Original article

Arterial blood gas tensions - Effect of storage time and temperature

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Abstract

Introduction: Arterial and venous blood gas analysis provide vital information on pH, oxygenation, ventilatory and acid-base status that guide clinician interventions. Factors such as syringe material, sampling technique, type of anticoagulant, storage time and temperature, and analysis may influence the accuracy of results. **Aim:** To determine the effect of storage time and temperature on blood gas analysis. **Method:** Nine 1-ml samples of arterial blood were collected by convenient sampling method involving 25 subjects in a single attempt from an in-dwelling arterial line. Four samples were stored in separate ice bags and four at room temperature. One sample was analysed immediately (*'reference'*). One sample from each storage group was analysed at 15-min intervals for 60-min. The agreement of the *'reference'* value with values of samples taken at 15-, 30-, 45- and 60-min were determined using Lin's concordance correlation and Bland and Altman's 95% limits of agreement (LOA). **Results:** The rate of decay of pH and $PaCO_2$ values over time were similar for samples stored at room temperature and ice. The average difference (95% LOA) in PaO_2 on samples stored at room temperature at 15, 30, 45 and 60 min when compared with baseline were 1.80 (-18 to 21.7), 3.84 (-20.5 to 28.2), 3.40 (-18.8 to 25.6) and 4.36 (-17.5 to 26.2) respectively. The decay in PaO_2 was less pronounced in samples stored in ice with differences of -2.04 (-18.3 to 14.2), -2.64 (-14.2 to 9.0), -3.08 (-20.0 to 13.5) and -2.40 (-12.2 to 7.4) at 15, 30, 45 and 60 min respectively. **Conclusion:** PaO_2 values reduce with time. The changes are more pronounced when samples are stored in room temperature than in ice. The wide LOA would imply that blood gas samples should, as far as possible, be processed immediately.

Keywords: Arterial blood gases, storage time, temperature.

Introduction

Gas analysis of arterial/venous blood provides vital and definitive information concerning the pH (hydrogen ion concentration), oxygenation, ventilatory and acid-base status of the patient. The variables, measured and derived, provide timely information to enable clinicians to intervene appropriately. Many factors such as syringe material, sampling, anticoagulant, storage time and temperature as well as analysis may influence the accuracy of results. Of these, storage time and temperature are thought to commonly impact the results. Particularly, time delay is a crucial factor in the intensive care unit (ICU) setting not only for the accuracy of results but also for the timely management of critically ill patients. There are situations where delays cannot be avoided, particularly when several samples are queued for analysis or when the technical staff are busy with attending to a critically ill patient. In this setting, it is important to ascertain if delay in analysis of the collected sample would impact the results. It is also unclear if such samples can be left in the ambient temperature or if it should be stored in ice to prevent time dependent decay of values.

The American Association for Respiratory Care in their clinical practice guide lines[1,2] and the American

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How to cite this article: Rajasekaran R, Arthur HS, Peter JV. Arterial blood gas tensions - Effect of storage time and temperature. Ind J Resp Care 2014; 3(1):350-6.

Thoracic Society pulmonary function laboratory management procedure manual[3] have advocated the best practices for sampling, handling and analysing arterial blood samples with a warning that blood specimens in plastic syringes are likely to yield inaccurate results, if cooling is done because of delayed analysis. The Clinical and Laboratory Standard Institute (formerly the National Committee for Clinical Laboratory Standards) recommends that it is proper to analyse the samples for arterial blood gases (ABG) immediately after collection. If analysis is delayed for more than 30-minutes, it further permits that samples collected in glass syringes could be stored in iced containers and samples collected in plastic syringes should be placed in room temperature. They specifically state 'Do not cool the specimen' – for plastic syringes.[4] It was advised in an editorial that since plastic syringes have nearly replaced glass syringes, blood sample may be collected in plastic syringes but not to place them in ice.[5] However, some text books recommend icing irrespective of the type of syringe and storage time.[6, 7]

The results of the several studies[8-17] have not been consistent because of the variations between the studies in their design, sample size, type and size of syringe. Some investigators used hospitalised patients and others used domiciliary patients. Similarly, for collection of samples, investigators either performed arterial punctures or used indwelling catheters or capillary sampling techniques. Some investigators used tonometers to create artificially arterialised blood. Some investigators used a special technique to simulate arterial blood specimens that have a known partial pressure of oxygen (PO_2) and partial pressure of carbon dioxide (PCO_2). These techniques allowed for specimens stored in glass or plastic, both cooled and at ambient temperature to be compared with expected values which give us a perspective on how large the differences, if any, can be under well–controlled conditions. It was also reported that presence of air bubbles in a 10% proportion was undesirable because of significant elevation in PO_2 values.[17]

In view of these controversies and as a quality assessment exercise, this study was designed to assess the effect of storage time and temperature on arterial blood gas analysis.

Methods

This study was done in the medical intensive care unit (ICU) of a tertiary care teaching hospital in India. Convenient sampling technique was used as the sampling was done only at the time of clinical need for ABG analysis. Twenty-five subjects were thus included for the study. The sampling was done through the indwelling lines and from each subject, nine 1-ml samples of arterial blood were collected in a single attempt. 3-ml BD A–line arterial blood collection syringes coated with dry calcium and lithium heparin were used. These syringes are specially designed for obtaining blood from arterial lines and manufactured by Becton Dickinson and company. UK. Venous Arterial blood Management Protection (VAMP) needleless shielded cannulas manufactured by Edwards Life Sciences DR, Haina, Dominician Republic were used for collection of blood samples from VAMP blood sampling system. Each sample was mixed thoroughly soon after collection. It was also ensured that no air bubbles were in the samples. Samples were labelled from 1 to 9. Four (S. No 2 to 5) samples were stored in separate ice bags and four (S. No 6 to 9) samples were stored at room temperature ranging from 22 to 24° Celsius. One sample (S. No 1) was analysed within 5-minutes of collection which served as a baseline indicator ('*reference sample*').

One sample from each storage group was analysed at 15-minute intervals till 60 minutes. Each stored sample was thoroughly mixed for not less than 20 sec both horizontally and vertically by rolling between the palms. This process was to mix the sample properly to be homogeneous and stabilise the temperature to uniformity. After mixing, the samples were inspected for homogeneity and also for any presence of clot.

The samples were analysed using GEM Premier 4000 analyser manufactured by Instrumentation Laboratory, USA. The GEM premier 4000 analyser is a portable critical care system for use by qualified health care professionals to rapidly analyse whole blood samples at the point of health care delivery

in clinical settings and in central laboratories. The instrument provides fast, accurate and quantitative measurements of pH, PCO_2, PO_2, sodium, potassium, chloride, ionised calcium, glucose, lactate, haematocrit and co-oximetry [total haemoglobin (tHb), oxyhaemoglobin (O_2Hb), carboxyhaemoglobin (COHb), hethaemoglobin (MetHb) and reduced haemoglobin (HHb)] parameters. Base excess (BEecf), haemoglobin content [tHb(c)], ionised calcium at pH 7.4 [Ca^{++}(7.4)], anion gap (AG), arterial PO_2 to inspired oxygen ratio, alveolar oxygen tension (PAO_2), oxygen saturation [SO_2(c)], serum bicarbonate [HCO_3^-(c)], HCO_3^-std, alveolar to arterial oxygen tension gradient (A-aDO_2) and ratio of arterial to alveolar oxygen partial pressure are the derived parameters obtained through this analyser. Intelligent Quality Management (iQM) is used as the quality control and assessment system for the GEM Premier 4000 analyser. iQM is an active quality process control program designed to provide continuous monitoring of the analytical process with real–time, automatic error detection, automatic correction of the system and automatic documentation of all corrective actions, replacing the use of traditional external quality controls. The primary component of the GEM Premier 4000 analyser is the GEM Premier 4000 PAK cartridge. The disposable, multi–use PAK houses all components necessary to operate the instrument once the cartridge calibration is validated. The components include sensors, solutions, sampler, co-ox optical cell and waste bag. Each time a new cartridge is inserted, the GEM Premier 4000, Calibration Valuation Product (CVP) testing was carried out. These products are external solutions intended to complete the calibration process and final accuracy assessment of the iQM cartridge calibration. This process also ensures the integrity of the new cartridge and overall the analysis system, providing a clear baseline for operation.

Analysis: Results were analysed using STATA® v.11 package. The accuracy of the ABG measurements was determined by the agreement (or lack thereof) between the '*reference*' ABG estimate done immediately and the samples that were analysed later at 15-, 30-, 45- and 60-minutes stored at room temperature and in ice. Agreement was summarised by the mean difference with Bland and Altman's 95% limits of agreement (LOA).[18] Lin's concordance correlation (p_c) which describes the relationship between paired measurements was also used.[19]

Results

The study cohort comprised of 25 subjects from whom samples were drawn from an indwelling arterial line. When the change in pH values were analysed over time, there was a very small and clinically insignificant change in the pH over time for samples stored at ambient temperature as well as those stored in ice *(Tables 1, 2)*. The concordance correlation coefficient was close to 1 *(Tables 3, 4)* and the LOA was also very small and clinically insignificant.

There was also a time-dependent reduction in arterial carbon dioxide tension values over time, with a maximum change of -1.5 mm Hg at 1-hour for samples stored at room temperature. The LOA was -3.2 to 0.2 at 1-hour *(Table 1)*. In samples stored in ice, the change in $PaCO_2$ was smaller with a maximum change of 1 mm Hg *(Table 2)*. The LOA were similar. The concordance correlation coefficients were again close to 1 *(Tables 3, 4)*.

Table 1: Average difference and limits of agreement between stat and samples stored at room temperature.

Variable	15 min	30 min	45 min	60 min
pH	0.007 (-0.02 to 0.04)	0.012 (-0.01 to 0.04)	0.016 (-0.01 to 0.04)	0.022 (0.00 to 0.041)
$PaCO_2$ mm Hg	- 0.32 (-2.4 to 1.8)	- 0.88 (-3.2 to 1.5)	- 1.12 (-3.3 to 1.0)	- 1.48 (-3.2 to 0.2)
PaO_2 mm Hg	1.80 (-18.1 to 21.7)	3.84 (-20.5 to 28.2)	3.40 (-18.8 to 25.6)	4.36 (-17.5 to 26.2)

Table 2: Average difference and Limits of agreement between stat and samples stored in ice:

Variable	15 min	30 min	45 min	60 min
pH	0.007 (-0.01 to 0.03)	0.009 (-0.02 to 0.04)	0.012 (-0.01 to 0.03)	0.012 (-0.01 to 0.03)
$PaCO_2$ mm Hg	- 0.64 (-2.4 to 1.4)	- 1.04 (-3.5 to 1.4)	- 1.08 (-3.4 to 1.3)	- 1.04 (-3.0 to 0.9)
PaO_2 mm Hg	- 2.04 (-18.3 to 14.2)	- 2.64 (-14.3 to 9.0)	- 3.08 (-20.0 to 13.5)	- 2.40 (-12.2 to 7.4)

The average difference (95% LOA) in PaO_2 on samples stored at room temperature at 15, 30, 45 and 60 min when compared with baseline were 1.80 (-18 to 21.7), 3.84 (-20.5 to 28.2), 3.40 (-18.8 to 25.6) and 4.36 (-17.5 to 26.2) respectively *(Table 1, Figure 1)*. The decay in PaO_2 was less pronounced in samples stored in ice with differences of -2.04 (-18.3 to 14. 2), -2.64 (-14.2 to 9.0), -3.08 (-20.0 to 13.5) and -2.40 (-12.2 to 7.4) at 15, 30, 45 and 60 min respectively *(Table 2, Figure 2)*. Although the concordance correlation coefficients were >0.95 for PaO_2, the wide LOA were of concern, particularly for samples that were stored in room temperature *(Figure 1)*.

Table 3: Concordance correlation coefficients between stat and samples stored at room temperature.

Variable	15 min	30 min	45 min	60 min
pH	0.995	0.994	0.992	0.989
$PaCO_2$ mm Hg	0.995	0.991	0.989	0.988
PaO_2 mm Hg	0.963	0.940	0.948	0.947

Table 4: Concordance correlation coefficients between stat and samples stored ice.

Variable	15 min	30 min	45 min	60 min
pH	0.997	0.995	0.995	0.995
$PaCO_2$ mm Hg	0.995	0.989	0.989	0.991
PaO_2 mm Hg	0.977	0.987	0.974	0.990

Discussion

Our study shows that changes occur in the ABG parameters when samples are not processed immediately. The rate of decay over time is small and clinically insignificant with respect to pH and $PaCO_2$ values. Although the mean change over time of PaO_2 is small, the LOA are wide, particularly for samples that were stored in room temperature when compared with those stored in ice.

Several standards of practice for arterial blood sampling and analysis for ABG are available. The American Association for Respiratory Care states that specimens should be analysed within 10–15

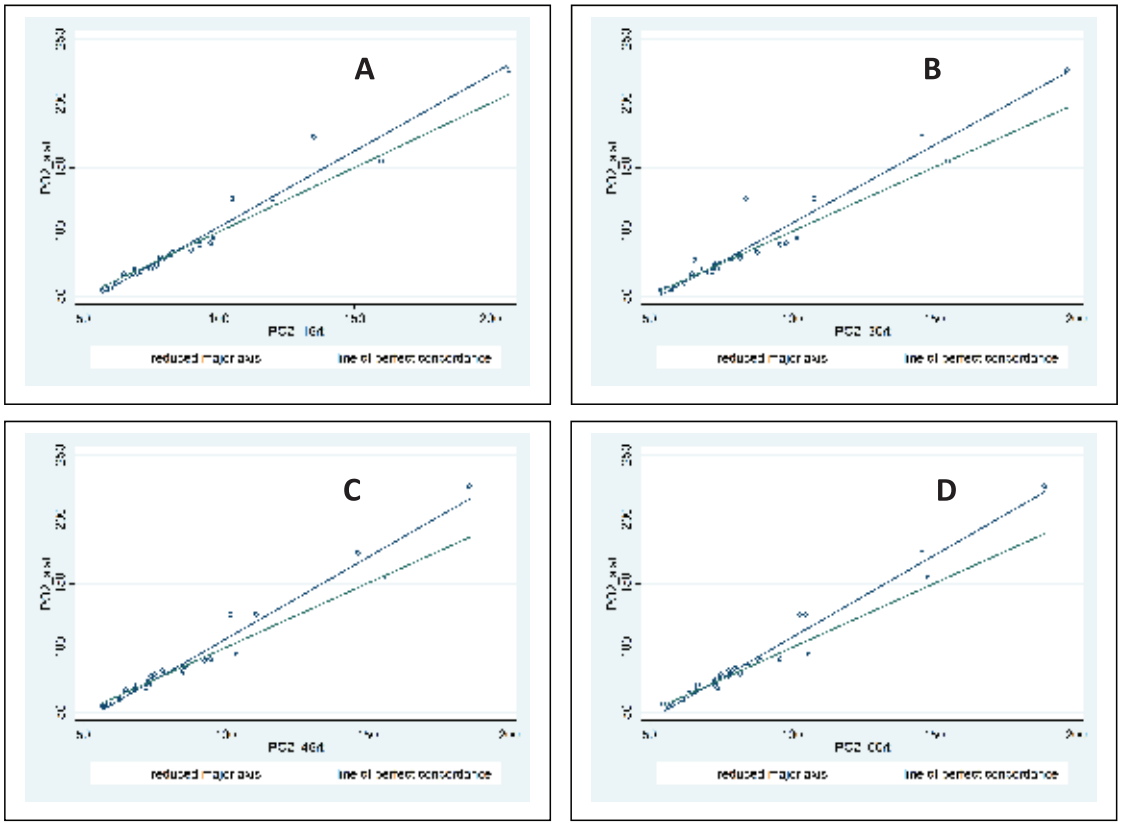

Figure 1: Relationship between baseline PaO_2 (reference) and samples stored in room temperature and analysed over time at 15 min [A], 30 min [B], 45 min [C] and 60 min [D]. Concordance for reference sample and 15, 30, 45 and 60 min samples demonstrated. Solid line shows the linear relationship with the dashed line indicating perfect concordance. Although the overall concordance correlation coefficient is high (>0.94), the graph depicts wide limits of agreement, particularly at high PaO_2 values.

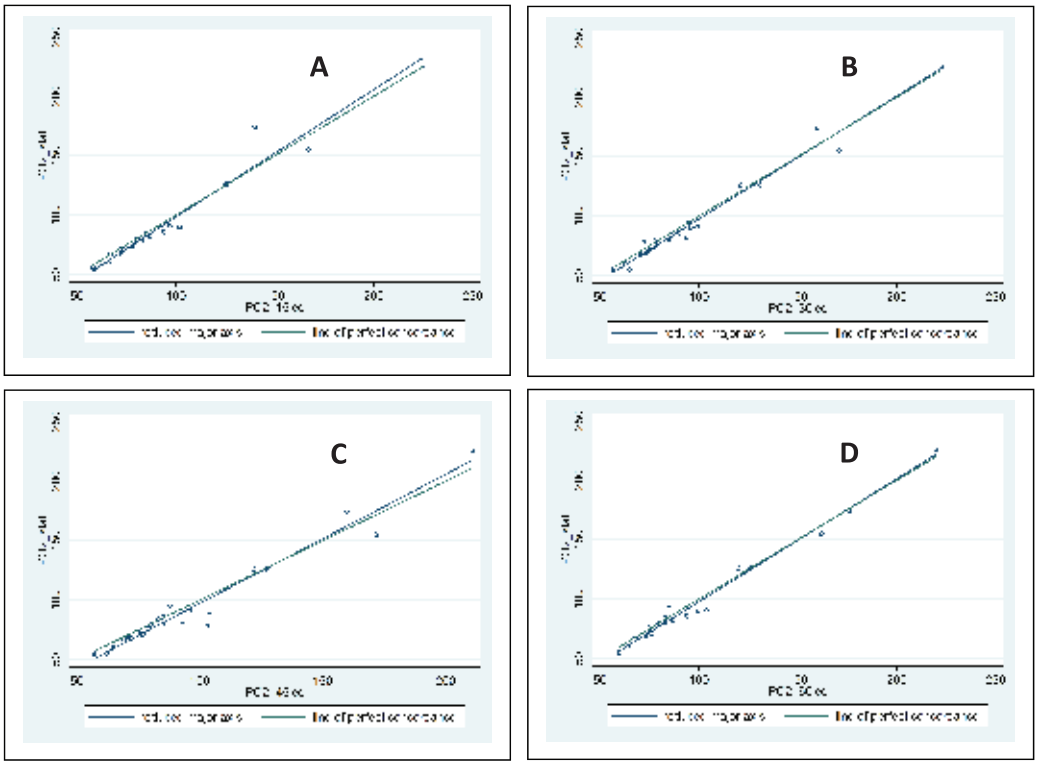

Figure 2: Relationship between baseline PaO_2 (reference) and samples stored in ice and analysed over time at 15 min [A], 30 min [B], 45 min [C] and 60 min [D]. Concordance for reference sample and 15, 30, 45 and 60 min samples demonstrated. Solid line shows the linear relationship with the dashed line indicating perfect concordance. The overall concordance correlation coefficient is high (>0.97) and the graph depicts small limits of agreement across the PaO_2 values.

minutes. If delays are anticipated, samples should be immediately chilled and can be analysed within 60 minutes.[1] The Clinical and Laboratory Standard Institute recommends analysis of ABG samples immediately after collection. If analysis is delayed for more than 30 minutes, samples collected in glass syringes should be stored in iced containers and the samples collected in plastic syringes should be at the room temperature. They specifically caution against cooling specimen collected in plastic syringes.[4] Greg, in his editorial, advised to analyse specimens collected in plastic syringes promptly and hold the ice because plastic syringes have nearly replaced glass syringes.[5]

In our study, samples stored at room temperature and ice were analysed at 15 minutes intervals. Collection of samples, labelling, storage and analysis were done by a qualified single professional under strict supervision. Samples were marked independently, thereby avoiding repeat analyses from left over samples. The blood gas analyser was in close proximity to the collection and storage area which enabled us to analyse the samples at the designated timings. Although there was no clinically significant change in the pH and $PaCO_2$ values over time, both in samples stored in room temperature and in ice, changes in PaO_2 were of concern. We observed that although the average difference in PaO_2 between the reference sample (baseline) and those that were analysed subsequently at different time intervals was small, the LOA were wide, particularly if the samples were not stored in ice.

Our results are consistent with those of Smeenk *et al* who found decreases in PaO_2 when plastic syringes were stored both in ice and at room temperature.[8] In their study, the initial analysis was delayed for more than 5 minutes because of transport of the samples to the laboratory, which added a further confounder of the effect of transport of blood gases to an area remote to the site of collection. They further stated that in glass syringes, there was no significant difference in PaO_2 at 30 minutes when stored in ice bath, but there was a statistically significant average decrease of 24.7 mm Hg when left at room

temperature. They estimated the rate of decline in PaO_2 using the glass syringe at room temperature method and it was 0.825 mm Hg/min and using the plastic syringe stored in ice water method, the decline was 0.375 mm Hg/min. In plastic syringes at room temperature, the rate of decline was 1.35 mm Hg/min. The decline in the PaO_2 was attributed to diffusion and metabolism. Our results are similar to those of Beaulieu *et al* who found decreases in PaO_2 when plastic syringes were stored at room temperature.[9] Our study shows that there was no significant difference in the iced sample whereas Beaulieu *et al* found that there was a significant increase in the samples which were stored in ice.

Pretto and Rochford reported that there was a decrease in PaO_2 when blood was stored in both plastic and glass at room temperature. They further stated that there was a decrease in PaO_2 when blood was stored in plastic and iced.[10] Srisan *et al* recommended that the blood sample should be analysed within 15 minutes after collection for better ABG and electrolytes results.[11] Dent *et al* reported that capillary stored blood at room temperature showed a statistically significant increase in the $PaCO_2$ but no change in PaO_2.[12] Knowles *et al* recommended that for accurate ABG, samples drawn in plastic syringes should be analysed immediately and if the analysis is going to be delayed, the sample should be drawn and stored in glass as he found that PO_2 stored in plastic syringes showed significant increase.[13] Mahoney *et al*, Liss and Payne, and Schmidt and Muller–Plathe reported that there was significant increase in PaO_2 when sample was iced in plastic syringes.[14-16] Our findings do not correlate with the findings of these studies.

Our study was limited by the small number of observations. A larger sample size is required to confirm the findings of our study. It is also possible that the type of plastic syringe used may impact the results and this needs further study.

Conclusion

The significant changes in PaO_2 over time with wide limits of agreement suggests that delay in processing ABG samples kept at room temperature may alter PaO_2 levels substantially that may be clinically significant. The decaying effect is believed to be because of continuing metabolic process that takes place in blood cells. A larger sample size would help to confirm this observation.

References

1. American Association for Respiratory Care. AARC clinical practice guideline: sampling for arterial blood gas analysis. *Respir Care* 1992; **37**(8):913-7.
2. American Association for Respiratory Care. AARC Clinical Practice Guideline: blood gas analysis and hemoximetry: 2001 revision and update. *Respir Care* 2001; **46**(5):498-505.
3. American Thoracic Society. Pulmonary function laboratory management and procedure manual, 2nd ed. 2005. Chapter 14; percutaneous arterial blood sampling.
4. Blonshime S, Fallon KD, Lehman CM, Sittig S. editors. NCCLS. Procedures for the Collection of Arterial Blood Specimens; Approved Standard—Fourth Edition. NCCLS document H11-A4. Pennsylvania.
5. Ruppel GL. Editorial. Of time and temperature, plastic and glass: Specimen handling in the blood gas laboratory. *Respiratory Care* 2006; **51**/7:717-8.
6. Scanlon CL, Wilkins RL. Analysis and monitoring of gas exchange. In: Wilkins RL, Stollar JK, Scanlan CL, editors, Egan's fundamentals of respiratory care, 8th ed. Mosby: St Louis; 2003:360.
7. White G. Basic clinical lab competencies for respiratory care, 4th ed. Clifton Park, New York: Delmar Publishers; 2003:132.
8. Smeenk F, Janssen J, Arends B, Harff G, van den Bosch J, Schonberger J, Postmus PE. Effects of four different methods of sampling arterial blood and storage time on gas tensions and shunt calculation in the 100% oxygen test. *Eur Respir J* 1997; **10**(4):910-3.
9. Beaulieu M, Lapointe Y, Vinet B. Stability of PO2, PCO2, and pH in fresh blood samples stored in a plastic syringe with low heparin in relation to various blood gas and hematological parameters. *Clin Biochem* 1999; **32**(2):101-7.
10. Pretto JJ, Rochford PD. Effects of sample storage time, temperature, and syringe type

on blood gas tensions in samples with oxygen partial pressures. *Thorax* 1994; **49**(6):610-2.
11. Srisan P, Udomsri T, Jetanachai P, Lochindarat S, Kanjanapattanakul W. *J Med Assoc Thai* 2011; **94**(3):S9-14.
12. Dent RG, Boniface DR, Fyffe J, Yousef Z. The effects of time delay and temperature on capillary blood gas measurements. *Respir Med* 1999; **93**(11):794-7
13. Knowles TP, Mullin RA, Hunter JA, Douce FH. Effects of syringe material, sample storage time, and temperature on blood gases and oxygen saturation in arterialized human blood samples. *Resp Care* 2006; **51**(7):732-6.
14. Mahoney J, Harvey J, Wong R, Van Kessel A. Changes in oxygen measurements when whole blood is stored in iced plastic or glass syringes. *Clin Chem* 1991; **37**(7):1244-8.
15. Liss HP, Payne C Jr. Stability of blood gases in ice and at room temperature. *Chest* 1993; **103**(4):1120-2.
16. Schmidt C, Muller-Plathe O. Stability of PO_2, PCO_2, and pH in heparinized whole blood samples: influence of storage temperature with regard to leukocyte count and syringe material. *Eur J Clin Chem Clin Biochem* 1992; **30**(11):767-73.
17. Madiedo G, Sciacca R, Hause L. Air bubbles and temperature effect on blood gas analysis. *J Clin Pathol* 1980; **33**(9):864-7.
18. Steichen TJ, Cox NJ. Sg84. Concordance correlation coefficient. *Stata Technical Bulletin Reprints* 1999; 8:137-45.
19. Lin LI. A concordance correlation coefficient to evaluate reproducibility. *Biometrics* 1989; **45**: 255-68.