In this method, the reduction of nitrate to nitrite is complete within 10 min, much shorter than the 90 min for the kinetic cadmium reduction method [13], 45 min for the enzymatic one-step assay [10], and 20 min for the enzymatic method of Moshage et al. [9]. Green et al. [12], by using a high-pressure reduction column coupled with a continuous-flow analyzer, reported a shorter reduction time (within 1 min), and an analysis rate of 30 samples per hour. Although our reduction time is longer, the analysis rate, considering the overall time spent on deproteinization, reduction, and measurement in a Hitachi 717 analyzer, amounts to >150 samples per hour. Recently, Yang et al. [11] described a very sensitive method for chemiluminescent detection of nitrate, but estimates for the analysis rate were 15–30 samples per hour. Moreover, the automated device by Green et al. [12] was specifically designed for analyzing nitrate concentrations and is not available to the majority of clinical chemistry laboratories. By contrast, Hitachi analyzers are widely used and allow simultaneous performance of other analyses. An additional advantage of this method is its low cost, because the cadmium may be regenerated and the reagents are cheaper than those used in enzymatic methods (nitrate reductase or β-NADPH).

The semiautomated method described here responds in a quantitative linear fashion to nitrate within the range of 0.8 to 600 μmol/L, whereas the other methods are linear only up to 200–300 μmol/L. The detection limits and the within-run and between-day imprecisions for this method are similar to those reported in the other methods [9, 10, 12, 13]. Values reported for serum are similar to those documented by Moshage et al. [9].

We conclude that this semiautomated procedure provides an easy, less time-consuming, inexpensive, and reliable method of analysis for nitrate concentration in biological fluids, and is therefore suitable for routine performance in clinical chemistry laboratories.

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References


Postpartum Determination of Umbilical Artery Blood Gases: Effect of Time and Temperature, Moshe Manor, Isaac Blickstein,* Ynon Hazan, Orna Flidel-Rimon,1 and Zion J. Hagay (Depts. of Obstet. and Gynecol. and 1 Neonatal., Kaplan Hosp., 76100 Rehovot, Israel (affiliated with Hadassah-Hebrew Univ. School of Med., Jerusalem); *author for correspondence: fax 972-8-9411944, e-mail blick@netvision.net.il)

Determination of cord blood gases and pH is recommended in all neonates with low Apgar scores to distinguish metabolic acidosis from hypoxemia or from other causes that might result in low Apgar scores [1]. Although the metabolic acidosis found in cord blood is a poor predictor of long-term neurological injury [2], assessment of umbilical cord blood gas is helpful to exclude intrapartum or birth events that cause acidosis and serves as legal evidence against any alleged association with poor outcome [3].

Textbook recommendations for postpartum umbilical cord blood sampling include immediate transport of the blood in a heparin-containing syringe placed in a plastic sack containing crushed ice [4]. Several studies have previously questioned the utility of this method [5–10]. Sato and Saling [5] evaluated pH values after only 30 min and at every hour up to 7 h in 30 blood samples stored at room temperature and in 30 different samples stored in a refrigerator. They concluded that if fetal blood has to be stored for >50 min, it must be kept refrigerated to inhibit autoxidation. Hilger et al. [6] evaluated the sequential changes in gases and pH in blood taken from the umbilical vein or from a superficial placental vein at 15-min intervals and at room temperature only. They found that as long as blood was taken from the cord vein, the gases and pH were not affected by as long as 1 h delay in sampling. Pel and Trefferes [7] and Sykes and Molloy [8] used blood samples collected into heparin-containing syringes and stored refrigerated for as long as 6 h and compared this with blood collected from an umbilical cord segment left at room temperature. The conflicting results of the latter studies were attributed to differences in the dose of heparin in the collecting syringes. Strick-
land et al. [9] evaluated pH and Pco₂ in cord blood stored at room temperature for variable intervals after delivery and concluded that blood drawn for determination of pH and Pco₂ can be kept at room temperature for up to 30 min. Duerbeck et al. [10] used blood taken in non-heparin-containing syringes and stored at room temperature; they reported that during the 60 min after delivery, there was no significant change in the tested parameters.

Obviously, the different methodologies used in previous studies obviate a true comparison between the results. As quoted, some studies used a rather small sample size that perhaps does not have enough statistical power to demonstrate a difference. Moreover, only a few studies demonstrated reproducibility of measurements of their samples [9] and instead relied on the precision quoted by the manufacturer [10].

Because the validity of umbilical artery gas measurements has obvious importance for both clinical and medicolegal aspects, we undertook a standardized evaluation of the effect of time and temperature on umbilical cord blood gases and pH.

The effect of time and temperature on blood gas determination was studied in a series of 50 random cases. Blood (6 mL) drawn from the umbilical artery within 2 min of cord clamping was immediately transferred to six 2-mL plastic syringes that had been flushed with a 1000 units/mL heparin solution (each syringe contained <0.1 mL of residual solution). Residual air was ejected and the needle was capped. The three pairs of syringes made up the three study groups. The blood in two syringes was immediately analyzed; two syringes were kept in room temperature (20–24 °C); and two were kept in the refrigerator (4 °C), to be analyzed after 1 h. The double sampling was used to assess the reproducibility of the measurements, and the average value of the two samples was used to assess the effect of time (immediate assessment vs 1 h) and temperature (room temperature vs refrigeration).

Blood Po₂, Pco₂, and pH were determined by the Radiometer ABL 228, which measures these analytes directly. The data were analyzed by True Epistat statistic software. We used Student’s paired t-test to examine four null hypotheses; P <0.05 was considered significant.

The first null hypothesis was that there was a difference between the two samples of each group, caused by the methodology of blood sampling and analysis. The data shown in Table 1, however, indicate no significant differences between the two samples; therefore, the rejection of the null hypothesis implies high reproducibility of the method. The second null hypothesis suggested a difference between samples tested immediately and those tested after storage for 1 h in the refrigerator, caused by the effect of time and temperature. The data shown in Table 1 indicate no significant difference between the mean values of all analytes tested in both groups. The third null hypothesis suggested a difference between samples examined immediately and after storage of 1 h at room temperature, caused by the effect of time. However, the data shown reject this hypothesis and suggest that a period of 1 h has no effect on the analytes tested. The
fourth null hypothesis was that temperature had an effect on the test results. The data shown in Table 1 also reject this hypothesis and suggest that temperature alone does not affect the tested variables.

There are almost no arguments against the value of umbilical artery blood gases analysis taken postpartum in cases indicated by ante- and intrapartum events. Because blood is a living tissue, it has been argued that metabolic changes will continue at room temperature, therefore making it necessary to examine the blood immediately or to minimize the metabolic changes by reducing the temperature [5]. We carried out the present study to examine the validity of blood gas measurements under the different situations that may occur in a busy practice, where shortage of staff and equipment, compounded with a shortage of staff and equipment, may sometimes prevent immediate or appropriate storage/shipment of the blood sample to the laboratory. Our data indicate that a time of 1 h, alone or in combination with temperature (with or without refrigeration), does not significantly affect the results for pH, PCO₂, and PO₂ of arterial cord blood.

Our conclusions were similar to those found in previous studies. However, our study examined the whole range of blood gas parameters, in comparison with only selected parameters in other studies. Moreover, we used standardized sampling, i.e., blood taken into heparin-containing plastic syringes directly from the umbilical cord, whereas other studies examined blood taken with or without heparin, directly or indirectly from the cord or from a cut segment. Finally, our study is among the few that assessed reproducibility. All in all, our study seems to be among the most complete of its kind.

The results we found provide two important clinical conclusions. First, the clinical laboratory should accept an umbilical cord blood sample within 1 h after storage at room temperature. Second, although uniformity of sampling (using minimally preheparinized syringes) and shipment procedures are to be preferred, the clinician working in the busy delivery room where no one is available to optimally handle the blood sample can be reassured that if the blood sample is taken immediately, he or she does not need to arrange for immediate shipment or for special storage. As long as the blood sample is examined within 1 h, the results are still valid for both clinical and medicolegal purposes.

Reference Intervals for 18 Clinical Chemistry Analytes in Fetal Plasma Samples Between 18 and 40 Weeks of Pregnancy, Maria Luisa Gozzo, Giuseppe Noia, Giuliano Barbarese, Luigi Colacicco, Maria Annunziata Serraino, Marco De Santis, Silvio Lippa, Cinzia Callà, Alessandro Caruso, Salvatore Mancuso, and Bruno Giardina (Ist. di Chim. e Chim. Clin. and Clin. Ostet. e Ginecol., Univ. Cattolica del S. Cuore, Facoltà di Med. e Chirurg. “A. Gemelli”, Largo Francesco Vito 1, 00168 Roma, Italy; "author for correspondence: fax 039-6-35501918, e-mail b.giardina@uniserv.ccr.cm.cnr.it"

The intrauterine biochemical assessment of the human fetus is important in fetal medicine, for both prenatal diagnosis and therapy of fetal diseases. Percutaneous umbilical blood sampling (PUBS) performed with ultrasound guidance is reliable for this, and the microvolume specimens obtained by this technique are readily analyzed by modern clinical chemistry analyzers to perform complete biochemical profiles with small blood volumes.

The interpretation of fetal biochemical tests requires age-gestational specific reference intervals to reveal possible pathological processes and to minimize the remarkable physiological variability observed with fetal growth and maturation, particularly in the last trimester of gestation. Various cord blood analytes have been investigated as potential indicators of fetal distress [1, 2], but no comprehensive report of reference intervals adjusted to various weeks of gestation has been available. Here, we present reference intervals for 18 analytes in plasma samples obtained by PUBS from 72 healthy fetuses between weeks 18 and 40 of pregnancy.

We analyzed plasma from 171 fetuses who underwent PUBS between the 18th and 40th week of gestation for various indications (Rh alloimmunization 79, maternal viral or parasitic infection 42, rapid karyotyping 25, and maternal thrombocytopenia 25). Informed consent was obtained from the parents. Gestational age at the time of sampling was determined from menstrual dates or ultrasound examination when the dates were uncertain. We selected 72 fetuses as “healthy” when the suspected pathologies were not confirmed and the subsequent progress of the pregnancy revealed no abnormalities; further, these babies were confirmed healthy at birth by pediatric examination and laboratory screening.

All fetal blood samplings were performed manually with ultrasound guidance by a single operator with heparin-