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INTERNATIONAL FEDERATION OF CLINICAL CHEMISTRY (IFCC)[1], [2]

Scientific Division
Committee on pH, Blood Gases and Electrolytes[3]

Approved IFCC Recommendations on Whole Blood Sampling, Transport and Storage for Simultaneous Determination of pH, Blood Gases and Electrolytes

Prepared for publication[4] *by*

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Summary: Pre-analytical variables, e. g., specimen collection, transport, and storage, can contribute significantly to inaccurate pH, blood gas, and electrolyte values. The International Federation of Clinical Chemistry (IFCC), through its Committee on pH, Blood Gases and Electrolytes, has developed specific recommendations to minimize the undesirable effects of pre-analytical variables. The Committee has drawn upon the experiences of its own members as well as published data by others. Specifically, the Committee has included pertinent guidelines and suggestions by the IFCC Working Group on Selective Electrodes (WGSE), the National Committee on Clinical Laboratory Standards (NCCLS), and the Electrolyte/Blood Gas Division of the American Association for Clinical Chemistry (AACC).

This paper will familiarize the reader with the effect of different types of specimen containers and anticoagulants. It discusses important aspects of specimen collection procedures including patients status and special precautions during specimen collection from indwelling catheters or cannulae. The paper also identifies different requirements in storage and transport of specimens for blood gas and electrolyte analysis.

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Contents

1 Introduction

Recent technical developments have made it possible to determine blood gases, pH, electrolytes, oxygen saturation, haemoglobin derivatives, and haematocrit directly in a single blood sample.

Each of these quantities is important for the control of vital functions of the body and for the supervision of therapies in critical situations. Since these quantities can change very rapidly, results have to be available with minimum delay. This can be best achieved by simultaneous measurement in whole blood, without any centrifugation or dilution steps.

Pre-analytically, the results of the determinations may be affected mainly by

- the nature of the specimen container,
- the preparation of the container with anticoagulant (effects of the anticoagulant itself and effects of dilution of the sample),
- the technique of specimen collection,
- the storage and transport of the specimen.

The purpose of the present paper is to recommend methods of sampling and storing which are suitable for gases, pH, and electrolytes (K^+, Na^+, actual Ca^{2+}, Cl^-)[6]. In addition, the sampling and storage procedures should allow for the determination of total haemoglobin concentration or haematocrit, and haemoglobin derivatives, which are often associated with blood gas analysis.

2. Specimen containers

Blood is collected either in syringes or in capillary tubes. Containers of either glass or plastic material are used.

2.1 *Glass containers*

Glass can be regarded as an inert material impermeable to gases. Thus, glass syringes and capillary tubes are suitable for sampling, storage, and transport of specimens for electrolyte and blood gas determinations. A well-stoppered glass syringe with a tightly fitting plunger can be regarded as gas-tight for at least 2 h. The same applies to correctly closed glass capillary tubes (2).

2.2 *Plastic containers*

Plastic materials are permeable to gases. Therefore, plastic syringes cannot be regarded as perfectly gas-tight (3–5). The permeability and imperfect gas-tightness may cause a shift of the original values of pO_2 and pCO_2 towards the corresponding values in the surroundings (5–7). The degree of this shift is dependent on the material and the syringe design, e. g., thickness of the wall, surface to volume relationship, and fitting of plunger and stopper.

pO_2 may change in different directions, according to the gradient between blood pO_2 and atmospheric pO_2. By examining blood samples in different plastic syringes stored in ice water slush, it was found experimentally that original pO_2 values around 12 kPa (88 mm Hg) are elevated by 0,13–1,20 kPa[7] (1–9 mm Hg) after 45 min, most of the alterations occurring in the first 20 min (8, 9).

There are no significant changes of pCO_2 in the first 30 min if the sample is stored in ice-water (5, 8).

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[6]) For the special pre-analytical problems concerning the determination of ionized calcium, refer to "IFCC Recommendation on sampling, transport and storage for the determination of the concentration of ionized calcium in whole blood, plasma and serum" (see Ref. 1).

[7])The decimal sign is a comma.

2.3 *Practical recommendation*

Plastic syringes with tightly fitting plungers and stoppers are suitable for the determination of acid-base status (pH, pCO_2, bicarbonate, base excess) and electrolytes (sodium, potassium, chloride, ionized calcium), if the storage interval is not longer than 30 min.

For determinations including pO_2 and oxygen saturation, plastic syringes should only be used if the measurement is performed within, maximally, 15 min after sampling. Thereafter the results may be in error. The magnitude of the error should be determined before use. If pO_2 values above about 27 kPa (200 mm Hg) are expected, glass syringes are recommended.

3. Anticoagulant

The recommended anticoagulant for blood gas and electrolyte determinations is heparin. Either the sodium or the lithium salt may be used but precautions must be taken to avoid a bias in electrolyte measurements.

3.1 *Interferences by heparin*

Dry sodium heparinate added to a blood sample typically causes the following interferences:

a) Increase in the sodium concentration
Up to 15 000 IU sodium heparinate per litre of blood, no alteration of the sodium values is detectable (10). Higher concentrations may cause an increase in the sodium concentration, which can be avoided by the use of lithium heparinate.

b) Decrease in pH, bicarbonate concentration, and base excess
Up to 50 000 IU heparinate per litre of blood, these effects are insignificant. Increments of 100 000 IU of heparinate per litre may cause the following alterations (11, 12):

pH	0 to −0,004
$cHCO_3^-$	0 to −0,3 mmol/l
BE	0 to −0,3 mmol/l

c) Decrease in ionized calcium
Calcium is bound to heparinate by chelation. Up to 4000 IU heparinate per litre of blood, the decrease in ionized calcium is below 0,01 mmol/l. Increments of 100 000 IU heparin per litre blood cause a decrease of about 0.13 mmol/l (13).

3.2 *Suitable heparin concentrations*

Because of the interferences, the amount of heparin should be kept as low as possible. Often heparin concentration much higher than necessary is used (14, 15).

The amount of heparin necessary for reliable anticoagulation depends on

a) whether dry or dissolved heparin is used. To compensate for the time of delay, which is necessary to dissolve and to distribute dry heparin, the initial amount of dry heparin available has to be greater than when a solution is used.

b) whether glass or plastic containers are used. Glass containers require more heparin than plastic containers (13). Coagulation factors and platelets are activated more rapidly by glass than by plastic surfaces. The higher heparin concentration compensates for the acceleration of the coagulation process.
The following heparin concentrations are recommended:

Container	Application	Final heparin concentration in blood
Glass	Dry heparin	40 000−60 000 IU/l
Glass	Heparin solution	8 000−12 000 IU/l
Plastic	Dry heparin	12 000−50 000 IU/l[8])
Plastic	Heparin solution	4 000− 6 000 IU/l

If heparin concentrations above 15 000 IU/l are applied, the use of calcium-titrated lithium heparinate is necessary to compensate for the calcium chelation (13) and to prevent increases in sodium values. If dry heparin is used, the construction of the sampling device should ensure that heparin is dissolved and distributed rapidly throughout the sample.

3.3 *Dilution effects*

If heparin solutions are used, the sample will be diluted. The concentration of electrolytes, bicarbonate, CO_2, haemoglobin, and other constituents will decrease. pO_2 and sO_2, in most case, will increase because pO_2 of the heparin solution is about 20 kPa (150 mm Hg). In order to keep the deviations small, the dead space of the syringe should never exceed 5% of the blood volume to be drawn.

The effects of a 5% dilution on blood gases and acid-base quantities are tolerable for most practical purposes (14, 16). For "normal" arterial blood, the following approximate deviations may be calculated:

pH	0
pCO_2	−0,27 kPa (−2,0 mm Hg)
$cHCO_3^-$ and base excess	−1,2 mmol/l
pO_2	+0,53 kPa (+4,0 mm Hg)

Because the sample dilution mainly applies to the

[8]) The amount of heparin necessary is highly dependent on how heparin is applied to and distributed in the sampling device.

plasma phase, a 5% dilution on electrolyte results, however, is significant, especially for the substance concentrations of sodium and ionized calcium, the reference ranges of which are quite narrow. Thus, for combined measurements of blood gases and electrolytes, a heparin solution is recommended which yields the heparin concentration mentioned in section 3.2 and which, additionally, compensates for electrolyte dilution.

The following concentration ranges are recommended for heparin solutions:

Sodium	120,0–150,0 mmol/l
Potassium	3,5– 4,5 mmol/l
Ionized calcium	1,2– 1,4 mmol/l
Chloride	100,0–130,0 mmol/l

The solution must not contain any calcium-binding anions, e. g., bicarbonate, phosphate, and sulfate. There is no calcium binding in blood detectable for formate up to 40 mmol/l; therefore, formate could be used for the composition of "electrolyte-balanced" heparin solutions (13). pH of the heparin solution should be between 6,0 and 8,0.

4. Specimen collection

4.1 *Status of the patient*

The patient should be in a steady state of ventilation before and during blood collection for blood gas analysis. Patients breathing spontaneously should be at rest for 15 min. The ventilatory settings of patients on artificial respiration should be unchanged for 30 min before blood collection, if possible. The same applies to patients receiving supplemental oxygen.

Pain and anxiety from the arterial puncture may influence the steady state of respiration and thus should be minimized. If necessary, local anesthesia should be given.

4.2 *General requirements for sampling*

Blood may be collected for blood gas and electrolyte determinations in syringes or capillary tubes. Independently from the mode of collection the following requirements have to be fulfilled:

The sample has to be drawn and handled without contact with air ("anaerobically"), in order to keep the gas tensions, the oxygen saturation, pH, and the concentration of ionized calcium unchanged.

The sample must be anticoagulated immediately and sufficiently, in order to avoid the formation of clots which may obstruct the analytical instrument.

Damage to the cells (haemolysis) must be avoided as far as possible. Haemolysis may cause a marked increase in potassium, and decrease in ionized calcium (17, 18).

If indwelling catheters or cannulae are used for sampling, it has to be ensured that infusion fluid or flush solutions are removed completely from the system by withdrawing a volume equal to three times the "dead space" of the catheter system prior to blood collection (19).

4.3 *Blood collection by syringe*

Blood is collected by syringe from the arterial or the venous system.

4.3.1 *Kinds of specimen:* From arterial blood a well-defined specimen can be obtained which reflects the gas exchange function of the lungs and the acid-base status of the body. It is also suitable for electrolyte, haemoglobin, and haematocrit determinations. The preferred arterial collection sites are the radial, the brachial, and the femoral arteries. Visible scalp arteries are sometimes used in young children. In neonatology, blood may be sampled from the umbilical arteries with a catheter. For repeated sampling, arteries may be cannulated.

Blood gas measurements with mixed-venous blood, preferably obtained from the pulmonary artery, are necessary to get insight into the oxygen extraction from the blood and the oxygen uptake of the organism. Important quantities in this respect are the mixed-venous pO_2 and the arterio-venous difference in oxygen concentration, $cO_2(a) - cO_2(v)$.

Prior to sampling of mixed-venous blood, complete removal of infusion fluid from the catheter is necessary. It is important that the catheter is not in a wedged position. Even then, the blood sample should be aspirated very slowly (approximately 1 ml per 5 seconds) to avoid backmixing of oxygenated pulmonary capillary blood.

In addition, venous blood for the purpose of detecting shunts between the arterial and venous system may be collected.

Peripheral venous blood, e. g., from the cubital vein, should not be used for measuring pO_2 and sO_2 because the results are not representative for the body as a whole. It should only be used for the determination of electrolytes, bicarbonate, base excess, and haemoglobin or haematocrit.

For the determination of actual ionized calcium, bicarbonate, and base excess, venous blood must be sampled anaerobically. Venous occlusion by tourniquet has to be

restricted to a maximum of 2 min. Muscular action of the extremity must be avoided.

4.3.2 *Sampling procedure:* To fill dead space of the syringe with heparin, 0,3–0,5 ml of the anticoagulant solution is aspirated into the syringe, thoroughly lubricating the inner wall of the syringe. The aspiration needle is replaced by the cannula for the puncture. With the needle upwards, the air is expelled and then, inverting the syringe, the excess anticoagulant is expelled. It is recommended that the volume of blood collected be at least 20 times the dead space.

The technique and hazards of blood collection from arteries are described in many textbooks of anesthesiology, intensive care, and pulmonary medicine. An Approved Guideline has been published by NCCLS (19).

Immediately after blood collection, the sample is checked for air bubbles, which have to be expelled at once (20–22). The needle is replaced by a tightly fitting cap. It is important to provide safe disposal of the needle. To ensure approximate mixing of blood and anticoagulant, the syringe is inverted five times and rolled between the palms for 5 seconds before being measured or stored in ice-water slush. Blood samples should be properly labeled including the following information: patient's name, date, collection time, site, and type of sample.

Recently, plastic blood gas syringes prepared with dissolved or dry heparin have been developed which fill by arterial blood pressure, the air being expelled through a vent in or beside the plunger. The instructions of the manufacturer as well as the outlines in the present paper concerning plastic syringes should be followed (see section 2.2).

4.4 *Blood collection by capillary tubes*

Heparinized capillary tubes are normally used to collect blood specimens by skin puncture. This procedure is routinely applied to neonates and young children, but may be used in adults too (e. g., ambulatory patients, sports medicine, industrial medicine).

4.4.1 *Skin puncture blood:* Skin puncture blood is collected from the medial or lateral plantar heel surface, from the palmar surface of the distal portion of a finger, or from the ear lobe.

In contrast to arterial blood, skin puncture blood is a mixture; it consists of blood from arterioles, venules, and capillaries, and contains small amounts of interstitial and intracellular fluid. Because of the arterial pressure, the proportion of blood from the arterioles and the arterial limb of the capillaries is dominant.

For blood gas and electrolyte analysis, the greatest similarity to arterial blood is intended. Therefore, it is necessary to increase the proportion of arterial blood as much as possible ("arterialization") and to avoid any pressure to the surrounding tissue which would increase the proportion of venous blood and interstitial fluid (see section 4.4.2).

The correlation of pH and pCO_2 between "arterialized" capillary blood and blood from an artery is excellent (23). In contrast, pO_2 values may differ considerably due to different proportions of venous blood in the specimen (24).

The results of ionized calcium are very similar (25). Very often a significant positive bias in the potassium concentration is observed (26). This is due to potassium from cells and will be enhanced by squeezing the tissue and by rigorous mixing inside the capillary tube. Note that newborns may have increased erythrocyte fragility and haematocrit values.

4.4.2 *Sampling procedure:* The exact sites, the technique, and the risks of skin puncture are described in many textbooks and in special guidelines (27, 28).

Arterialization is achieved by warming the skin. The puncture site and its surroundings are covered by a hot, moist towel at a temperature between 39 and 42 °C for 3 min or, if appropriate, immersed in water of 42 °C. After cleaning with a neutral antiseptic liquid, the site is punctured with a lancet, the tip of which has a length of 3–4 mm, or for infants up to 3 months, 2.4 mm (29).

The first drop is removed. Blood should flow without any squeezing to form a drop of 3–4 mm in diameter. Placing the tip of the capillary deeply into the drop and holding the capillary horizontally or slightly downwards from the puncture site, the tube is filled completely. The end which was in the blood drop is sealed immediately with a plastic cap. Then a metal mixing bar ("flea") is inserted, and the opposite end of the capillary is sealed. Blood and heparin are mixed by moving the bar with a magnet from end to end, five times.

5. Storage and transport

5.1 *Processes during storage and transport*

Specimens for blood gas and electrolyte measurements may be affected during storage by the following processes.

5.1.1 *Metabolism of the blood cells:* Glycolysis – mainly in the red cells – causes formation of lactic acid and shifts pH, $cHCO_3^-$, and base excess towards the range of metabolic acidosis. Oxygen consumption in

leukocytes and platelets decreases pO_2 and increases pCO_2. The fall in pO_2 is accelerated if the original sample pO_2 is elevated (30).

For a sample remaining at room temperature for 30 min, the following alterations (SD) were found (12):

pH	−0,021 ±	0,008
pCO_2	+0,11 ±	0,14 kPa
	+0,86 ±	1,02 mm Hg
$cHCO_3^-$	−0,44 ±	0,50 mmol/l
Base excess	−0,77 ±	0,41 mmol/l
pO_2 normal range	−7,5 ±	3,7% of original value (8,0−16,0 kPa or 60−120 mm Hg)
pO_2 high range	−30,1 ±	2,9% of original value (18,7−26,7 kPa or 140−200 mm Hg)

These data were ascertained from severely diseased patients in an intensive care unit (12). In patients without anaemia and leukocytosis, smaller decreases of pO_2 are usually found (30).

The metabolic processes are reduced by cooling the sample. Cooling is necessary if the sample cannot be analyzed within 15 min after sampling. The sample is placed in ice-water slush. Large cubes of ice do not cool appropriately. The sample must be immersed totally.

5.1.2 *Ion release from the blood cells:* Prolonged storage, vibration during sample transport (e. g., with some pneumatic dispatch systems), extremely fragile red cells, and severe thrombocytosis are factors which may contribute to an increase of potassium and phosphate and a decrease in ionized calcium in the plasma.

During the first hour of storage in ice water, the average increase in the plasma potassium concentration is 0.1 mmol/l (8, 31).

5.1.3 *Leakage of gases:* As mentioned in section 2.2, containers of plastic material are not absolutely gastight; alterations of pO_2 become detectable after several minutes. Glass capillaries and glass syringes are gastight (3−8) for several hours.

5.2 *Recommended storage procedures*

A blood sample in a closed glass container (syringe or capillary) cooled by ice-water slush can be stored for at least 2 h to measure pH, blood gases, oxygen saturation, haemoglobin or haematocrit, and ionized calcium. If potassium is included, the storage interval should not exceed 1 h.

If pO_2 and sO_2 measurements are included, blood samples collected in plastic syringes should not be stored in ice, but kept at room temperature and analyzed within 15 min. However, room temperature storage may cause additional pre-analytical errors when extreme leukocytosis ($> 50 \times 10^9$/l), thrombocytosis ($> 600 \times 10^9$/l), or anaemia (Hb < 75 g/l) is present; in cases of severe anaemia, the ability of haemoglobin to "buffer" the endogenous oxygen influx is reduced. Immediate analysis is preferred when the history of the patients is unknown.

Blood samples, the pO_2 of which is supposed to be above 27 kPa (200 mm Hg), should be collected in glass syringes. They must be analyzed as soon as possible.

Blood samples with leukocyte concentrations above 50×10^9/l or platelet counts above 600×10^9/l should be analyzed immediately.

If only acid-base status and electrolytes are to be measured, the sample can be stored in slushed ice-water for 30 min.

If only electrolytes are to be measured, the specimen should not be stored longer than 1 hour to avoid release of potassium from red cells. If ionized calcium is included, the sample must be refrigerated (4 °C).

6. Mixing before measurement

After any storage period, careful remixing of the samples prior to analysis is necessary, especially if determinations of haemoglobin concentration, haematocrit, and oxygen concentration are intended.

Specimens in glass capillaries are remixed by moving the metal wire ("flea") from end to end for 5−10 s.

Specimens in glass or plastic syringes are remixed by inverting the syringe 10 times and then rolling it horizontally for 10 s.

Remixing of blood in syringes can be facilitated by use of a metal washer (thickness of 1 mm), which is somewhat smaller in diameter than the internal width of the syringe. The platelet is introduced into the syringe before filling the dead space with heparin solution.

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