The Optimal Time for Plasma Separation from Lithium-Heparinized Whole Blood for Glucose Testing

เวลาที่เหมาะสมสำหรับปั่นแยกพลาสมาจากตัวอย่างเลือดชนิดลิเทียมเฮปาริน สำหรับใช้ในการตรวจวัดกลูโคส

Warayupa Thompat (วรยุพา ถมปัด)* Narong Nuanmuang (ณรงค์ นวลเมือง)*
Renu Wiriyaprasit (เรณู วิริยะประสิทธิ์)* Kanokwan Mohprasit (กนกวรรณ เหมาะประสิทธิ์)**
Dr.Anchalee Chittamma (ดร.อัญชลี จิตธรรมมา)*** Dr.Wanvisa Boonlert (ดร.วันวิสาข์ บุญเลิศ)****

ABSTRACT

Lithium-heparin is currently used for anticoagulation to reduce serum preparation time. We were investigated the optimal time point for preparing plasma from blood, which was stored at room or refrigerated temperature, and compared glucose concentrations of plasma samples obtained from lithium heparin or NaF-Oxalate treated blood. The results showed that initial plasma glucose levels (0 hour) of lithium heparin-treated blood and NaF-treated blood were not significantly different ($p>0.05$). Plasma glucose concentrations determined of lithium heparinized blood were significantly decreased after a storage time of 2 hours at room temperature and after 6 hours under the storing conditions in a refrigerator ($p<0.05$). In conclusion, plasma samples should be prepared from lithium-heparinized blood before 2 hours after storing at room temperature or before 6 hours after storing in a refrigerator.

บทคัดย่อ

ปัจจุบันลิเทียมเฮปารินถูกเลือกใช้เป็นสารกันเลือดแข็งเพื่อลดระยะเวลาการเตรียมซีรั่ม คณะผู้วิจัยได้ทำการศึกษาเพื่อหาเวลาที่เหมาะสมสำหรับการเตรียมพลาสมาจากเลือดที่เก็บ ณ อุณหภูมิห้องและอุณหภูมิตู้เย็น และเปรียบเทียบความเข้มข้นของกลูโคสที่เก็บโดยใช้สารกันเลือดแข็งชนิดลิเทียมเฮปารินกับโซเดียมฟลูออไรด์ผสมกับออกซาเลท ผลการศึกษาพบว่าระดับความเข้มข้นของกลูโคส ที่เก็บในสารกันเลือดแข็งชนิดลิเทียมเฮปารินและโซเดียมฟลูออไรด์ผสมกับออกซาเลทที่เวลา 0 ชั่วโมง ไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p > 0.05$) และระดับความเข้มข้นของกลูโคสที่เก็บในสารกันเลือดแข็งชนิดลิเทียมเฮปารินลดลงอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) ที่เวลา 2 ชั่วโมง ณ อุณหภูมิห้อง และที่เวลา 6 ชั่วโมง ณ อุณหภูมิตู้เย็น สรุปได้ว่าพลาสมาที่เตรียมจากลิเทียมเฮปารินที่เหมาะสมควรเก็บและทำการทดสอบภายใน 2 ชั่วโมง ณ อุณหภูมิห้อง และทำการทดสอบภายใน 6 ชั่วโมง เมื่อเก็บไว้ในตู้เย็น

Key Words: Antiglycolytic agent, anticoagulant, clinical chemistry testing
คำสำคัญ: สารต้านการสลายกลูโคส สารกันเลือดแข็ง การตรวจทางเคมีคลินิก

*Master degree students, Biomedical Sciences Master Program, Faculty of Allied Health Sciences, Naresuan University.
** Medical technologist, Clinical Chemistry Laboratory Medicine, Pathology Consortium, Budhachinaraj Hospital, Phitsanulok.
*** Lecturer, Clinical Chemistry Division, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University.
**** Assistant Professor, Faculty of Allied Health Sciences, Naresuan University.

Introduction

Lithium heparin (LH) is often used for blood collection in clinical chemistry testing because plasma can be immediately separated from blood cells (Greiner Bio-One 2002; Smith et al., 1987). However, the lithium heparin alone does not inhibit glycolysis of blood cells to maintain plasma glucose level (Le Roux et al., 2004). Several laboratories used lithium heparin plasma for all routine biochemical testing including plasma glucose especially in case of emergency or in pararel with sodium fluoride (NaF) plasma. Previous study showed that heparin can preserve glucose level and could be used in other tests (Landt, 2000). Moreover, heparin usage trends to increase spending in laboratory in Thailand and mobile public medical check-up services. However, time of plasma separation and storage conditions may effected to accuracy of glucose measurements and reach into a mis-interpretation in a diagnosis of the diabetes mellitus.

This study was to determine the optimal time points to prepare plasma at room and refrigerated temperatures from lithium-heparin-treated blood samples for plasma glucose determination.

Materials and Methods

Subjects

A total of 40 participants (20 diabetic patients and 20 healthy volunteers) were recruited from the Tapho Primary Care Unit and Clinical Chemistry Research Unit, Department of Medical Technology, Faculty of Allied Health Sciences, Naresuan University. The health demographic data was based on their medical history and a physical examination. All subjects gave written informed consent, and the study protocol was approved by the Ethics Committee of the Naresuan University. The recovery (%) and precisions of the Cobas c 111 were performed using two levels of control materials purchased from Roche Diagnostic. The recovery was tested by mixing 2 control materials at ratio 1:1 and the mixing was sampled for glucose measurements (n=20) by the Cobas Integra 600 (Roche Diagnostic, Germany) as the reference analyzer. The recovery was calculated in %. Ten milliliters of fasting blood samples were obtained from 20 healthy volunteers and 20 diabetic patients. One milliliter was aliquot into LH and NaF commercial tubes (Greiner Bio-One, Austria). The tubes were incubated at room temperature or kept in a refrigerator for 0, 1, 2, 4, 6 hours. The plasma samples were separated at the end of each setting times. Zero hour means the immediately separated of plasma samples after blood drawn by centrifuge 5 minutes. Each obtained plasma sample was measured for glucose concentrations by the Cobas c 111 Analyzer. Plasma glucose concentration was duplicately measured at each time point by the Cobas c 111 Analyzer (Roche Diagnostic, Switzerland) and measurement of plasma glucose was relied on the Hexokinase G6P-Dehydatase principle (Marks and Lloyd, 1963). Decreasing in glucose concentrations at each time point were compared to initial time (0 hour) and calculated in percentages. The room temperature was ranged from 26 to 28 $^{\circ}$C under the air condition control at the Clinical Chemistry Laboratory Research Unit, Department of Medical Technology, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok, Thailand.

The temperature of a refrigerator was ranged from 2 to 8 °C.

Statistical analysis

Statistic analysis was performed using the SPSS computer program version 11.0 (SPSS, Chicago, IL). Means of plasma glucose concentrations were calculated. The descriptive statistics such as means, standard deviations, and percentages were used to present obtained results. Means of glucose at various times of room temperature or kept in a refrigerator were compared using the ANOVA. All analyzes were undertaken using $\alpha < 0.05$ (two-tailed) as the significant statistic.

Results

The recovery (%) and precisions of the Cobas c111 were performed using 2 levels of control materials. The recovery was closed to 100% (Table 1). The imprecision results were <1.1% for within run and between day run (Table 2). The ranges, means, and standard deviations of initial plasma glucose levels (0 h) obtained from LH and NaF treated blood were in Table 3. The comparisons of glucose means between LH and NaF in healthy and diabetic mellitus subjects were not significantly different (p>0.05) at 0 hours. Plasma glucose concentrations obtained from LH blood was significantly decreased after 2 hours storing at room temperature and at least 6 hours under the storing conditions in a refrigerator (p<0.05).

TABLE 1. The recovery (%) of glucose measurements by the Cobas c111 by the mixing method.

Cobas Integra Level I (mg/dL)	Cobas Integra Level II (mg/dL)	Expected 1:1 (mg/dL)	Cobas c 111 1:1 (mg/dL)	Recovery %
92.0	249.0	170.5	173.0	101.5

TABLE 2. The precisions of glucose measurements by the Cobas c 111.

Level	Assay Range	Within-run n=20			Between-day n=30		
		Mean	SD	CV (%)	Mean	SD	CV (%)
I	79.1 – 107.3	95.5	0.5	0.5	91.7	0.8	1.0
II	222.0 – 300.0	249.5	1.4	0.5	248.6	2.7	1.1

TABLE 3. Comparison of glucose concentrations between LH and NaF plasma samples at 0 hour using healthy and diabetic patients (n=40).

Types of subjects	LH range mg/dl	LH mean (SD) mg/dl	NaF range mg/dl	NaF mean (SD) mg/dl	Bias mg/dl	*P*-value
Healthyn=20	77-93	85 (8.3)	78-94	86 (7.9)	1.0	0.423
Diabetic patients n=20	90-128	109 (19.3)	83-122	105 (19.8)	4.0	0.062

Note: LH= Lithium heparin, NaF= Sodium fluoride

TABLE 4. Comparison of plasma LH glucose levels at 0, 2, 4, and 6 hours at room and refrigerator temperature.

Hours	RT		Refrigerator	
	mean (SD)	Decreasing (%)	mean (SD)	Decreasing (%)
0	94 (5.5)	0.0	94 (5.5)	0.0
1	91 (5.4)	3.2	92 (6.5)	2.1
2	85 (8.4)	9.6	89 (7.1)	5.3
3	74 (6.4)*	21.2	88 (6.8)	6.4
4	64 (7.5)*	31.9	87 (7.6)	7.5
6	50 (8.2)*	46.8	86 (7.3)	8.5
p-value	<0.001*		0.572	

Note: *Significance

Discussion

Lithium heparinized plasma samples are useful for clinical chemistry testing because of the plasma samples can be simultaneously separated after blood collection and LH has less affects on the biochemical results when compare to other anticoagulation (Landt, 2000). This study found that means of initial plasma glucose levels (0 h) obtained from LH and NaF treated blood in healthy and diabetic mellitus subjects were not significantly different at 0 hours. Therefore LH could be used instead of NaF plasma when immediately separated plasma from blood cells. However, the use of lithium heparin alone does not inhibit glycolysis to maintain glucose level. The plasma separation from LH blood for glucose measurement should be finished within 2 hours at room temperature (26-28 °C). If longer than 2 hours, the antiglycolytic agents such NaF plus anticoagulant should be used for blood additives. The NaF can preserve glucose levels more than 8 hours (Christopher and O'Neill, 2000).

The decreasing of glucose concentrations at the refrigerator temperature was less than those at room temperature. The temperature at 2 to 8 °C can decrease plasma glucose uptake by glycolysis pathway of blood cells (Burtis and Ashwod, 2006). The current study present that plasma samples could be incubated with blood cells for 6 hours if kept the blood samples in the refrigerator and did not affect to significantly low of glucose concentrations. The decreasing percentages of glucose concentrations were higher after 2 hours and almost 50% decreased from initial concentration when incubated with blood cells for 6 hours at 26-28 °C. The glycolysis can be stimulated if temperature is higher than 28 °C as found in atmosphere of Thailand. Therefore, the collection of blood samples out site hospitals should be avoided for falsely low of glucose concentrations.

Conclusions

Plasma samples should be separated from LH blood before 2 hours after storing at room temperature or before 6 hours after storing in a refrigerator to avoid significantly decrease of glucose concentrations.

Acknowledgement

The Authors would like to thank the undergraduate and graduated students of the Department of Medical Technology, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok, Thailand

and Medical Technologists in the Clinical Chemistry Laboratory Medicine, Pathology Consortium, Budhachinaraj Hospital. We do thank you the Roche Diagnostic, Thailand for their collaboration and support of the Cobas c111 Analyzer.

References

Burtis, C.A. and Ashwod, E. R. 2006. 4th edition. **Tietz Textbook of Clinical Chemistry and Molecular Diagnostics.** St. Louis: Saunders.

Christopher, M.M. and O'Neill, S. 2000. Effect of specimen collection and storage on blood glucose and lactate concentrations in healthy. **Vet Clin Pathol** 29: 22-28.

Greiner Bio-One. 2002. 7th edition. **Product Manual of Vacuette.** Autria: Kremsmunster.

Landt, M. 2000. Glyceraldehyde preserves glucose concentrations in whole blood specimens. **Clin Chem** 46: 1144-1149.

Le Roux, C.W., Wilkinson, S.D., Pavitt, D.V., Muller, B.R. and Alaghband-Zadeh, J. 2004. A new antiglycolytic agent. **Ann Clin Biochem** 41: 43-46.

Marks, V. and Lloyd, K. 1963. Preservation of blood sample for glucose analysis by glucose-oxidase. **Clin Chem Acta** 8: 326.

Smith, J.C. Jr., Lewis, S., Holbrook, J., Seidel, K. and Rose, A. 1987. Effect of heparin and citrate on measured concentrations of various analytes in plasma. **Clin Chem** 33: 814-816.