of Laboratory Medicine, Shimane University Faculty of Medicine, 89-1 Enya-cho, Izumo, Shimane 693-8501, Japan.
E-mail: syano@med.shimane-u.ac.jp
Received December 21, 2023; accepted September 9, 2024
*1 Laboratories Division, Shimane University Hospital
*2 Department of Laboratory Medicine, Shimane University Faculty of Medicine
*3 ME Center, Shimane University Hospital
*4 Department of Internal Medicine 3, Shimane University Faculty of Medicine

ABSTRACT

In recent years, testing equipment located inwards has been managed in a central laboratory, and the same is true for blood gas analyzers. We investigated the time course of blood gas analysis (BGA) using actual samples and attempted to apply it to the inter-instrument difference test of blood gas analyzers. Patients admitted to our hospital between October 2021 and March 2022 with BGA samples taken were analyzed after data collection. 5 mL of blood was collected into a blood collection tube containing heparin sodium, and continuous BGA testing was performed approximately every 2.5 minutes. The reference range for quality control was calculated from (the average) ± 2 × (standard deviation) of the amount of change obtained in 10 consecutive measurements. While pH and pO_2 increased over time, pCO_2, HCO_3^-, Ca^{2+}, and glucose decreased. On the other hand, no obvious changes were observed in Na^+, K^+, Cl^-, and Lactate. In continuous BGA using 2 devices, the HCO_3^- of all 3 patients and the pCO_2 of 2 out of 3 patients decreased beyond the reference range, suggesting differences between the devices, where improvement was observed after the maintenance of the electrode. From the above, the time course of BGA was clarified. Furthermore, it was considered that the present results could be applied to the inter-instrument difference test of blood gas analyzers using actual samples.

〔Lab Med Int 2024; 3(4): 118-124〕

Key Words
Blood gas analysis, Quality control, and Inter-instrument difference

I. Introduction

In hospital laboratory departments, all testing equipment should be able to perform regular maintenance and return constant test results regardless of the person performing the measurement or the time of measurement. For this reason, daily quality control is essential. Furthermore, when there are multiple testing devices, it is also required that the results between the measuring devices or reagents be the same. The same applies to blood gas analysis (BGA). Our hospital has a total of 10 blood gas analyzers, and we monthly conduct quality control using control samples.

The end of this study is to investigate whether an actual blood sample can be used as the quality control for BGA instead of control samples. Most errors in laboratory diagnostics fall outside the analytical phase, and BGA may be vulnerable to errors, especially in the pre-analytical

phase [1),2)]. On the other hand, patient-based real-time quality control has recently been getting attention, especially in point-of-care testing (POCT) [3)-5)]. However, at least in our search, no study has been reported to apply actual blood samples to inter-instrument difference verification for BGA. Unlike a control sample, an actual blood sample contains a large amount of blood cells and plasma components. Thus, a significant difference may be detected using patient blood samples, even if no difference is observed in a control sample. Generally, O_2 is dissolved in the arterial blood of a healthy person at 70 to 100 mmHg, and CO_2 is dissolved in about 35 to 45 mmHg. In the atmosphere, they are approximately 160 mmHg and 0 mmHg, respectively, so if the sample comes into contact with the atmosphere, it is expected that O_2 will increase and CO_2 will decrease over time. According to previous reports, it has been pointed out that even if air bubbles are mixed in by more than 1 to 2% of the blood sample amount, pCO_2 may decrease [6)]. However, we do not know the details of the time course changes in BGA measurement.

Therefore, we conducted a single-center retrospective observational study using the results of continuous BGA measurements performed by clinical engineers or clinical laboratory technicians during extracorporeal circulation, etc. Furthermore, based on the results, we attempted to examine the agreement or differences between the two inspection devices to address quality control.

II. Methods

This clinical study was conducted with the approval of the clinical research committee of Shimane University (Approval number: 20220914-3), under the ethical standards established by the institution in which the experiments were performed or following the Helsinki Declaration.

The study design was a single-center retrospective observational study. The subjects were patients admitted to our hospital between October 2021 and March 2022 and had blood gas samples taken. The following data collected from medical records was used: date and time of blood sampling, measuring equipment, measurement end time, pH, pO_2, pCO_2, HCO_3^-, Na^+, K^+, Cl^-, Ca^{2+}, Glucose, and Lactate. ABL825FLEX (Radiometer Co. Tokyo, Japan) was used as the analyzer, and VP-H050K (Terumo Co. Tokyo, Japan) was used as the vacuum blood collection tube. The room temperature in our facility was monitored all the time and kept at 22-24℃.

1) Continuous BGA measurement

5 mL of blood was collected into a blood collection tube containing heparin sodium, and measurements were performed 10 times at regular intervals. The measurement interval was approximately 2 minutes and 30 seconds, which is the time for one analysis. Before each measurement, the sample was mixed by inversion. A total of 10 items including measured items: pH, pO_2, pCO_2, Na, K^+, Cl^-, Ca^{2+}, Glucose, and Lactate, and calculated item: HCO_3^- (calculated from measured pCO_2 and H^+) were assessed.

2) Application to inter-instrument difference test

A reference range was calculated from the average value and twice the standard deviation (SD) of the amount of change after 2.5 minutes obtained in the above continuous measurements. Next, using the same sample, we continuously measured in ABL-1 (operating room) and ABL-2 (same equipment as ABL-1) to determine whether there are differences between the devices based on whether the data is within the above reference range or not.

3) Statistics

The average value and SD of the fluctuations in the measured values of each item were calculated using the spreadsheet software Excel (Office 2019, ©Microsoft).

III. Results

1) Continuous BGA measurement (Figure 1)

pH and pO_2 increased over time, and pCO_2, HCO_3^-, Ca^{2+}, and glucose decreased over time. That is, during the 2.5 minutes from one measurement to the next, pH increased by an average of 0.028, pO_2 increased by 1.7 mmHg, pCO_2 decreased by 3.7 mmHg, HCO_3^- decreased by 0.5 mmol/L, and Ca^{2+} decreased by 0.01 mmol/L, glucose showed a decrease of 1 mg/dL (**Figure 1 and Table 1**). On the other hand, no obvious changes were observed in Na^+, K^+, Cl^-, and Lactate. Hemoglobin and hematocrit levels in the blood sample were 7.4g/dL and 23.0%, respectively.

2) Application to inter-instrument difference test

The average value and SD of the amount of change after 2.5 minutes obtained in continuous measurements are shown (**Table 1**). All the measurement results except one (pH 7.552 at the last time point with underline) were within the control limits in our facility. After excluding this data, the quality control reference range (minimum and maximum values) was calculated from the delta values.

Next, continuous measurement data for three people is shown (**Table 2**). In both cases, measurements were taken with ABL-2 and then ABL-1. As a result, it can be seen that among the three patients (A-C), the difference

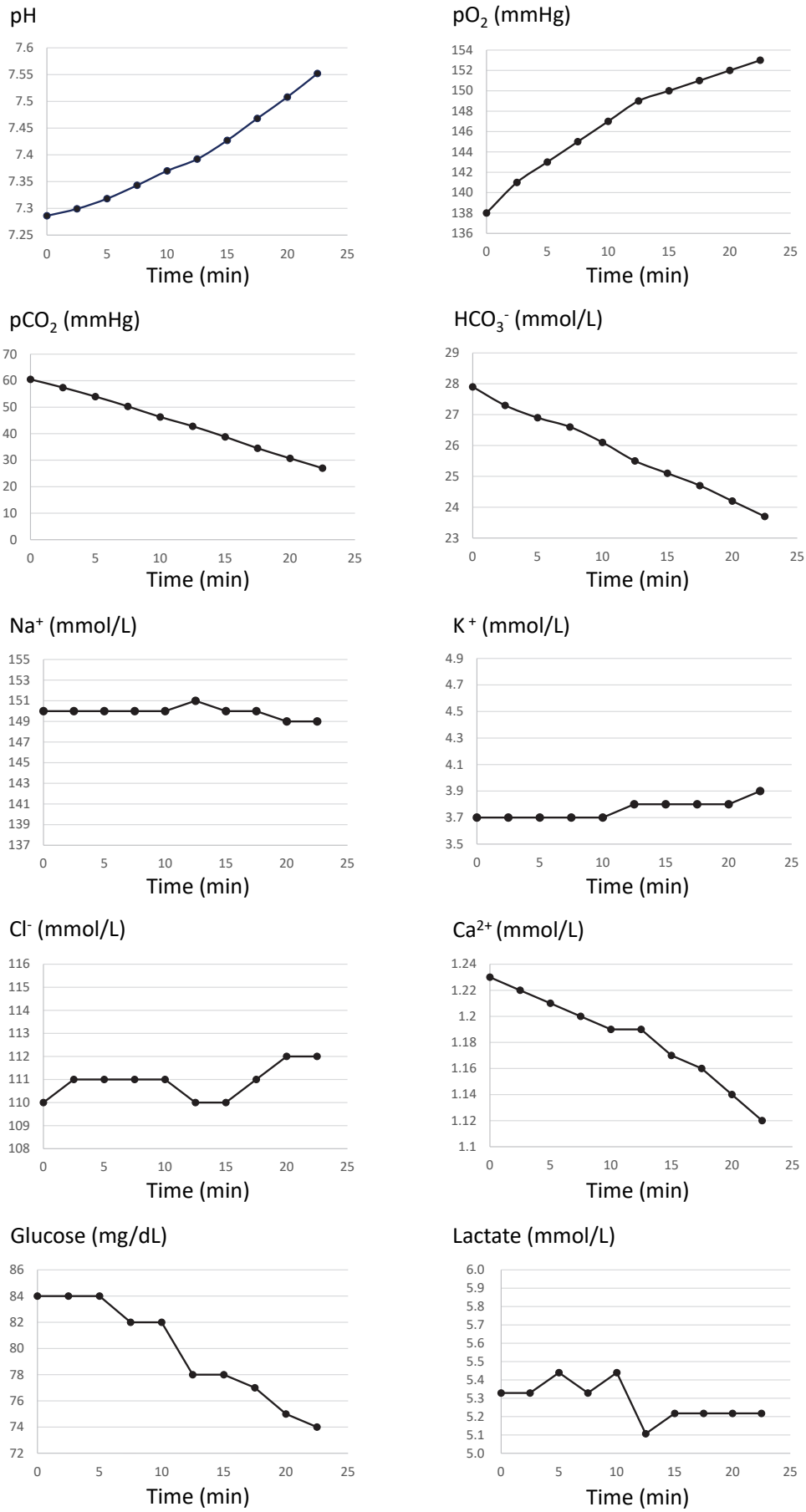

Figure 1 Results of blood gas analysis in 10 continuous measurements.

Table 1 Results from continuous measurements of blood gas analysis, calculated reference range for quality control (Delta), and control limits in our facility for the variables

Time (min)	0	2.5	5	7.5	10	12.5	15	17.5	20	22.5	Delta Mean	Delta SD	Delta Min	Delta Max	Control limits
pH	7.286	7.299	7.318	7.343	7.37	7.392	7.427	7.468	7.508	7.552	0.028	0.010	0.008	0.048	6.850-7.550
pCO_2 (mmHg)	60.5	57.4	54	50.3	46.3	42.8	38.8	34.5	30.7	27	-3.722	0.360	-4.442	-3.003	17.0-160.0
pO_2 (mmHg)	138	141	143	145	147	149	150	151	152	153	1.667	0.707	0.252	3.081	20-580
HCO_3^- (mmol/L)	27.9	27.3	26.9	26.6	26.1	25.5	25.1	24.7	24.2	23.7	-0.467	0.100	-0.667	-0.267	NA
Na^+ (mmol/L)	150	150	150	150	150	151	150	150	149	149	-0.111	0.601	-1.313	1.091	120-180
K^+ (mmol/L)	3.7	3.7	3.7	3.7	3.7	3.8	3.8	3.8	3.8	3.9	0.022	0.044	-0.066	0.110	2.0-8.0
Cl^- (mmol/L)	110	111	111	111	111	110	110	111	112	112	0.222	0.667	-1.111	1.556	95-150
Ca^{2+} (mmol/L)	1.23	1.22	1.21	1.2	1.19	1.19	1.17	1.16	1.14	1.12	-0.012	0.007	-0.026	0.001	0.51-2.20
Glucose (mg/dL)	84	84	84	82	82	78	78	77	75	74	-1.111	1.364	-3.840	1.617	9-270
Lactate (mmol/L)	5.328	5.328	5.439	5.328	5.439	5.106	5.217	5.217	5.217	5.217	-0.012	0.141	-0.294	0.269	0.5-15

NA: not applicable

Table 2 Serial measurements of blood gas analysis before maintenance

Patient	A			B			C		
Equipment	ABL1	ABL2	Delta	ABL1	ABL2	Delta	ABL1	ABL2	Delta
pH	7.329	7.317	0.012	7.309	7.297	0.012	7.287	7.277	0.010
pCO_2 (mmHg)	51.5	59.3	-7.8	47.3	52.6	-5.3	50.0	53.7	-3.7
pO_2 (mmHg)	39.8	38.5	1.3	30.0	28.7	1.3	27.5	26.4	1.1
HCO_3^- (mmol/L)	26.3	29.5	-3.2	23.1	24.9	-1.8	23.1	24.3	-1.2
Na^+ (mmol/L)	140	141	-1	140	141	-1	141	142	-1
K^+ (mmol/L)	4.6	4.7	-0.1	4.8	4.9	-0.1	4.7	4.8	-0.1
Cl^- (mmol/L)	106	106	0	108	107	1	109	108	1
Ca^{2+} (mmol/L)	1.29	1.32	-0.03	1.26	1.29	-0.03	1.21	1.23	-0.02
Glucose (mg/dL)	111	111	0	132	134	-2	146	152	-6
Lactate (mmol/L)	1.443	1.554	-0.111	1.443	1.443	0	1.665	1.776	-0.111

Table 3 Serial measurements of blood gas analysis after maintenance

Patient	D			E			F		
Equipment	ABL1	ABL2	Delta	ABL1	ABL2	Delta	ABL1	ABL2	Delta
pH	7.326	7.312	0.014	7.31	7.297	0.013	7.27	7.26	0.01
pCO_2 (mmHg)	50.3	53	-2.7	44.4	47.1	-2.7	50.7	52.8	-2.1
pO_2 (mmHg)	42.2	41.2	1	38.1	36.3	1.8	32.5	31.7	0.8
HCO_3^- (mmol/L)	25.5	26	-0.5	21.7	22.3	-0.6	22.5	22.9	-0.4
Na^+ (mmol/L)	141	142	-1	141	141	0	142	143	-1
K^+ (mmol/L)	4.5	4.5	0	4.7	4.7	0	4.6	4.7	-0.1
Cl^- (mmol/L)	106	106	0	107	107	0	108	108	0
Ca^{2+} (mmol/L)	1.3	1.32	-0.02	1.27	1.29	-0.02	1.23	1.25	-0.02
Glucose (mg/dL)	102	100	2	121	123	-2	136	139	-3
Lactate (mmol/L)	2.442	2.553	-0.111	2.664	2.886	-0.222	2.886	3.108	-0.222

of pCO_2 (A and B) and HCO_3^- (A, B, and C) between the instruments exceeds the above reference range. Based on this result, it was determined that there was a difference between ABL-1 and ABL-2, and maintenance was performed on both devices. Specifically, the pCO_2 electrode was washed with sodium hypochlorite for 15 minutes, and after washing with water, the membrane was replaced. Furthermore, the pCO_2 and Cl electrodes on the main body side were cleaned with a cotton swab containing water.

The results of continuous measurements on three people after the maintenance are shown (**Table 3**). In all three samples (D-F), the difference of pCO_2 and HCO_3^- became less than 4.44 mmHg and 0.66 mmol/L, respectively. It was thought that the differences between the two instruments that were initially observed disappeared after maintenance. Before maintenance, many results were seen outside the reference range for K, Ca, and glucose, but after maintenance, there was a significant improvement. In addition, when the same specimen was measured at the same time, ABL-1 and ABL-2 showed almost the same in all items (data not shown). Hemoglobin and hematocrit levels in 6 samples of A-F showed 10.2-12.3g/dL and 31.6-37.9%, respectively.

IV. Discussion

This study revealed the time course changes in blood gas measurements. Variables except Na^+, K^+, Cl^-, and Lactate were significantly changed; pH and pO_2 increased over time, and pCO_2, HCO_3^-, Ca^{2+}, and glucose decreased. Our findings indicate that an increase in pH of 0.008-0.048 and a decrease in pCO_2, of 3.003-4.442 mmHg during 2.5 min are clinically acceptable in the continuous measurement (**Table 1**). Furthermore, our findings suggested the feasibility of quality control of BGA using patient samples. Results from continuous measurements of the same blood sample in 2 different analyzers show the outside of the range described above, indicating the presence of inter-instrument difference.

The increase in pO_2 and decrease in pCO_2 over time observed in continuous BGA measurements were thought to be due to contact of the blood sample with the atmosphere. Generally, O_2 is dissolved in the arterial blood of a healthy person at about 70 mmHg to 100 mmHg, and CO_2 is dissolved at about 35 to 45 mmHg. In the atmosphere, they are approximately 160 mmHg and 0 mmHg, respectively, so if the sample comes into contact with the atmosphere, PO_2 will increase over time, CO_2 will diffuse into the atmosphere, and pCO_2 will decrease.

It is thought that a decrease in blood pCO_2 leads to a decrease in bicarbonate and hydrogen ions through acid-base balance, leading to an increase in pH as shown by the Henderson-Hasselbach equation [7].

Acid-base balance:

$$CO_2 + H_2O \rightleftarrows H_2CO_3^- \rightleftarrows H^+ + HCO_3^-$$

Henderson-Hasselbalch equation:

$$pH = 6.1 + \log([HCO_3^-] / 0.03 \times [pCO_2])$$

The temporal changes in gas analysis results when a blood sample comes in contact with the atmosphere have long been studied [8)-10)]. In 1980, Madiedo et al. added 10% volume of air bubbles to an arterial blood sample from an ICU patient collected using a glass syringe, left it at 4℃ for 20 minutes, and measured blood gases. They reported that pO_2 increased significantly (average increase of 11 mmHg) [8]. In addition, Biswas et al. mixed 0.1, 0.2, and 0.5 mL of air into a 2 mL arterial blood sample and measured blood gases every minute. They reported that pCO_2 decreased significantly after 3 minutes of mix with even 0.1mL of air [9]. Toffaletti et al. investigated gas changes when 40 μL of air bubbles (air) were mixed into 1.2 mL of blood sample every 4 minutes [5]. After 20 minutes, pO_2 changed from 70 to 180 mmHg, pCO_2 from 34 to 31 mmHg, and the pH from 7.243 to 7.255. The effect of bubble inclusion was strong when the initial pO_2 was 80-160 mmHg, and an increase of 20-30 mmHg was observed even when the bubble volume was about 20-40 μL. They also reported the effect of Hb level was negligible [10].

On the other hand, there is a report that examined temporal changes in gas analysis results when blood samples do not come into contact with the atmosphere [11]. 500 mL of fresh human whole blood was converted into arterial blood at 37℃ with 12% O_2 and 5% CO_2, and 90 samples were collected and measured under 6 conditions with different containers, temperatures, and times. With the plastic syringe, pO_2 values were significantly higher at both 4℃ and 22℃ after 30 minutes (11.9-13.7mmHg) compared to the measured values immediately after blood collection. On the other hand, no significant changes were observed after 30 minutes with the glass syringe. Therefore, it is recommended that blood gases be measured immediately after blood collection when the sample is collected with a plastic syringe.

Among the electrolytes examined in this study, only Ca^{2+} was observed to decrease significantly over time. This factor is thought to be mainly due to an increase in pH. In other words, at equilibrium, approximately 50% of

the calcium in the blood is dissolved as free ionized calcium (Ca^{2+}) with approximately 1.25 mmol/L, and 40% is bound to proteins such as albumin and globulin. The remaining 10% exists as bound calcium, which is chemically bound to bicarbonate ions, lactate ions, and phosphate ions. This equilibrium is affected by temperature, ionic strength, pH, etc., so as pH increases (alkalosis), binding with proteins becomes stronger, and Ca^{2+} concentration is thought to decrease [12]. Glucose was considered to be degraded and consumed by glycolysis and decreased over time. On the other hand, no change was observed in the lactate concentration, at least during the observation period.

This study demonstrated that the method can be applied to inter-instrument difference testing using human samples based on the change after continuous measurement. Based on the BGA results from 6 patients before and after the device maintenance, the importance of daily maintenance was realized to guarantee the laboratory data. For accurate testing, in addition to maintaining quality control of BGA, the pre-analytical errors including the time to the measurement, removal of air bubbles from the syringe, plugging it immediately, and sufficient mixing of the sample (at least for 40 seconds) before the measurement, and the post-analytical errors such as input error, should be minimized [13),14)]. According to recent studies, all parameters of blood gas and electrolytes remain stable for 30-minute storage at room temperature [15)-17)]. If the delay exceeds 30 minutes, it is recommended that the sample is placed at 4℃ for Lactate measurement [17)]. On the other hand, no strategies are available to prevent changes over time, because air usually touches the blood sample in the current BGA instrument. A completely closed-type instrument may avoid such changes. The limitation of this study is that the reference range may vary depending on the conditions, including the BGA measuring device, temperature, amount of blood collected, and blood cell counts. Leukocytosis may affect BGA results to show pseudo-hypoxemia. However, we have not measured the blood cell counts and thus, described hemoglobin and hematocrit levels from BGA in the results.

V. Conclusion

This study revealed the time course changes in blood gas measurements in our hospital's laboratory. Furthermore, the feasibility of testing differences between blood gas analyzers using patient samples was suggested.

Acknowledgments: We would like to express our sincere gratitude to all the staff, who supported this work.

Funding: This research received no external funding.

Authorship contributions: FI designed the study, prepared and analyzed the data, and was a major contributor to writing this manuscript. SY designed the study, prepared and analyzed the data, and was a major contributor to writing this manuscript. KA and HU contributed to the conception of the study and data acquisition. TG and AN contributed to the interpretation of the analysis results and revisions of the manuscript.

Disclosure of Conflicts of Interest: The authors declare no conflict of interest.

Informed Consent Statement: Not applicable.

References

1) Lippi G, Becan-McBride K, Behúlová D, et al. Preanalytical quality improvement: in quality we trust. Clin Chem Lab Med 2013; 51 (1): 229-41.
2) Salvagno GL, Lippi G, Gelati M, et al. Hemolysis, lipaemia, and icterus in specimens for arterial blood gas analysis. Clin Biochem 2012; 45 (4-5): 372-3.
3) Badrick T, Bietenbeck A, Cervinski MA, et al. Patient-based real-time quality control: review and recommendations. Clin Chem 2019; 65 (8): 962-71.
4) Badrick T, Bietenbeck A, Katayev A, et al. Patient-based real time QC. Clin Chem 2020; 66 (9): 1140-5.
5) Westgard JO, Cervera J. Intelligent Quality Management 2 with IntraSpect™ technology for quality control of GEM® Premier™ 5000 blood gas analyzers− A novel application of the patient sample as its own control. Pract Lab Med 2022; 30: e00273.
6) Williams AJ. ABC of oxygen: Assessing and interpreting arterial blood gases and acid-base balance. BMJ 1998; 317 (7167): 1213-6.
7) Takai D: Acid-base equilibration tests. In: Okumura N, Tozuka M, and Yatomi Y, editors. Kanai›s Manual of Clinical Laboratory Medicine. 33rd ed. (in Japanese) Tokyo: Kanehara-Shuppan Co.; 2010, p.627-33.
8) Madiedo G, Sciacca R, Hause L. Air bubbles and temperature effect on blood gas analysis. J Clin Pathol 1980; 33 (9): 864-7.
9) Biswas CK, Ramos JM, Agroyannis B, et al. Blood gas analysis: Effect of air bubbles in syringes and delay in estimation. Br Med J (Clin Res Ed) 1982; 284 (6320): 923-7.
10) Toffaletti JG, McDonnell EH: Effect of small air bubbles on changes in blood pO2 and blood gas parameters: calculated

vs. measured effects. 2012 [cited 2023 Dec 10]. Available from: URL: https://acutecaretesting.org

11) Knowles TP, Mullin RA, Hunter JA, et al. Effects of syringe material, sample storage time, and temperature on blood gases and oxygen saturation in arterialized human blood samples. Respir Care 2006; 51 (7): 732-6.

12) Committee on Blood Gases and Electrolytes, Japan Society of Clinical Chemistry. Approved recommendation (1993) on JSCC methods on the correction of results using certified serum reference materials for the determination of the concentration of ionized calcium in blood. Rinsho Kagaku (in Japanese). 1993; 22: 291-9.

13) Grenache DG, Parker C. Integrated and automatic mixing of whole blood: an evaluation of a novel blood gas analyzer. Clin Chim Acta 2007; 375 (1-2): 153-7.

14) Kageyama N, Shibata Y, Iino K, et al. Validity of the method of sample mixing in blood gas analysis -Based on CLSI guideline-. J Jap Soc Emerg Med (in Japanese). 2014; 17 (4): 509-14.

15) Çuhadar S, Özkanay-Yörük H, Köseoğlu M, et al. Detection of preanalytical errors in arterial blood gas analysis. Biochem Med (Zagreb). 2022; 32 (2): 020708.

16) Zavorsky GS, van Wijk XMR. The stability of blood gases and CO-oximetry under slushed ice and room temperature conditions. Clin Chem Lab Med. 2023; 61 (10): 1750-9.

17) Puravet A, Rieu B, Phere C, et al. Impact of storage temperature and time before analysis on electrolytes (Na+, K+, Ca2+), lactate, glucose, blood gases (pH, pO2, pCO2), tHb, O2Hb, COHb, and MetHb results. Clin Chem Lab Med. 2023; 61 (10): 1740-9.