

The use of heparin in preparing samples for blood gas analysis

April 2007



Chris Higgins

Little Acre, Main Road
Shurdington
Nr Cheltenham
Gloucester
GL51 4XF, UK
E-mail: cjhiggins@hotmail.co.uk

The significance of good practice during the pretesting phase of clinical laboratory investigation cannot be overemphasized.

The production of high-quality, accurate results which are clinically useful depends as much on practice before the patient's sample reaches the laboratory as it does on the analytical phase within the laboratory.

There are few tests that better exemplify this general truth than blood gas analysis. Less than scrupulous adherence to protocol for blood collection, as well as that for the handling and timely transport of specimens, can invalidate blood gas results.

This article addresses one aspect of the pretesting phase of blood gas analysis: the use of heparin to anticoagulate blood samples. The main focus will be the potential errors in measurement of blood gas analyzer

parameters that can arise as a result of the necessary addition of heparin to blood, and how these can be avoided or at least minimized.

The article begins with a brief overview of heparin itself.

Heparin overview

Heparin is a naturally occurring anticoagulant present in all mammalian species, so called because it was first isolated in 1916 from liver tissue [1]. It is synthesized in mast cells and basophils and stored in the secretory granules of these cells.

Since mast cells are present in many tissue types, heparin can be sourced from a range of extrahepatic tissues. Commercial preparations are now most commonly derived from the mucosal intima of pig (porcine) intestine.

1. Structure

Heparin belongs to a family of complex carbohydrates, known as the glycosaminoglycans (or mucopolysaccharides). In essence, glycosaminoglycans are long unbranched chains of repeating disaccharide units, each comprising an N-acetyl hexosamine and either a hexose or hexuronic acid.

Within this general format many different disaccharide subunits are possible and these can be joined in a multiplicity of sequences to a range of chain lengths. These variables account for the great molecular heterogeneity displayed by both glycosaminoglycans as a family and family members such as heparin.

Heparin, then, is a heterogeneous "mixture" with regard both to chain composition and chain length so that molecular weight ranges from 3kDa to 30 kDa (mean around 15 kDa).

One of the features that distinguishes heparin from other glycosaminoglycans is that it contains an unusually high proportion of sulphated disaccharide units. A particular, unique highly sulphated pentasaccharide sequence that is present in 30 % of heparin molecules accounts for the anticoagulant effect of heparin [2].

2. Anticoagulant action and therapeutic use

Heparin prevents blood from clotting because the unique pentasaccharide sequence contained within its structure binds avidly to antithrombin III. Antithrombin III is a plasma protein that inhibits blood clotting by binding to and thereby inhibiting the enzymic action of several activated blood clotting factors, including Factors XIa, Xa, IXa and IIa (thrombin).

The physiological function of antithrombin III, in common with other inhibitors of the blood-clotting cascade, is to prevent in vivo blood clotting and thereby maintain fluidity of blood within intact vessels. The effect of heparin binding is to increase the activity of antithrombin III more than 1000 fold [3].

As a consequence, fibrin formation by the clotting cascade, a necessary requisite for blood clot formation, is prevented. This anticoagulant effect of heparin occurs both in vitro and in vivo.

Since the late 1930s the in vivo effect of administered heparin to artificially lower the coagulability of blood has been used therapeutically [4]. It is not absorbed from the gastrointestinal tract, so it must be administered by intravenous or subcutaneous injection.

Despite this limitation, heparin remains one of the most widely prescribed antithrombotic drugs, used principally for the treatment and prevention of venous thrombosis and pulmonary embolism.

Unfractionated heparin (UFH) presented as the sodium salt is the traditional, once the only available form of heparin. In more recent years, however, more refined low-molecular-weight heparin (LMWH) preparations with mean molecular weight in the range of 3-5 kDa have replaced UFH (mean molecular weight around 15 kDa) as the drug of choice in many clinical contexts, principally because these new preparations are associated with fewer adverse side effects [5].

An even more recent development is a class of synthetic heparin drugs based on analogues of the crucial antithrombin III-binding pentasaccharide sequence. Fondaparinux (Atrixia) is the best established of these "synthetic heparin" drugs.

3. Standardization of heparins – salts of heparin

Heparin activity (concentration) is measured in either International Units (IU) defined by the World Health Organization (WHO) International Standard [6], or United States Pharmacopeia (USP) units.

The USP unit is defined as the amount of heparin that prevents 1.0 mL of citrated sheep plasma clotting for 1 hour after addition of 0.2 mL of 1 % CaCl₂ solution [7]. This is of the order of 0.005 mg heparin.

There is a small (7-10 %) difference between the IU and USP unit [5], and there are currently moves to harmonize the two units [8]. Sodium heparin is the naturally occurring salt of heparin used medicinally and in the laboratory.

Lithium heparin, which is used exclusively in the laboratory as an in vitro anticoagulant, is prepared from sodium heparin by cation-exchange chromatography.

In vitro use of heparin and blood gas analysis

The in vitro anticoagulant action of heparin has been exploited in the preparation of blood samples for laboratory chemical analysis for well over 50 years.

Most chemical analyses are performed on the liquid (non-cellular) portion of venous blood that is recovered following centrifugation of blood. Anticoagulation allows immediate separation of this liquid portion (plasma), thereby avoiding delay of an hour or more, required for clot retraction, in separation of the liquid portion (serum) from blood that has no added anticoagulant.

The recommended method of anticoagulation for most plasma-borne chemical analytes is lithium heparin at a final heparin concentration of 10-30 USP units/mL blood [9].

This is recognized to be an excess of heparin for effective anticoagulation, and experience over several decades has demonstrated that at this concentration addition of heparin has no effect on a range of the most commonly requested blood analytes (i.e. no clinically significant bias compared with serum).

In this instance anticoagulation is not essential, but provides a speedier, more convenient means of sample recovery.

There are some blood tests, by contrast, that can only be performed on a homogeneous whole-blood sample.

For these tests, which include pH, $p\text{CO}_2$ and $p\text{O}_2$, the

traditional parameters measured by all blood gas analyzers, an anticoagulated blood sample is essential (unless analyzed within 1-2 minutes of collection before the process of in vitro coagulation is sufficiently underway [25]), and heparin in one form or another has always been the anticoagulant of choice for blood gas analysis.

It is essential that anticoagulation is complete, not only for a homogeneous specimen necessary for accurate results, but also to avoid the presence of microthrombi (not necessarily visible to the naked eye) that can block the sample pathway of blood gas analyzers.

To address the issues surrounding the use of heparin it is useful to take an historical perspective because new problems associated with heparin use emerged over time as a result of the increased repertoire of tests available on modern blood gas analyzers.

Initially, when the blood gas analyzer repertoire was confined to blood gas ($p\text{CO}_2$, $p\text{O}_2$) and pH measurement, samples for blood gas analysis were anticoagulated with sodium heparin solution.

1. The early years – use of liquid sodium heparin solution

In 1960 Siggaard-Andersen determined that as long as the heparin concentration in blood is maintained below 1 mg/mL (i.e. around 200 IU/mL blood), it has no effect on measured acid-base parameters (pH and $p\text{CO}_2$). For every mg/mL of blood above this heparin concentration he showed that pH falls by 0.003 units and $p\text{CO}_2$ rises by 0.1 mmHg (0.013 kPa) [10].

Since the heparin concentration required to achieve anticoagulation is considerably less than 1 mg/mL, and heparin in solution is easily mixed with blood, sodium (and later lithium) heparin solution (1000 IU/mL) was adopted as the conventional means of anticoagulating arterial blood for measurement of pH, $p\text{CO}_2$ and $p\text{O}_2$. Just 0.2 mL of sodium (lithium) heparin (1000 IU/mL) added to 5 mL of blood will give a final heparin concentration of 40 IU/mL blood, sufficient for anticoagulation.

The principle disadvantage of liquid heparin is a potential for error if blood is over-diluted with heparin. This potential error is due to the considerable difference in pH, $p\text{CO}_2$ and $p\text{O}_2$ of liquid heparin compared with that of arterial blood.

Approximate values for heparin solution are pH 6.4; $p\text{CO}_2$ 7.5 mmHg (1 kPa) and $p\text{O}_2$ 160 mmHg (21 kPa), reflecting the fact that heparin is an acidic solution in equilibrium with air. A number of studies [11-14] have examined the effect on measured blood pH, $p\text{CO}_2$ and $p\text{O}_2$ of increasing sample dilution with heparin.

In summary, these studies have demonstrated that measured pH is resistant to this dilution effect. Even if heparin and blood are mixed in equal volumes, i.e. 50 % dilution of blood, pH remains constant, presumably due to the buffering capacity of blood. $p\text{O}_2$ is also relatively resistant to the dilution effect.

In some studies no effect on $p\text{O}_2$ was observed, whilst in others an increase in $p\text{O}_2$ was observed at high (35-50 %) dilution. $p\text{CO}_2$ is the most susceptible parameter. As long as dilution is less than 10 % (e.g. 0.5 mL heparin added to 5.0 mL of blood), $p\text{CO}_2$ is not significantly affected, but dilutions above 10 % are associated with an increasingly significant decline in $p\text{CO}_2$ values.

Results of one study [13] are representative. This demonstrated that if blood with $p\text{CO}_2$ of 51 mmHg (6.8 kPa) is diluted 10 %, 20 % and 40 %, measured $p\text{CO}_2$ is reduced to 44 mmHg (5.9 kPa), 39.0 mmHg (5.2 kPa) and 31 mmHg (4.1 kPa), respectively.

That is an approximate 1 % decline in $p\text{CO}_2$ for every 1 % increase in dilution. Calculated acid-base parameters, bicarbonate and base excess that are derived from measured $p\text{CO}_2$ are affected to the same magnitude.

It should be emphasized that the dilution effect described above is not due to heparin per se but rather to the fact that liquid (equilibrated with ambient air) is being added to blood. Exactly the same effect is seen if saline is added to blood.

To avoid the potential dilution errors in $p\text{CO}_2$ and $p\text{O}_2$ associated with the use of liquid heparin, it is important that the volume of heparin is certainly less than 10 % and preferably closer to 5 % or less of the total sample volume.

This is achieved by ensuring that syringes only contain sufficient heparin to wet the wall of the syringe and fill the dead space occupied by the syringe hub. This dead-space volume is around 0.1 mL for a standard 2.0-mL syringe and a dilution of 0.1 mL heparin (1000 IU/mL) in 2.0 mL of blood results in a dilution of 5 % and a final heparin concentration of 50 IU/mL of blood.

The risk of error in $p\text{CO}_2$ and $p\text{O}_2$ measurement associated with incorrect use of liquid sodium (or lithium) heparin is entirely eliminated if samples are collected into a syringe containing lyophilized (dried) heparin.

The disadvantage of dried heparin is that it is less easily mixed with blood, posing the theoretical risk that its use is associated with incomplete anticoagulation unless heparin concentration is increased [15].

However, in a study comparing dried with liquid sodium heparin at exactly the same concentration (33 IU/mL blood) there was no difference in measured pH, $p\text{CO}_2$ or $p\text{O}_2$ and no difficulties of clotting were encountered with the use of dried heparin [16].

2. Extended test repertoire poses new challenges

Clinically demand-driven advances in technology allowed the development of blood gas analyzers capable of measuring a number of additional chemical analytes (e.g. sodium, potassium, ionized calcium, bilirubin, glucose) on the same arterial blood specimen used to measure pH, $p\text{CO}_2$ and $p\text{O}_2$.

It is now important that the heparin used to anticoagulate blood gas samples does not interfere with only pH, $p\text{CO}_2$ and $p\text{O}_2$, but also does not interfere with any of these additional analytes.

Measurement of electrolytes, particularly ionized

calcium, posed a particular problem because the conventional heparin preparations used to anticoagulate blood for pH, $p\text{CO}_2$ and $p\text{O}_2$ measurement cause significant errors in the measurement of ionized calcium.

2.1 Heparin and ionized calcium

Two types of error in the measurement of ionized calcium can arise as a result of using heparin as anticoagulant. The first is a straightforward dilution error, resulting from the use of conventional liquid heparin preparations.

The magnitude of the negative bias in ionized calcium that results from the use of liquid heparin is directly proportional to the dilution of blood by heparin and can be as high as 5 % [17].

This error, which theoretically applies to some extent to all analytes, is minimized by maintaining the ratio of heparin to sample volume as low as is practicably possible. It can with scrupulous technique be just 21 μL heparin: 2 mL blood, i.e. 1 % dilution.

At this dilution there is a small but almost clinically insignificant bias in ionized calcium [18]). A safer strategy, which ensures that there can be no dilution error, is to use dried (lyophilized) heparin. This is the strategy recommended by CLSI (NCCLS) guidelines [19].

The second source of potential error arises as a result of the calcium-binding property of heparin. Sodium (and lithium) heparin binding of calcium artefactually reduces the ionized calcium concentration, the magnitude of the reduction being directly proportional to the heparin concentration.

A lithium heparin concentration of 15 IU/mL blood, for example, causes an approximate 0.03 mmol/L reduction in ionized calcium. This negative bias rises to 0.15 mmol/L with a heparin concentration of 50 IU/mL and to 0.19 mmol/L if the heparin concentration is 100 IU/mL [15,17].

One of two basic strategies has been adopted to combat the problem of calcium binding by lithium heparin:

- reducing the concentration of conventional lithium heparin or
- abandoning conventional lithium heparin in favor of alternative novel heparin products (calcium-balanced heparin, zinc heparin and blended zinc/lithium heparin)

To reduce the negative bias in ionized calcium induced by heparin binding to insignificant levels, the lithium heparin concentration must be less than 10 IU/mL blood [20].

Some experts, however, caution that “while 10 IU/mL heparin is clearly advisable to minimize the side effects of heparin, it should be expected that this low concentration might not eliminate the clotting process within all analyzers.

We have noted, with certain blood specimens, that even concentrations as high as 150 IU/mL are insufficient” [23]. The potential problem of inadequate anticoagulation associated with the use of a low heparin concentration has spawned novel technology in which dried lithium heparin is prepared in a “puff” of inert filler that disperses readily in blood following mixing.

This technology has been used in a commercial blood gas sampler that allows a lithium heparin concentration of just 2.8 IU/mL blood. The approach has been validated in several trials [20-22] that showed no bias in measured electrolytes (including ionized calcium) when compared with blood without added anticoagulant.

Calcium (electrolyte) balanced heparin is a mixture of lithium and sodium heparinates to which calcium chloride has been added, so that the final ionized calcium concentration is 1.25 mmol/L, the mid-point in the concentration range of ionized calcium for healthy adults.

This novel heparin preparation eliminates the effect of calcium binding at a calcium concentration of 1.25 mmol/L, but as the concentration deviates from 1.25 mmol/L, a slight (clinically insignificant) bias occurs [24, 25].

This bias is positive if the concentration is less than 1.25 mmol/L and negative if the concentration is greater than

1.25 mmol/L. So, for example, if the true ionized calcium concentration is 0.75 mmol/L, bias at this concentration is +3 % and measured ionized calcium would be 0.77 mmol/L.

Conversely, if the true ionized calcium concentration is 2.5 mmol/L, bias is -2 % and measured ionized calcium is 2.45 mmol/L. Around 90-95 % of ionized calcium results lie in the range of 0.9-1.8 mmol/L, and for all results within this range the bias is less than 2 % and clinically acceptable.

The advantage of this approach is that the heparin concentration can be relatively high (70 IU/mL blood) so that the risk of inadequate anticoagulation is much reduced.

Several studies [21,22,24,25] have validated the use of calcium (electrolyte) balanced heparin for all analytes measured on blood gas analyzers. The addition of calcium-containing anticoagulant to the sample renders it unsuitable for total calcium determination in the laboratory.

The rationale for the use of zinc heparin is based on the premise that the heparin sites where calcium would normally bind are occupied by zinc. This effectively reduces calcium binding by heparin and thereby the associated error in ionized calcium measurement.

In fact, zinc heparin was shown to increase ionized calcium slightly [26]. Since lithium heparin decreases ionized calcium by the same amount that zinc heparin increases it, a 1:1 blend of the two was trialed and shown to eliminate bias in ionized calcium across the concentration range seen in health and disease.

It was also shown to have no effect on a range of other analytes, including total calcium [27]. Several studies have validated the use of a zinc-lithium heparinate blend as a suitable anticoagulant for blood gas analysis [21, 28].

Summary

Heparin is the only anticoagulant used to prepare

samples for blood gas analysis. There are two ways in which heparin can interfere with results. The first is high heparin concentration in blood and the second is heparin dilution of blood if liquid rather than dried (lyophilized) heparin is used.

Traditional blood gas analytes (pH, $p\text{CO}_2$ and $p\text{O}_2$) are less affected than electrolytes (particularly ionized calcium), also measured on modern blood gas analyzers. The sample requirements as far as heparin is concerned are thus less exacting if only pH, $p\text{CO}_2$ and $p\text{O}_2$ are to be measured.

For these analytes it is still essential that the heparin (either sodium or lithium) concentration is less than 200 IU/mL blood and that the blood is not diluted more than 5 %. The inclusion of electrolytes in the test repertoire excludes the use of sodium heparin in favor of lithium heparin.

The inclusion of ionized calcium in the test repertoire demands that the heparin should be lyophilized and the concentration should be not exceed 10 IU/mL blood, unless a specialized heparin that eliminates the effect of calcium binding by heparin is used.

Whatever the heparin formulation, it is essential for accurate results that the correct volume of blood is sampled to achieve a correct heparin concentration (and dilution, if liquid heparin is used), and that blood and anticoagulant are well mixed immediately after sampling.

One of the most common practical problems associated with blood gas analysis is inadequate anticoagulation and the formation of small blood clots that can block the sample pathway of blood gas analyzers and invalidate results. Inadequate mixing of specimen with heparin is usually the problem.

Clearly, the lower the heparin concentration the greater is the risk that poor mixing technique will give rise to inadequate anticoagulation and the associated problems.

References

1. Marcum J. The origin of the dispute over the discovery of heparin. *J Hist Med Allied Sci* 2000; 55: 37-66.
2. Lindahl U, Backstrom G, Hook M *et al.* Structure of the antithrombin binding site in heparin. *Proc Natl Acad Sci* 1979; 76: 3198-3202.
3. Bjork I, Lindahl U. Mechanism of the anticoagulant action of heparin. *Mol and Cell Biochem.* 1982; 48: 161-82.
4. Crafoord C. Heparin and post-operative thrombosis. *Acta Chirurgica Scand.* 1939; 82: 319-22.
5. Baglin T, Barrowcliffe TW, Cohen A *et al.* Guidelines on the use and monitoring of heparin. *Brit J Haematol*, 2006; 133: 19-34
6. Gray E, Walker D, Mulloy B *et al.* A collaborative study to establish the 5th International Standard for Unfractionated Heparin. *Thromb Haemost* 2000; 84: 1017-22
7. Vickers MD, Morgan M, Spencer, Read M. *Drugs in Anaesthetic and Intensive Care practice.* 8th ed London: Butterworth-Heinemann 1999
8. Personal communication UK National Institute for Biological Standards March 2007.
9. National Committee for National Clinical Laboratory Standards NCCLS Tubes and additives for venous blood specimens 5th edition H1-A5 NCCLS: Pennsylvania USA 2003.
10. Siggaard-Andersen O. Sampling and storing of blood for determination of acid-base status. *Scand J Clin & Lab Invest.* 1961; 13:196-204
11. Hamilton R, Crockett A, Alpers J. Arterial blood gas analysis; potential errors due to the addition of heparin. *Anaesth Intensive Care* 1978; 6: 251-55.
12. Dake MD, Peters J, Teague R The effect of heparin dilution on arterial blood gas analysis. *Western J Med* 1984; 140: 792-93.
13. Hutchinson A, Ralston S, Dryburgh F *et al.* Too much heparin: possible source of error in blood gas analysis. *BMJ* 1983; 287: 1131-32.
14. Gayed A, Marino E, Dolinski E. Comparison of the effects of dry and liquid heparin on neonatal arterial blood gases. *Am J Perinatol* 1992 9: 159-61.
15. Toffaletti J. Use of novel preparations of heparin to eliminate interference in ionised calcium measurements: have all the problems been solved? *Clin Chem* 1994; 40: 508-09.
16. Madieto G, Sciacco R. Use of syringes containing dry lyophilized heparin in sampling blood for pH measurement and Blood-Gas analysis. *Clin Chem* 1982 28: 1727-29.
17. Sachs Ch, Rabouine Ph, Chaneac M *et al.* Preanalytical errors in ionized calcium measurements induced by the use of liquid heparin. *Ann Clin Biochem* 1991; 28: 167-73
18. Shin CS, Chang CH, Kim J-H. Liquid heparin anticoagulant produces more negative bias in the determination of ionized magnesium than ionized calcium *Yonsei Med J* 2006; 47: 191-95.
19. National Committee for Clinical Laboratory Standards (NCCLS) Procedures for collection of Arterial Blood Specimens 4th edition H11-A4 NCCLS:Pennsylvania USA 2004
20. Swanson J, Heeter C, Limbocker M *et al.* Bias of ionized calcium results from blood gas syringes. *Clin Chem* 1994; 40 : 669-70
21. Toffaletti J, Widerman R. The effects of heparin anticoagulants and fill volume in blood gas syringes on ionized calcium and magnesium measurements. *Clin Chim Acta* 2001; 304: 147-51
22. Sachs C, Rabourne P, Dautzenberg M-D. Evaluation of syringes for ionized calcium measurements. *Scand J Clin Lab Invest* 1996 56 (suppl 224): 193-01.
23. Anon. Summary of Comments and Working Group responses C46-P in : National Committee for National Clinical Laboratory Standards (NCCLS) C46-A Blood gas and pH analysis and related measurements; Approved Guideline NCCLS: Pennsylvania 2001.
24. Sachs Ch, Rabouine Ph Chaneac M *et al.* In vitro evaluation of a heparinised blood sampler for ionized calcium measurement. *Ann Clin Biochem* 1991; 28: 240-44.
25. Toffaletti J, Ernst P, Hunt P *et al.* Dry electrolyte-balanced heparinized syringes evaluated for determining ionized calcium and other electrolytes in whole blood. *Clin Chem* 1991; 37: 1730-33.
26. Lyon ME, Guajardo M, Laha T *et al.* Zinc heparin introduces a pre-analytical error in measurement of ionised calcium concentration. *Scand J Clin Lab Invest* 1995; 55: 61-5
27. Toffaletti J Thompson T Effects of blended lithium-zinc heparin on ionized calcium and general clinical chemistry tests *Clin Chem* 1995; 41: 328-29.
28. Landt M Hortin G Smith C *et al.* Interference in ionized calcium measurements by heparin salts. *Clin Chem* 1994; 40: 565-570