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Validation protocol for multiple blood gas analyzers in accordance with laboratory accreditation programs

Protocolo de validação de múltiplos equipamentos para análise de gases sanguíneos em conformidade com os programas de acreditação laboratorial

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ABSTRACT

Introduction: The results of blood gas analysis using different instrumentation can vary widely due to the methodological differences, the calibration procedures and the use of different configurations for each type of instrument. **Objective:** The objective of this study was to evaluate multiple analytical systems for measurement of blood gases, electrolytes and metabolites in accordance with the accreditation program (PALC) of Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial (SBPC/ML). **Materials and methods:** 20 samples were evaluated in three ABL800 Flex (Radiometer Medical ApS, Denmark) blood gas analyzers, and the results were compared with those of the device in use, which was considered the reference. The analysis of variance (Anova) was applied for statistical purposes, as well as the calculation of mean, standard deviation and coefficient of variation. **Results:** The *p* values obtained in the statistical analysis were: pH = 0.983, pO_2 = 0.991, pCO_2 = 0.353, lactate = 0.584, glucose = 0.995, ionized calcium = 0.983, sodium = 0.991, potassium = 0.926, chlorine = 0.029. **Conclusion:** The evaluation of multiple analytical systems is an essential procedure in the clinical laboratory for quality assurance and accuracy of the results.

Key words: gas analysis; harmonization; laboratory accreditation; pH; pO_2; pCO_2; lactate; glucose; Na; K; Cl; Ca^{2+}.

INTRODUCTION

Blood gas analysis is aimed at assessing oxygen uptake in the lungs and tissue oxygenation, besides the study of acid-base balance. Thus, measurement of partial pressures of gases allows the adoption of therapeutic interventions to correct disturbances in pulmonary ventilation, tissue oxygenation, and acid-base balance. Depending on the nature of the sample, whether blood is arterial or venous, results are distinct, and for the proper interpretation of results, it is important that the sample type be identified in the medical order. When one is interested in assessing oxygen uptake in the lungs, arterial blood is the specimen of choice, because the result will allow obtaining information on gas exchange and the calculation of the amount of oxygen being delivered to tissues. However, investigation of just the metabolic components can be done with the use of a venous sample.

Validation of analytical systems aims at studying and documenting performance of multiple instruments prior to their implementation into routine use. The Clinical Laboratory Accreditation Program (PALC) of Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial (SBPC/ML)[1], as well as the guidelines of the Clinical and Laboratory Standards Institute (CLSI)[2,3] and the rule ABNT NBR NM ISO 15189:2008[4], determine the necessity of comparing multiple analyzers used in the laboratory routine, regardless of whether the devices have the same brand.

Gas analysis results obtained from different instruments may present wide variation due to factors such as different principles of analysis and calibration procedures, or use of different instrument configurations[1,2]. Oliveira and Mendes highlight that performance evaluation carried out based on analytical validation allows to measure present errors and securely determine whether

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these errors affect results, permitting to know if the system works in the expected manner and if it produces adequate results[5].

OBJECTIVE

The objective of this work was to analyze the performance of multiple blood gas analyzers in the measurements of the following parameters: pH, pO_2, pCO_2, ionized calcium, sodium (Na), potassium (K), glucose, lactate and chlorine (Cl), by comparing their results.

MATERIALS AND METHODS

Venous blood samples were drawn from outpatients into blood gas-dedicated syringes containing lithium heparin; samples with air bubbles and clots were eliminated. At least 20 samples were tested for each analyte, using three ABL800 Flex (Radiometer Medical ApS, Denmark) analyzers in comparison with the used reference instrument. Evaluation of assay performance was based on CLSI protocols[2, 6].

In the ABL800 analyzers, one-point automatic calibrations were performed each four hours or for each 10 samples; two-point calibrations, each eight hours. In the reference equipment, a one-point calibration was performed each 60 minutes; a two-point calibration, each 12 hours, all according to instructions by the manufacturer.

Method performance was evaluated for precision, accuracy, linearity, analytical sensitivity, carryover contamination and stability of the sample. In the precision study, we compared the performance of each device based on simultaneous analyses of the same materials in all analytical systems. Inter-assay precision was determined with two control solutions (high and low) by means of two daily repetitions during 20 days; intra-assay precision, with 40 aliquots, was determined with two control solutions (high and low) by means of two daily repetitions. Sensitivity and linearity of the methods were tested with samples acquired from the College of American Pathologists (CAP), of the United States. Recovery tests were carried out by addition of control solutions with values known in patients' blood samples. According to the rules of Agência Nacional de Vigilância Sanitária (Anvisa) and CLSI, recovery must be within the 80%-120% range[3]. Stability of the sample was examined based on a same sample analyzed one, two and three hours after the storage at 2ºC-8ºC. Acceptable variation was set at less than 10%. Carryover was determined via a sequence analysis with 21 aliquots, interspersing high- and low-concentration samples. The acceptable limit of error was three times the standard deviation (SD) of the mean obtained from the low-concentration sample in the sequential measurement of low-concentration samples followed by another low-concentration sample (low-low). The reference range was calculated using venous blood samples of adult volunteers considered healthy ($n = 20$). Statistical analysis was performed by the calculation of mean, SD, coefficient of variation, linear regression, Pearson's correlation, calculated error rate and analysis of variance (Anova). We also evaluated if the values obtained from healthy volunteers presented Gaussian distribution, aiming at validating reference ranges in relation to those described in the literature. Calculations were made using statistical packages EP Evaluator version 9.3 and Minitab version 5.0.

RESULTS

Table 1 describes the results of pH, pO_2, pCO_2, ionized calcium, sodium, potassium, chlorine, glucose, and lactate for assessment of precision together with the mean values obtained from the three ABL Flex 800 analyzers. The acceptable levels were defined based on the literature data. The yielded results demonstrated that the instruments exhibit precision.

Table 2 displays data observed with the purpose of evaluating sensitivity, linearity, analytical measurement range, recovery of the method and stability of the sample. The obtained recovery range was 80.5%-102.5%, within the 80%-120% acceptable interval. Stability of parameters for samples of different levels was tested in the interval of zero to three hours, at temperatures of 2ºC to 8ºC.

The mean, limit error, and carryover (**Table 3**) observed in the four instruments showed that the method does not suffer from the influence of high-concentration samples over low-concentration ones. The differences between high-low and low-low means were compared with the calculated limit error, which amounts to three times the SD obtained in the measurements of low-concentration samples following a low-concentration sample.

In order to assess agreement among methods, error was estimated by dispersion graphs for measurements (**Figure 1**). Clinically or statistically significant difference was not verified among the studied instruments. The obtained errors are all within the statistical and clinical limits, when compared with the total error allowable (TEa) described in the literature for each analyte. This demonstrates that the instruments may be used interchangeably without affecting clinical care and without causing harm to the patient, as shown in **Tables 4** and **5**, and in **Figure 2**.

TABLE 1 – Results obtained for assessment of intra- (n = 40) and inter-assay (n = 40) precision of blood gas analyzers

Parameter	Intra-assay						Inter-assay						
	Mean (low level)	SD	CV (%)	Mean (high level)	SD	CV (%)	Mean (low level)	SD	CV (%)	Mean (high level)	SD	CV (%)	Acceptable CV (%)
pH	7.168	0.004	0.1	7.57	0.001	0	7.17	0.049	0.1	7.567	0.022	0.1	2.63
pCO_2 (mmHg)	23.5	0.48	2	63.9	0.67	1.1	23	0.56	2.4	73.9	1.3	1.8	3.6
pO_2 (mmHg)	73.4	0.38	0.5	138.7	0.49	0.4	74	0.8	1.1	137	1.3	0.9	10
Ionized calcium (mg/dl)	2.44	0.01	0.51	6.19	0.02	0.39	2.52	0.04	1.59	6.28	0.33	0.5	3.6
Sodium (mEq/l)	120	0.2	0.2	155	0.3	0.2	120	0.2	0.2	155	0.2	0.1	1.9
Potassium (mEq/l)	2.9	0.001	0.03	6.7	0.1	0.12	2.9	0.05	1.7	6.7	0.04	0.7	3.6
Chlorine (mEq/l)	74	0.8	0.8	112	0.4	0.4	74	0.7	0.9	112	0.5	0.5	2.21
Glucose (mg/dl)	107	0.9	0.8	375	0.7	0.2	112	2.2	2	391	3.7	0.9	4.88
Lactate (mg/dl)	11.3	0.5	4.8	82.4	1	1.2	11.9	0.3	2.7	81.5	1.4	1.7	20.4

SD: standard deviation; CV: coefficient of variation.

TABLE 2 – Results for evaluation of linearity, analytical sensitivity, recovery, and stability

	pH	pO_2 (mmHg)	pCO_2 (mmHg)	Ionized calcium (mg/dl)	Sodium (mEq/l)	Potassium (mEq/l)	Chlorine (mEq/l)	Lactate (mg/dl)	Glucose (mg/dl)
Analytical sensitivity	6.87	43	14	0.41	88	1.1	66	0.6	11
Linearity	7.8	426.5	83	2.87	172	9.4	140	15.8	409
Recovery (%)	99.2	80.5	82.5	94.5	102	98.5	85.2	102.5	94.5
Stability (hours)	2	1	1	1	3	3	3	3	3

TABLE 3 – Carryover study

	pH	pO (mmHg)	pCO_2 (mmHg)	Ionized calcium (mg/dl)	Sodium (mEq/l)	Potassium (mEq/l)	Chlorine (mEq/l)	Lactate (mg/dl)	Glucose (mg/dl)
Mean high-low concentration	7.18	75.4	23.6	2.55	121	2.9	74	11.6	111
Mean low-low concentration	7.17	75.7	23.2	2.55	121	2.9	74	11.6	111
Error limit (3 SD of mean low-low concentration)	0.0075	3.5	1.8	0.05	2.1	0.1	1.3	1.6	3.9
Carryover	0.003	-0.24	0.36	-0.002	0.4	-0.02	-0.4	0	0.6

SD: standard deviation.

TABLE 4 – Results of comparison between data obtained from ABL 800 Flex instruments and the reference equipment

	Mean of ABL 800 Flex	Mean of the reference analyzer	Total error allowable* (%)	Estimated error rate	p**
pH	7.36	7.37	3.9	< ± 1	0.983
pO_2 (mmHg)	45	46	10	< ± 1	0.991
pCO_2 (mmHg)	41.9	42.6	8	< ± 1	0.353
Ionized calcium (mg/dl)	4.6	4.6	2.1	< ± 1	0.983
Sodium (mEq/l)	138	138	5	< ± 1	0.991
Potassium (mEq/l)	4.3	4.2	5.8	< ± 1	0.926
Chlorine (mEq/l)	102	104	5	< ± 1	0.029
Lactate (mg/dl)	16.5	18.4	20	< ± 1	0.584
Glucose (mg/dl)	133	136	10	< ± 1	0.985

**total error allowable as described by CLIA; **data obtained by means of analysis of variance (Anova); CLIA: Clinical Laboratory Improvement Amendments.*

TABLE 5 – Verification of reference values in individuals considered healthy for venous blood

	Interval suggested by the manufacturer	Obtained interval	Obtained mean	Obtained median	Total analyses/excluded	Verification
pH	7.35-7.45	7.35-7.45	7.4	7.41	20/0	Verified
pO_2 (mmHg)	80-100	80.6-100	91.7	91.8	20/0	Verified
pCO_2 (mmHg)	35-45	35.4-45	40.23	40.3	20/0	Verified
Ionized calcium (mg/dl)	4.49-5.29	4.54-5.2	4.79	4.74	20/0	Verified
Sodium (mEq/l)	135-145	136-143	139.17	139	20/0	Verified
Potassium (mEq/l)	3.5-4.5	3.5-4.5	3.96	3.9	20/0	Verified
Chlorine (mEq/l)	98-107	98-107	104.43	106	20/0	Verified
Lactate (mg/dl)	4-20	6-19	11.26	11.5	20/0	Verified
Glucose (mg/dl)	60-99	62-99	89.9	92	20/0	Verified

In the verification of reference values, we analyzed pH, pCO_2, ionized calcium, sodium, potassium, chlorine, lactate, and glucose in 20 healthy individuals, and we obtained, as a result, 100% of these individuals within reference ranges.

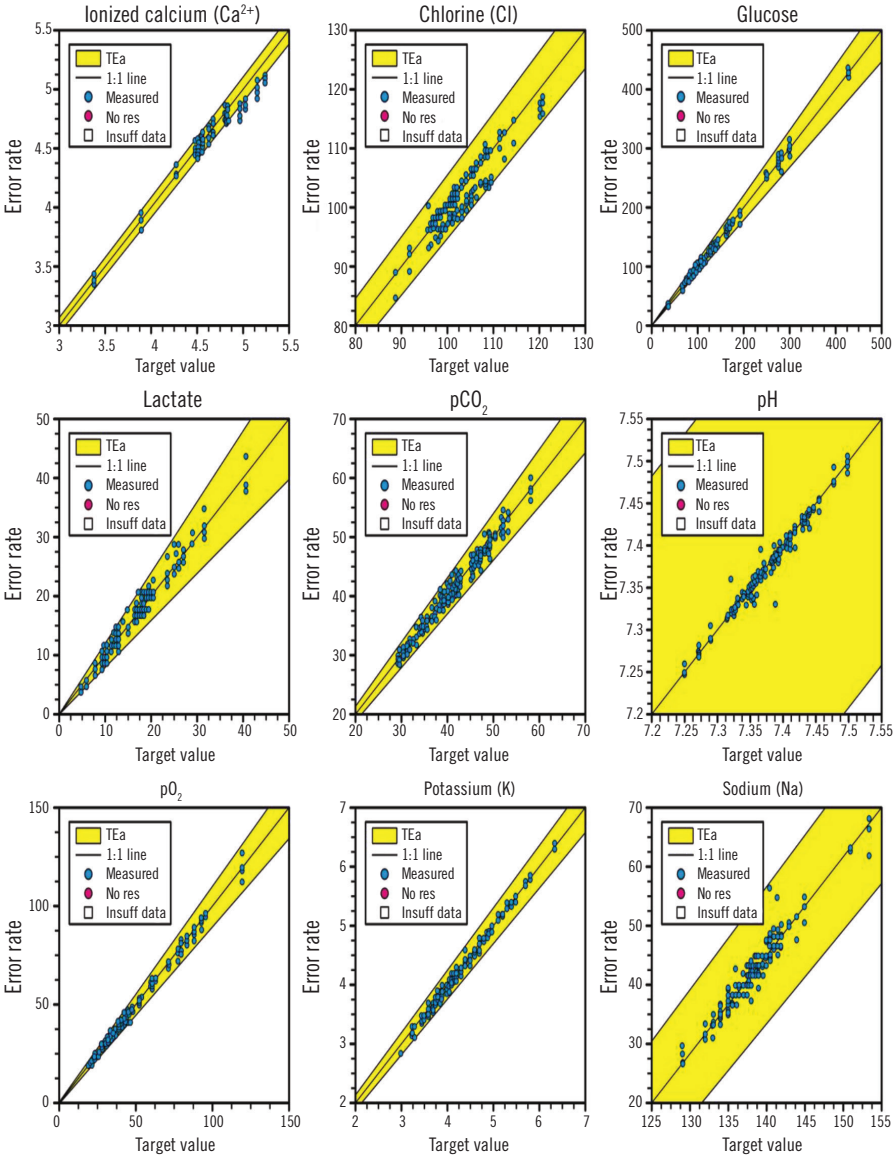

FIGURE 1 – *Analysis of linear regression of parameters*

TEa: total error allowable.

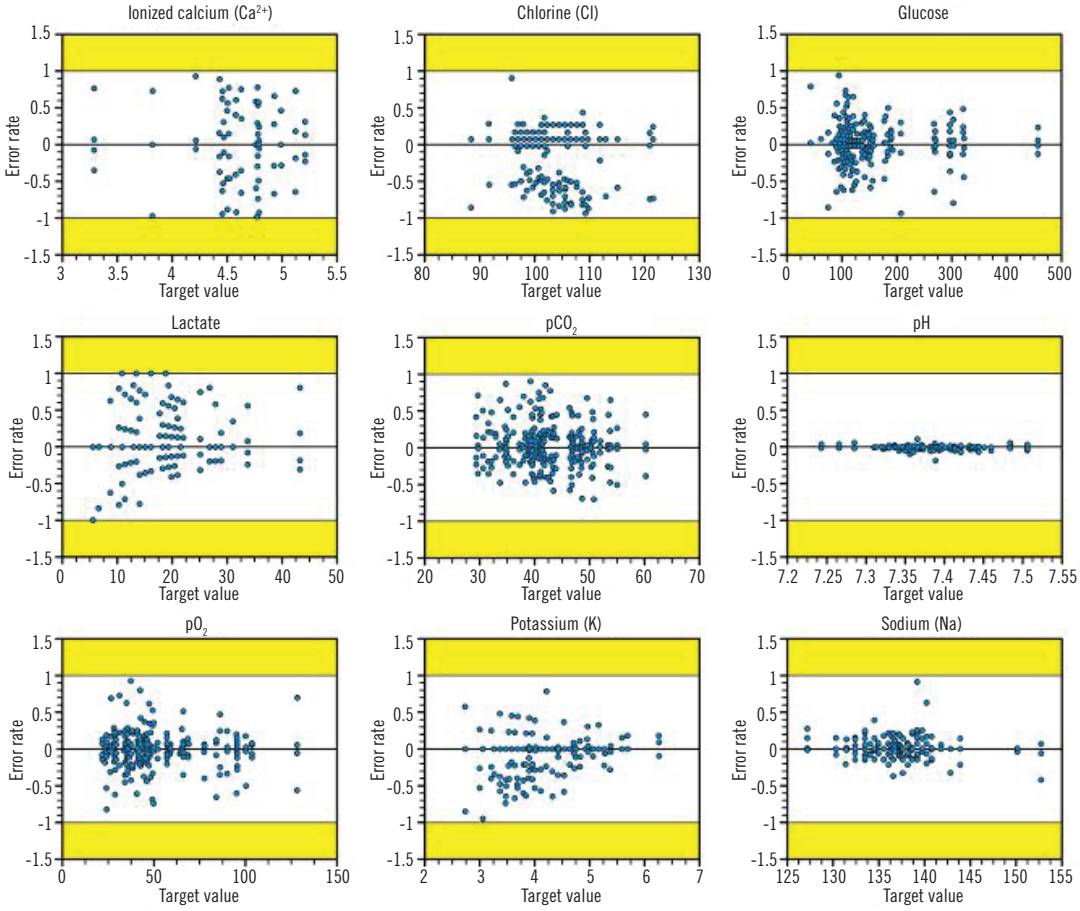

FIGURE 2 – *Evaluation of error rate*

DISCUSSION

Quality of analytical results is assured by the creation of mechanisms for failure prevention through process design and auditing. In order to ensure process quality in blood gas analysis, it is important that pre- and post-analytical phases also be within specifications, acting preventively on the possible sources of error and TEa[5, 7, 8].

Reducing the possibility of error during the analytical phase is essential when one is selecting processes and defining procedures that allow for an adequate performance. It is necessary that test limitations be known, as well as their parameters of clinical and analytic performance, to provide reliable results, always aiming at higher safety levels for patients. In this context, it is crucial that the sample type (venous or arterial blood) be identified in the medical order. If the patient is on assisted ventilation, it is necessary that essential data about this condition be also described in the order. Besides, one must verify if the sample was collected into a syringe containing balanced heparin, if the sample volume is suitable for the conduction of the exam, if the syringe is adequately sealed, if the ideal conditions of storage were met, if transportation was carried out within the stipulated time, and if material was correctly identified[7].

In the analytical phase, besides internal quality control and proficiency assays, there are other general recommendations that directly influence quality of the performed analyses. The first step is the development of a validation protocol that ensures method reliability, assessing performance and identifying potential errors. Input screening and validation of different batches, including controls and calibrators, help a lot in the management of the whole process, and minimize differences between the different used batches.

It is really troublesome to establish a process to evaluate performance of gas analysis methods due to sample instability, especially pCO_2 and pO_2, and the difficulty to run dilutions in these samples for sensitivity and linearity tests. For these reasons, we chose to study sensitivity and linearity of the samples provided by CAP.

A crucial point is the selection of reagent material of the same batch, and the samples that will be used, that is, these materials must encompass all ranges of important clinical values to avoid a biased evaluation of the obtained statistical results. This is one of the most arduous phases during the process of equipment validation. Thus, biological samples for gas analysis must be measured in all the studied instruments just after collection. Because the stabilization time of the analytes is extremely short, degradation or oxidation over time may result in the obtainment of wrong results.

Activities of process control in the analysis of blood gases are important for clinical decision making. Confidence in the method is crucial and may be assessed by means of equipment validation. Another important factor to be taken into account is the choice of the most appropriate instrument for the laboratory routine.

García-Payá *et al.* compared performance of quality control in patients of four hospitals, and used the sigma metrics for evaluation of precision and accuracy of the method[8].

The intra-assay and inter-assay variation coefficients in our work resulted in values below the limits of analytical quality specifications for the studied analytes[9]. The value of TEa adopted was based on the criteria of the Clinical Laboratory Improvement Amendments (CLIA)[9, 10].

It is important to relate the results of daily quality control to the criteria or the acceptable performance standards described in the literature. Our reference was the biological variation, which became a determining factor of how exact and precise a test can be. By means of determination of systematic (bias) and random (variation coefficient) error, we may estimate the total error permitted for each analyte, and the capacity to foresee how many errors can occur in a million opportunities through sigma calculation[11].

The limitations of our study were: analysis of patients' samples with values within the reference interval, small intra-individual variability, difficulty in obtaining enough sample volume, and short sample stability.

The systems involved in human interactions and decisions are prone to error. Therefore, it is necessary to standardize process activities to avoid errors, or, at least, make them tolerable. Error detection in each sample provides additional safety against random errors[12]. Thus, it is fundamental that methods be validated following the presented criteria, assuring quality of results for patients' safety.

CONCLUSION

In this work, differences between results obtained from the studied instruments were not statistically and clinically significant. The gathered data presented good accuracy and precision. Analytical measurement intervals satisfied the needs of the clinical body. The obtained results permit to conclude that validation of multiple instruments admits determination of the same laboratory parameters using distinct devices. These findings are fundamental for quality assurance and reliability of results.

At last, quality is a comprehensive and multifaceted concept, whose dimensions vary in importance, depending on the situation: technical competence, accessibility, effectiveness, interpersonal relationship, efficiency, continuity, safety and adequate facilities.

RESUMO

Introdução: *Os resultados da análise dos gases sanguíneos utilizando diferentes equipamentos podem apresentar grandes variações decorrentes das diferenças metodológicas, dos procedimentos de calibração e da aplicação de configurações distintas para cada tipo de instrumento.* ***Objetivo:*** *O objetivo deste trabalho foi avaliar múltiplos sistemas analíticos para teste de gases sanguíneos, eletrólitos e metabólitos, em conformidade com o Programa de Acreditação de Laboratórios Clínicos (PALC) da Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial (SBPC/ML).* ***Materiais e métodos:*** *Foram avaliadas 20 amostras em três analisadores de gases sanguíneos ABL800 Flex (Radiometer Medical ApS, Dinamarca) em relação ao equipamento em uso, que foi considerado referência. A análise de variância (Anova) foi aplicada para fins de estudo estatístico dos resultados obtidos nos quatro equipamentos, bem como o cálculo da média, do desvio padrão e do coeficiente de variação.* ***Resultados:*** *Os valores de* p *obtidos na análise estatística foram: pH = 0,983, pO_2 = 0,991, pCO_2 = 0,353, lactato = 0,584, glicose = 0,995, cálcio ionizado = 0,983, sódio = 0,991, potássio = 0,926 e cloro = 0,029.* ***Conclusão:*** *A avaliação de múltiplos sistemas analíticos é procedimento essencial no laboratório clínico para garantia da qualidade e da exatidão dos resultados.*

Unitermos: *gasometria; harmonização; acreditação laboratorial; pH; pO_2; pCO_2; lactato; glicose; Na; K; Cl; Ca^{2+}.*

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