

PERFORMANCE CHARACTERISTICS OF HEMOX-ANALYZER FOR ASSESSMENT OF THE HEMOGLOBIN DISSOCIATION CURVE

Roberta Guarnone*, Esther Centenara*, Giovanni Barosi^o

*Istituto di Medicina Interna e Oncologia Medica, ^oLaboratorio di Informatica Medica, IRCCS Policlinico S. Matteo, Pavia, Italy

ABSTRACT

The Hemox-Analyzer (TCS, Medical Products Division, Southampton, PA) is an automatic system for determining the oxyhemoglobin dissociation curve (ODC) and P50 values. The ODC is recorded during deoxygenation with nitrogen gas and plotted on graph paper; the oxygen tension is detected by a Clarke electrode while the oxyhemoglobin fraction (%HbO₂) is evaluated by a dual-wavelength spectrophotometer. Even though this instrument has been commercially available for more than 20 years, its performance characteristics have never been assessed. We evaluated the performance characteristics of the Hemox-Analyzer. P50 was tested in 28 healthy volunteers, in 16 anemic and in 9 polycythemic patients. To test its precision we evaluated both inter- and intra-assay variability. The system shows good precision: standard deviation was 0.39 for assays in duplicate, CV=1.9% for intra-assay measurements and CV=3.0% for inter-assay. The mean P50 values were 25.3±1.5 mmHg in normal volunteers and 27.3±1.4 mmHg in anemic patients. The Hemox-Analyzer is a simple, quick and reliable instrument for recording the oxyhemoglobin dissociation curve. Both the P50 value and observation of the fine structure of the curve can furnish information about the delivery of oxygen to tissues.

Key-words: P50, oxyhemoglobin dissociation curve, Hemox-Analyzer

The oxyhemoglobin dissociation curve (ODC) for whole blood relates hemoglobin percentage saturation with oxygen to the partial pressure of the oxygen with which the blood is in contact. It describes how oxygen binds to hemoglobin and how it is released, as can be imagined in the lungs and capillaries, respectively. The ODC has a sigmoidal shape and is characterized by the P50 value, i.e. the partial pressure of oxygen at which hemoglobin is 50% saturated with oxygen. The primary reason for an abnormal ODC position is the presence of pathologic hemoglobins with altered oxygen affinity, but modifications are also caused by disease states which affect the factors that displace the curve, mainly pH, temperature, 2,3-DPG and pCO₂.¹ Moreover, P50 is a useful parameter for studying the regulation of erythropoiesis in different pathological and experimental conditions.^{2,3} Thus the perfor-

mance characteristics of the instruments employed and the reliability of the measurements obtained are important for both diagnostic accuracy and research results.

For routine P50 measurements laboratories are usually equipped with a blood gas analyzer and a tonometer for the determination of pO₂, pCO₂, pH and sO₂. P50 is then calculated through different algorithms that interrelate these parameters.⁴ In recent years new machines that combine a multi-wavelength blood oxymeter and a blood gas analyzer have become available. These instruments automatically provide *in vitro* determination of the oxygen status of blood and P50.⁵

The Hemox-Analyzer (TCS, Medical Products Division, Southampton, PA) is an apparatus that determines oxygen partial pressure and degree of hemoglobin saturation with oxygen by means of a Clark oxygen electrode and spec-

Correspondence: Giovanni Barosi, M.D., Laboratorio di Informatica Medica, IRCCS Policlinico S. Matteo, 27100 Pavia, Italy.

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trophotometry, respectively. While this device has been commercially available for more than 20 years, its performance characteristics have never been examined.

In the present study we evaluate the characteristics of the measurements provided by the Hemox-Analyzer and report preliminary data obtained in a series of patients with various types of anemia in order to evaluate the sensitivity with which the instrument records changes in P50 at different Hb levels.

Materials and Methods

P50 measurement

The Hemox-Analyzer determines the ODC by exposing 50 μL of blood or hemolysate to an increasing partial pressure of oxygen and deoxygenating it with nitrogen gas. A Clark oxygen electrode detects the change in oxygen tension, which is recorded on the x-axis of an x-y recorder. The resulting increase in oxyhemoglobin fraction is simultaneously monitored by dual-wavelength spectrophotometry at 560 nm and 576 nm and displayed on the y-axis.

For all the experiments, blood samples were taken from the antemedial vein, anticoagulated with heparin and kept at 4°C on wet ice until the assay. Fifty μL of whole blood were diluted in 5 μL of Hemox-solution, a manufacturer-provided buffer that keeps the pH of the solution at a value of 7.4 ± 0.01 . The sample-buffer is drawn into a cuvette and the temperature of the mixture is equilibrated and brought to 37°C; the sample is then oxygenated to 100% with air. After adjustment of the pO₂ value the sample is deoxygenated with nitrogen; during the deoxygenation process the curve is recorded on graph paper. The P50 value is extrapolated on the x-axis as the point at which O₂ saturation is 50%. The time requires for a complete recording is approximately 30 minutes.

Intra- and inter-assay variations

For intra-assay variation determination, 35 blood samples drawn from normal subjects (12 samples) and patients with anemia (23 samples) were tested in duplicate. For inter-assay variabil-

ity determination, two subjects were tested one day apart for 4 days at the same hour. The same lot of diluent and buffer was used for both intra- and inter-assay variability.

Stability of the sample

The manufacturer's instructions suggest determining the ODC as soon as possible after blood has been drawn. In practice, it is sometimes convenient or necessary to postpone the assay. Thus, we recorded the variations in the measurement results after allowing the samples to remain at different temperatures for varying periods of time.

Samples were collected at 8 a.m. from a normal male subject and from a patient with iron deficiency anemia (Hb=10 g/dL). Aliquots of each sample were divided into twelve tubes and six were maintained at room temperature and six on ice. The determinations were carried out immediately after blood was drawn and after 1, 2, 5, 12 and 24 hours.

Subjects

P50 was evaluated in 28 healthy volunteers, 14 males and 14 females, aged from 20 to 35 years. Sixteen patients with anemia and 9 with erythrocytosis due to myeloproliferative disease were also tested. The anemic patients represented a group of consecutive patients with Hb lower than 12 g/dL who were admitted to our Clinic from December 1993 to March 1994. Five presented hypochromic microcytic anemia secondary to chronic hemorrhage, 4 anemia of chronic disease, 2 anemia of chronic renal failure and 5 showed anemia consequent to hematologic malignancies. Hemoglobin and other red cell indices were measured with an automatic Coulter-S counter.

Statistical analysis

Statistical analysis was performed with MICROSTAT (Ecosoft, Inc.) on an IBM-PC. The Student's t-test was used for comparisons between means, Pearson's test to calculate correlation coefficients and linear regression analysis to assess the correlation between variables. A p value less than 0.05 was considered significant.

Results

Performance characteristics

In the 35 samples tested in duplicate (intra-assay variability), P50 ranged from 15 to 29.2 mmHg. Standard deviation of the duplicate assays was 0.39, which represents a 1.9 percent coefficient of variation (Table 1). The standard deviation of the duplicate values was not correlated with the P50 or Hb level of the patient assayed.

The inter-assay variability study yielded P50 values ranging from 28 to 29.6 mmHg, with a median value of 28.5 mmHg, a SD of 0.88 and a coefficient of variation of 3%. When the samples were allowed to remain at room temperature or on ice, a decline in the P50 value was observed in both conditions. When samples from normal subjects were tested, P50 decreased from a basal value of 28 mmHg to 27.4 mmHg at the 5th hour and to 26.9 at the 24th hour on ice, and from 28 mmHg to 25.7 mmHg at the 5th hour and to 22.5 mmHg at the 24th hour at room temperature. These values represent a respective decrease of 2.1% at the 5th hour and 3.9% at the 24th hour for samples stored on ice, and a respective decrease of 8.2% at the 5th hour and 19.6% at the 24th hour for samples kept at room temperature.

For the anemic patient the decline was from 29.5 mmHg to 28.8 mmHg at the 5th hour and to 27 mmHg at the 24th in the samples on ice (a decrease of 2.3% at the 5th and 8.4% at the 24th hour), and from 30 to 27 mmHg at the 5th hour and to 25 mmHg at the 24th hour in those at room temperature (a decrease of 10% at the 5th and 16.6% at the 24th hour).

Table 1. Precision of the Hemox-Analyzer (intra-assay variability)

<i>N. of pairs of results</i>	<i>SD of duplicates (mmHg)</i>	<i>Range of values (mmHg)</i>	<i>CV (%)</i>
35	0.39	15-29.2	1.9

Study populations

The range of P50 values for normal subjects was 23-28.5 (mean 25.3±1.5) mmHg. There was no significant difference between males and females in spite of the significantly lower hemoglobin levels in the latter (a mean of 15.4 g/dL, range 14.3-16.8, versus a mean of 13.4 g/dL, range 12.6-15.2).

Anemic patients showed hemoglobin values ranging from 3.5 to 10.5 g/dL. The mean P50 value in the anemic population was 27.3 mmHg (range 23 to 32 mmHg) and was significantly higher than in normal subjects ($p < 0.01$).

Patients with erythrocytosis showed normal P50 values (median 25.4, range from 23.6 to 29.5 mmHg) (Table 2). The correlation between Hb and P50 did not reach statistical significance in either the normal subjects or the anemic ones. On the contrary, a significant inverse correlation was demonstrated between the two parameters when the two populations were considered together ($r = -0.5$; $p < 0.01$) (Figure 1).

Discussion

Changes in hemoglobin oxygen affinity may play an important role in determining the amount of oxygen released from arterial blood.

Table 2. P50 values in different populations of subjects.

	<i>N. of subjects</i>	<i>P50 median (mmHg)</i>	<i>SD (mmHg)</i>	<i>P50 range (mmHg)</i>	<i>Hb range (g/dL)</i>
Normal subjects	28	25.3	1.5	23-28.5	14.1 (12.6-16.8)
men	14	25.1	1.5	23-28.5	15.4 (14.3-16.8)
women	14	25.5	1.6	23-28.5	13.4 (12.6-15.2)
Anemic patients	16	27.3	1.4	23-32	7.5 (3.5-10.5)
Patients with erythrocytosis	9	25.4	1.7	23.5-29.5	18.1 (17-22)

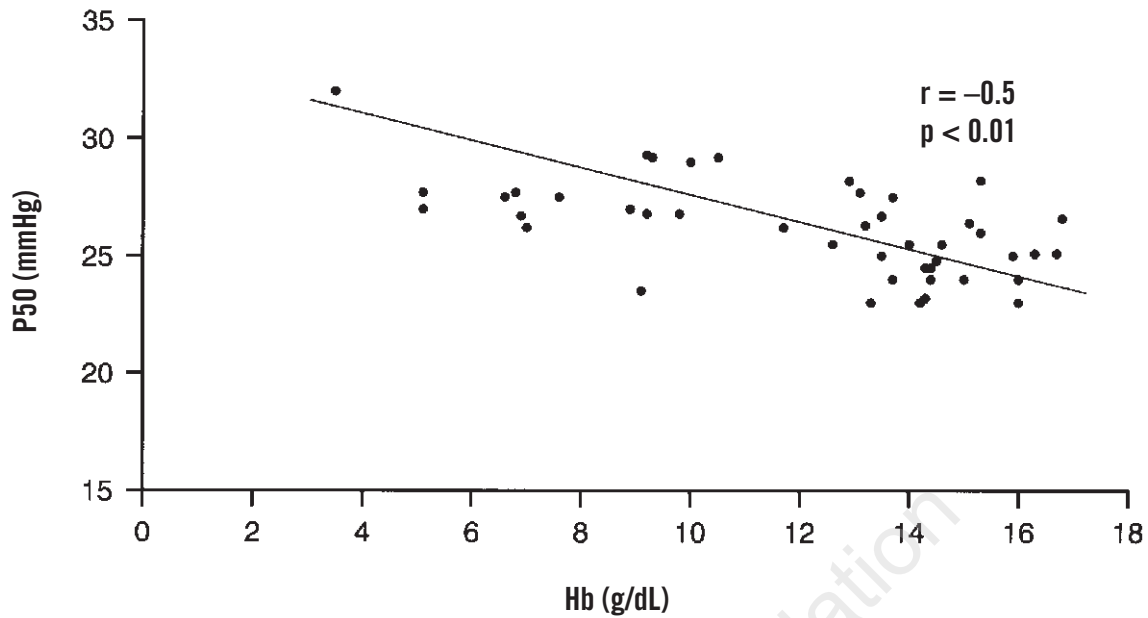


Figure 1. The relationship between hemoglobin and P50 value in normal and anemic subjects.

Following the work by Drabkin⁶ on the optical properties of hemoglobin and its derivatives, methods based on light absorption were developed that allowed exact determination of the ODC with an automatic apparatus. The Hemox-Analyzer is one such device for recording the ODC. The operating time required for the determination is about 30 minutes; the first curve of the day takes more time because it is necessary to first test the functionality of the oxygen membrane. This time is very similar to that reported for other instruments based on spectrophotometry, such as the Hemo-scan (30-40 minutes), while manual or automated methods such as the CO-Oximeter take much more time (from 100 to 120 minutes).^{4,5,7,8} Since it is well known that a number of factors, e.g. temperature and pH, may influence the results and since one application of the instrument involves sequence measurements of samples for experimental purposes, we decided to test some performance characteristics that had never been assessed before.

The range of P50 values for normal subjects was 23-28.5 mmHg, similar to that reported for instruments based on a spectrophotometric methods and slightly lower than that reported

by authors who utilized different techniques.^{9,10}

P50 values obtained with the Hemox-Analyzer were found to be extremely reproducible in both intra- and inter-assays. The respective coefficients of variation were 1.9 and 3%. Regarding intra-assay variation, the literature reports SD values for duplicate experiments of 1.87 mmHg for the manual method, 0.94 mmHg for the CO-Oximeter, 0.78 mmHg for the Hemo-scan;^{7,9} the Hemox-Analyzer with a SD=0.39 mmHg shows good reproducibility.

It is also well known that P50 decreases with time due to increased formation of methemoglobin and a decline in 2,3-DPG content, so the manufacturer recommends testing the sample immediately after blood collection. Given the practical consideration that some samples are not immediately available after blood collection, we also tested the stability of samples maintained both on ice and at room temperature. The best reproducibility was obtained with samples stored on wet ice and tested the day of collection; in fact, the curve shifts to the left in samples stored for more than 24 hours (even if maintained on ice), while samples kept at room temperature show a notable shift of the ODC to the left a few hours after blood collec-

tion. However, sample stability proved to be fairly good up to the 5th hour since the variation in p50 during this period is in the range of the intra-assay variability.

Our study also evaluated the reliability of the measurements by assessing how anemia influences P50. It is known that there is a physiological adaptation to anemia-induced hypoxia which is mediated by increases in 2,3-DPG. Samples from anemic patients showed P50 values that were significantly higher than those from normal subjects, and a correlation was observed between hemoglobin and P50 when normal subjects and anemic patients were considered together.

There was an even greater variability in p50 values in the anemic group, suggesting that different adaptive mechanisms take place in the red cell when hemoglobin levels fall. Such mechanisms are partly independent of hemoglobin levels and differ in the various forms of anemia.

In conclusion, the main advantages of the Hemox-Analyzer system are the stability of the measurements and the reproducibility of the results. Our apparatus seems to represent a good system for recording the ODC, and our data coincide well with those of other investiga-

tors who utilized spectrophotometric methods under the same conditions.

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