บทความปริทัศน์

Using heparin tube for clinical chemistry, an application of evacuated blood collection system

วิโรจน์ ไววานิชกิจ[1]

Abstract:

Using heparin tube for clinical chemistry, an application of evacuated blood collection system

Wiwanitkit V.

Department of Laboratory Medicine, Chulalongkorn University, Bangkok, 10330, Thailand

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Heparin is a common blood anticoaulant. The heparinized vacuum tube is an application of the evacuated blood collection system. Using the vacuum heparinized tubes can bring similar results compared to the routine plain tubes for clinical chemistry laboratory. It can decrease the turnaround time of the tests. However, due to its high cost, it seem suitable for the laboratories with high workloads only.

Key words: *heparin, clinical chemistry*

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[1]**M.D., Lecturer, Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, 10330, Thailand**

รับต้นฉบับวันที่ 11 พฤศจิกายน 2545 รับลงตีพิมพ์ 3 มิถุนายน 2546

บทคัดย่อ:

เฮปารินเป็นสารกันเลือดแข็งที่ใช้บ่อย หลอดที่ถูกฉาบเคลือบเฮปารินเป็นหลอดที่ผลิตขึ้นเพื่อการประยุกต์ใช้อีกรูปแบบหนึ่งของการเจาะเลือดด้วยระบบสุญญากาศ ในปัจจุบันมีแนวคิดที่จะนำหลอดชนิดนี้มาใช้ในงานด้านเคมีคลินิกโดยทั่วไป เนื่องจากการใช้หลอดชนิดนี้มีผลรบกวนน้อยและหลอดเฮปารินยังช่วยลดระยะเวลารอคอยผลการตรวจได้อีกด้วยเนื่องจากไม่ต้องผ่านขั้นตอนการรอการแข็งตัวของเลือดเพื่อให้ได้น้ำเหลือง การใช้หลอดดังกล่าวสามารถให้ผลการตรวจที่สอดคล้องกับหลอดที่ไม่บรรจุสารกันเลือดแข็งที่ใช้กันทั่วไปในทางเคมีคลินิก อย่างไรก็ตาม ราคาของหลอดดังกล่าวค่อนข้างสูงจึงเหมาะสำหรับห้องปฏิบัติการที่มีภาระงานสูงเท่านั้น

คำสำคัญ: *เฮปาริน, เคมีคลินิก*

Introduction

The venipuncture procedure is complex, requiring both knowledge and skill to perform. Each phlebotomist generally establishes a routine that is comfortable for her or him. Several essential steps are required for every successful collection procedure.[1-2] The selection of the additive for each test is important.

Heparin[2] is a common anticoagulant, available as an ammonium, lithium or sodium salt, and its action is thought to prevent the transformation of prothrombin to thrombin and thus not allow a fibrin clot from forming. It is a good anticoagulant because it causes minimal interference in clinical chemistry (Table 1). Unfortunately, it is expensive. In addition, it is not recommended for the preparation of blood smears when using Wright's stain because it causes a blue background to form on the smear. Therefore, at present, it is recommended for only some special tests.

Heparin is a naturally occurring anticoagulant stored in, and released from, the mast cells lying beneath the vascular endothelium. Commercial heparins are extracted from animal tissues, purified and concentrated, and have molecular weights of 3,000 to 57,000.[2] Apart from the routine heparin, there are some special heparin subtypes such as low molecular weight heparin (LMWH) and unfractionated heparin.[2-3]

- Low Molecular Weight Heparin (LMWH)

This subtype is heparin with a mean molecular weight of about 5,000 - commercially prepared by enzymatic depolymerization of conventional porcine heparin, LMWH does not prolong the activated partial thromboplastin time (APTT) as much as high-molecular weight heparin and binds less to platelets.

- Unfractionated Heparin

Unfractionated heparin is produced from routine heparin by further processing by enzymatic digestion and other methods to yield unfractionated heparin, also used as an anticoagulant.

Table 1 Heparin comparing with the other anticoagulants

Anticoagulant	Mechanism	Pros	Cons
Heparin	Prevent the transformation of prothrombin to thrombin	Few interference on chemistry analytes	High cost
EDTA	Binds calcium, forming insoluble calcium salts	Well preserve of the blood cell	Interference on chemistry analytes
Citrate	Binds calcium, forming insoluble calcium salts	Few interference on clotting factors	Interference on chemistry analytes

High sodium values can result from sodium heparin salt interference. High lithium values can result from lithium heparin salt interference and high BUN values can result from ammonium heparin salt interference. Apart from the interference, the other two main problems from using heparin as additive are calcium binding and dilution effects. The International Federation on Clinical Chemistry (IFCC) Scientific Division, Working Group on Ion-Selective Electrodes (WGSE)[1] has recommended the following solutions.

- Ca 2+ binding by heparin can be minimized by using either of the following: 1) a final concentration of sodium or lithium heparinate of 15 IU/mL blood or less; or 2) calcium-titrated heparin with a final concentration of less than 50 IU/mL blood.

- Dilution effect can be avoided by the use of dry heparin in capillaries or syringes.

In general, heparinised tube is recommended for most haemolytic studies, cytogenetics virology, collection of white cells for cytomegalovirus (CMV) isolation, clinical chemistry tests and antibiotic assays (minimum volume > 1 mL) at present (Table 2).

Heparinized vacuum tube

Since it is difficult to hold and prepare in-house routine heparin tubes heparinized tubes for usage are commercially available (Table 3). The heparinized vacuum tube is an application of the evacuated blood collection system. Usually the heparinized tubes are classified as plasma tubes. The inner wall of the tube is coated with lithium heparin, ammonium heparin or sodium heparin. The anticoagulant heparin activates antithrombins, thus blocking the coagulation cascade and producing a whole blood/plasma sample instead of clotted blood plus serum.[1-2]

Most blood tests listed in clinical chemistry (Table 3) can be carried out on a single specimen of 5 mL of blood in a heparin tube. Currently the heparinized tube contains lithium heparin as an anticoagulant, and is used for certain tests in hematology, chemistry and virology (Table 1). The specific stopper color of the heparinized tube is dark green.[4] Concerning heparinized tubes' characteristic, the concentration of additive is about 14.3 USP Units and volume of blood drawn is about 2-10 ml without minimun volume effect (the acceptable range of collected blood volume is ± 10%). Concerning the order of draw, heparinized tube is usually the last draw.[4]

Table 2 Examples of blood tests that can use the heparinized tube as blood collection tube

Types of use	Tests for
Clinical chemistry	Insulin
	Catecholamines
	Cortisol
	Dexamethasone
	Galactose l Phosphate Uridyl transferase
	Porphyrins
	Vitamin BI, B2, B6
	Emergency general chemistry tests
Toxicology	Heavy metals (except Pb)
Hematology	Carboxy haemoglobin
Other tests	Chromosome studies

Table 3 Steps in preparation of homemade heparinized tube*

Step	Brief descriptions
1. Glass tube preparation	Clean the used tube as the cleaning protocol and prepare for the further process
2. Heparin preparation	Prepare heparin power or solution according to the calculated ratio to expected col lected blood
3. Filling of the tube	Fill prepared heparin into the prepared glass tube
4. Incubation	Wet incubation for drying and sterility in the hot air oven

*However, these processes are not recommended since it is very difficult to control the proper quantity of prepared heparin and the practitioners have risk in prepartion.

Recently, there is a new application of the vacuum tube. Plasma tubes with lithium heparin and gel containing a barrier gel in the tube are produced. The specific gravity of this material lies between the blood cells and plasma. During centrifugation the gel barrier moves upwards providing a stable barrier separating the plasma from cells. Plasma may be aspirated directly from the collection tube, eliminating the need for manual transfer to another container. This barrier allows for the stability of certain parameters in the primary tube under the recommended storage conditions for 48 hours. This application is helpful for present clinical chemistry laboratory.

Using heparin tube as a general tube for clinical chemistry

Indeed, heparin is a good additive for blood collection and has less interference compared to the other additives.[5] In contrast, heparin has no osmotic effect; concentrations of most of the analytes measured in the heparinized plasma are statistically no different from those measured in the corresponding serum.[5-6] However, it is expensive. Therefore, in most general laboratories, the plain tube with no additive is in usage.

Presently, the concept of increasing the laboratory value through satisfaction management of the patients is under discussion. An important method to improve patient satisfaction is to decrease the waiting time for the laboratory results.[7] Also, clinical laboratories are under constant pressure to reduce costs and at the same time provide faster delivery of laboratory test results. Clinical service areas such as the operating room, emergency room, and intensive care unit are demanding availability of information within minutes of realizing the need for it. Obviously, in the practice of critical care medicine, faster turnaround time is crucial for rapid clinical decision-making. In clinical chemistry, the turnaround time is usually longer than the other types of tests since most of the tests must wait for the clotting of the collected specimen before performing the test. There is an attempt to produce a clot activator tube, but it does not improve the turnaround time much because waiting for a clot is still necessary (Table 4).

There are some ideas on using the heparinized tube for general clinical chemistry tests. The ideas are based on the fact that the heparinized plasma can give a simlar result compared to serum,[5-6] but it needs no clotting time, therefore, the turnaround time by using the heparinized tube may be shorter than with the routine plain tube. There are some previous studies to verify this idea (Table 5), ie.

a) Studies to prove the similarity of the results by using heparin and plain tube

Donnelly et al.[6] have compared the values of 27 biochemical analyses performed with a Kodak Ektachem 500 analyzer of the collected blood by using lithium heparin plasma and serum as additives. According to this study, when the concentration of lithium heparin was increased to three and five times normal (reflecting incomplete Vacutainer tube filling), alanine aminotransferase, amylase, aspartate aminotransferase, lipase, and potassium all exhibited some changes compared with serum. The main recommendation from this study is that the anticoagulant tubes should be completely filled to prevent spurious intraindividual variations in serial specimen analysis.

Table 4 Comparison between heparinized and serum separator tube

Items	Heparinized tube	Serum separator tube
1. Mechanisms	As anticoagulant; prevent the transformation of prothrombin to thrombin	Forms a barrier between the cell and serum layers, thus making it easier to pour-off and reducing reactivity between cell and serum components.
2. Effect on turnaround time	Decrease up to 30 minutes since sample preparation by centrifugation is not required	About 15 minutes is still required for separation mechanism before further analysis

Table 5 Some previous reports on using heparinized tube in routine clinical chemistry tests

Author	Details
Donnelly et al[6], 1995	The authors compared the values of 27 biochemical analyses by heparin plasma and serum performed with a Kodak Ektachem 500 analyzer. For 16 of the 27 tests, there were statistical differences between the mean results obtained from the analysis of heparinized plasma and those obtained from the analysis of serum.
Racine et al[8], 1996	The authors report the feasibility of using heparinized blood samples in clinical chemisty analysis using Beckman Synchron CX analyzers.
Doumas et al[9], 1989	The authors measured 25 analytes in heparinized plasma and serum from the same blood specimen, using the Kodak Ektachem 700 XR Analyzer. For 22 of the analytes, values for plasma were practically the same as those for serum; for the other three, differences between concentrations in plasma and serum were significant, both statistically and medically.

Table 6 Comparison between plain and heparinized tubes

Items	Heparinized tube	Plain tube
1. Cost	About 6 Baht	About 4 Baht
2. Waiting time for clot	0 minute	About 30 minutes
3. In-house preparation	Is not recommended	Can be performed, reuse of the tube is possible
4. Safety	User have chance in contact with heparin	No chance in contact with additive
5. Proper workload*	More than 500 test/days	Less than 500 tests/days

*also depends on the other factors such as the number of analytical machines, the number of the workers, the expected turnaround time by the patients, budget of the laboratory and etc.

Acceptable results from using a heparin instead of a plain tube were also reported by the study of Racine et al.[8] In another study, of Doumas et al,[9] an additional finding that values for the same analytes in plasma from regular heparinized tubes were essentially indistinguishable from those for plasma obtained by use of Plasma Separator Tubes (PST) was reported.[9] The most recent work of He et al.[10] also pointed out the similarity of the test result of thyroid tests from the specimens collected by heparin and plain tubes. In conclusion, based on these studies, it can be said that there is similarity between using heparin and plain tube for routine clinical chemistry tests.

b) Studies to compare the turnaround time between using heparin and plain tube

Donnelly et al.[6] have reported a shorter turnaround time for samples from heparin tubes compared to plain tubes. Although there is no other paper to support this point it can be said that the turnaround time should be shortened because using the heparin tube does not require waiting for clotting as does the plain tube.

Although the heparin tubes seem to be useful, some points should be considered. First, it is expensive, therefore it seems cost effective for a setting with a high workload only (Table 6). Secondly, some analytes cannot be done based on heparinized blood such as zinc, copper, ammonia lactate, electrophoresis, lithium, acid phosphatase and troponin.

Conclusion

Using the vacuum heparin tube can be a new application of evacuated blood collection system for clinical chemistry laboratory. It can decrease the turnaround time of the tests. However, due to its high cost, it seems suitable for the laboratories with high workloads only.

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