Performance Evaluations of the Reduced Phenolphthalein Testing and the Application for Blood Contamination Detection on Glucose Meters

การประเมินผลการทดสอบของน้ำยารีดิวซ์ฟีลนอลฟทาลีน และใช้ในการตรวจหาการปนเปื้อนของเลือดบนเครื่องกลูโคสมิเตอร์

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ABSTRACT

The objectives of this study were to evaluate the performances of reduced phenolphthalein testing and to introduce reduced phenolphthalein testing for detection any blood contaminations on glucose meters. The testing was determined for sensitivity, precisions, specificity, and stability on sample degradations. Blood contamination on randomed glucose meters was investigated using reduced phenolphthalein testing. There were 55.56% (60/108) of blood contamination found on randomed meters and most of contaminations 30% were found on the side of the meters. There were 50.0% of hospital glucose meters and 56.75 % of primary care unit (PCU) glucose meters that were contaminated. Reduced phenolphthalein testing represented good performances and could be applied for blood contamination detection on glucose meters.

บทคัดย่อ

การวิจัยนี้มีวัตถุประสงค์ เพื่อประเมินผลการทดสอบของน้ำยารีดิวซ์ฟีลนอลฟทาลีนและ ใช้ในการตรวจหาการปนเปื้อนเลือดบนเครื่องกลูโคสมิเตอร์ ศึกษาความไว ความแม่นยำ ความจำเพาะ และทดสอบคุณสมบัติของน้ำยา รีดิวซ์ฟีลนอลฟทาลีนต่อการทิ้งคราบเลือดไว้ ในการศึกษานี้ทำการสุ่มตัวอย่างเครื่องกลูโคสมิเตอร์เพื่อตรวจหาการปนเปื้อนของเลือดด้วยน้ำยารีดิวซ์ฟีลนอลฟทาลีน ผลการวิจัย พบว่า มีการปนเปื้อนของเลือดบนเครื่องกลูโคสมิเตอร์ถึงร้อยละ 55.56 (60/108) ตำแหน่งที่พบการปนเปื้อนมากที่สุด คือ ด้านข้างคิดเป็น ร้อยละ 30 พบการปนเปื้อนของเลือดบนเครื่อง กลูโคสมิเตอร์ที่ใช้ในโรงพยาบาล ร้อยละ 50 และสถานีอนามัย (สถานบริการระดับปฐมภูมิ) ร้อยละ 56.75 ดังนั้นน้ำยารีดิวซ์ ฟีลนอลฟทาลีน มีประสิทธิภาพ เพียงพอเหมาะสมที่จะนำมาใช้ประโยชน์เพื่อการตรวจหาการปนเปื้อนของเลือดบนเครื่องกลูโคสมิเตอร์ ได้เป็นอย่างดี

Keywords: reduced phenolphthalein, blood testing, glucose meter
คำสำคัญ : รีดิวซ์ฟีลนอลฟทาลีน การทดสอบเลือด กลูโคสมิเตอร์

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Introduction

There are several methods that used to identify blood in Forensic Sciences (Forensic Analysis 2006). Reduced phenolphthalein testing or the Kastle-Meyer test is a forensic presumptive blood test in which the chemical indicator phenolphthalein is used. This method is widely used, because of the reagent can be prepared in laboratory and the reduced phenolphthalein reagent is stable for several days. The principle relies on the colorless reduced phenolphthalein is oxidized by the hydrogen peroxide breakdown from heam in red blood cell which changes it into a bright pink color phenolphthalein (Forensic Analysis 2006; Forensic Analysis 2009).There are several factors involving in false positive and false negative reading results by reduced phenolphthalein method. Therefore, the efficiency of reduced phenolphthalein testing should be investigated. (Lee JB, Levy M, et al 2005; Lowe AH, Bagg J, et al 2002).

Glucose meter is one of the most widely used forms of point-of-care testing (POCT) handheld devices at primary care unit settings in Thailand. Heavy utilization from skill and non skill medical persons is attributed to ease of operation, user convenience, and minimal blood loss at the site of patient care. The application of reduced phenolphthalein reagent on detection of blood contamination on medical or laboratory devices and analyzers may be useful for biohazard control and reduce a potential source of spreading infection agents (Louie, F. Richard, B.S., et al. 2005; Louie RF, Lau MJ, Tran NK. 2003)

The objectives of this study were to determine sensitivity, precisions, specificity, and stability of reduced phenolphthalein testing on fresh and degradation blood samples and to introduce reduced phenolphthalein testing to detect blood contamination on glucose meters used at community hospitals and Primary Care Units in Uttaradit and Pichit Provinces, Thailand.

Materials and Methods

Samples and Reagents

Blood samples were taken from an anonymous donor. All equipment used to extract and manipulate the blood for the experiments was sterile, substances tested include free hemoglobin such as tomato juice, melon juice, detergent (LIPON-F), sodium dichoro-S-triazinetrione, 70%alcohol, red color, and glucose meters. The modified reduced phenolphthalein was prepared by Kastle-Meyer method. (Shanan S. Tobe, Msc,Nigel Watson, et al. 2007).

The modified reduced phenolphthalein testing as POCT was used during field surveys. The method was investigated for sensitivity, within-day and between-day precision, and sample degradation. Whole blood and 0.9% sodium chloride were used as positive and negative control of reduced phenolphthalein test (Louie, F. Richard, B.S., et al. 2005).

The detection of blood by modified reduced phenolphthalein methods. The filter paper was used as the slide contaminate blood; the testing follow as, drop working reduced phenolphthalein solution 1-2 drop on filter paper, drop 3 % hydrogen peroxide 1-2 drop and observe the change of a color on the filter paper within 10 second. For positive result the filter paper changes from colorless to pink and negative result the filter paper doesn't change (Glaister J. 1996).

The sensitivity of reduced phenolphthalein method that determined by testing normal serial dilution of EDTA blood sample using 0.9 % normal saline(NaCl) as diluents and used to create a series of dilute blood sample(1:10 -1:10^6). To drop each dilution 10 µl into slide follows the testing method reduced phenolphthalein.

The triplicate testing were done on each dilution., Sensitivity of reduced phenolphthalein in whole blood high dilution order result at least two in three positive in a round of the experiment (Louie, F. Richard, B.S., et al. 2005).

The specificity of reduced phenolphthalein method is determined by testing of substances tested include free hemoglobin such as tomato juice, melon juice, detergent (LIPON-F),70%alcohol, sodium, dichoro-S-triazinetrione, red color and cabbage. Using whole blood and 0.9% NaCl as positive control and negative control respectively the next step follows the testing method of modified reduced phenolphthalein reagent. By testing are 160 samples. Specificity of reduced phenolphthalein is a chance will present are true negative result the test is correct (Shanan S. Tobe, Msc,Nigel Watson, et al. 2007).

Precision of the reduced phenolphthalein method was determined by testing of whole blood and 0.9 % NaCl. To drop 10 ul of 0.9% NaCl positive and negative control and concentration of blood with positive of sensitivity testing into 3 slides (triplicate testing). The precision testing was done in 20 measurements for within day and between days. Precision is also called reproducibility or repeatability, the degree to which further measurements show the same results (Louie, F. Richard, B.S., et al. 2005; Shanan S. Tobe, Msc,Nigel Watson,et al. 2007).

The investigation of sample degradation the concentration of blood with positive of sensitivity testing were used. The whole blood and 0.9% NaCl was used as positive control and negative control To drop whole Blood, Normal saline and sample on slide 10 µl each (20 set or 800 slide). For twenty minutes, keep in a box, at twenty four hours do the tested one set (1 set or 10 slides). Test repeated, until twenty fully days (20set) and recorded.

We assessed blood contamination on 108 randomly selected glucose meters by using the reduced phenolphthalein method at the site of patient care in 15 hospitals and 40 PCUs. Each glucose meter was inspected visually for blood on the outside surface, test strip insertion site, and meter storage area before sampling.

Meters with absence of visible gross blood contamination were sampled by using a piece of Whatman filter paper No. 1 (Whatman International Ltd., Maidstone, England). Remoistened a piece of filter paper with 1 or 2 drops of 95% ethanol and used this paper to sample each of 5 inspected areas (front, back, side, strip insertion site, and meter storage area).

Results

Type of sample	Sensitivity	
	Positive (%)	Negative (%)
Whole blood (Positive control)	20/20 (100)	0/20 (0)
0.9% NaCl (Negative control	0/20 (0)	20/20 (100)
Dilution blood 1: 10	20/20 (100)	0/20 (0)
Dilution blood 1: 10^2	20/20 (100)	0/20 (0)
Dilution blood 1: 10^3	20/20 (100)	0/20 (0)
Dilution blood 1: 10^4	19/20 (90)	1/20 (10)
Dilution blood 1: 10^5	0/20 (0)	20/20 (100)
Dilution blood 1: 10^6	0/20 (0)	20/20 (100)

Table 1 the sensitivity of reduced phenolphalein

Type of sample	Precision	
	Within day	Between day
Whole blood (Positive control)	100	100
0.9% NaCl (Negative control	0	0
Dilution blood 1: 10^4	95	95

n=20

Table 2. The precision of reduced phenolphthalein

The sensitivity of the modified reduced phenolphthalein test ranged between $1:10^3$ and $1:10^4$ hemoglobin concentrations in blood dilutions, or between 0.00128g/dl and 0.0128 g/dl hemoglobin with the reduced phenolphthalein test (Table 1). Within-run and between-day precision of reduced phenolphthalein testing were highly reproducible (Table 2).

Specificity was 96.25% with substances tested include free hemoglobin, it found false positive 3o % with same vegetable juice. (Table 3)

	Hb.	Results	
		Positive.	Negative
Whole Blood (positivecontrol)	17 g/dl	20/20 (100)	0/20 (0)
0.9% Nacl (negative control)	0 g/dl	0/20 (0)	20/20 (100)
Substances Free Hb.	0 g/dl	0/140 (0)	140/140 (100)
Vegetable juice	0g/dl	6/20 (30)	14/20 (70)
Specificity		96.25 %	

n=160

Table 3 the specificity of reduced phenolphthalein

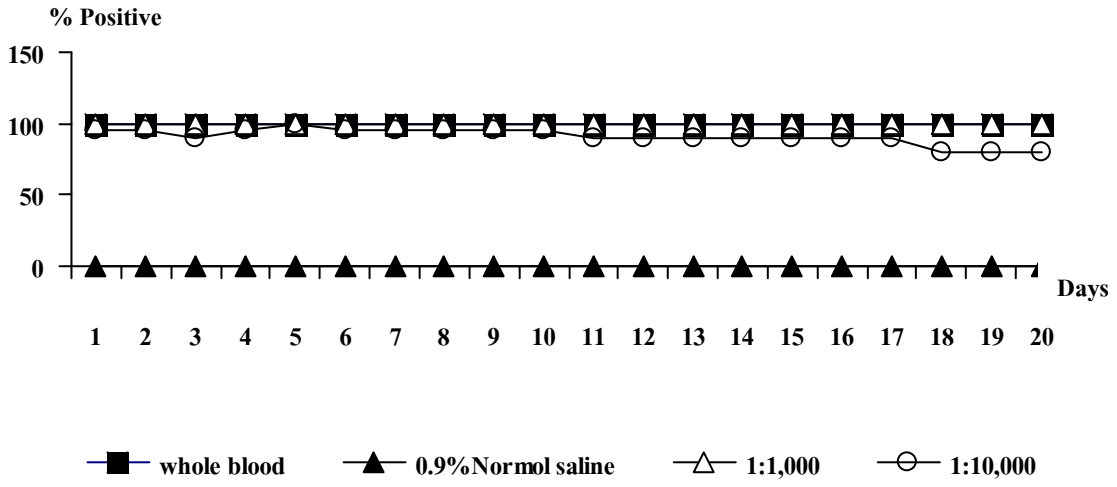

Figure 1. The effect sample degradation on the performance of reduced phenolphthalein assay

The performance of the reduced phenolphthalein assay on dry samples did not change when testing samples that had been stored for 20 days at room temperature (28 ^{O}C) (Figure 1)

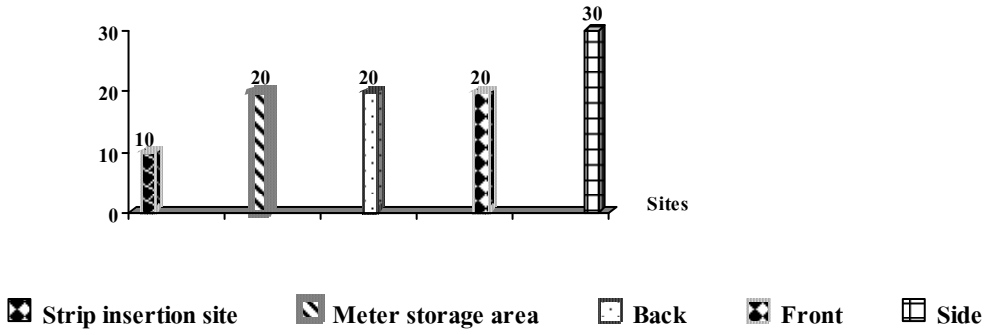

Figure 2.The site of blood contaminate on glucose meter

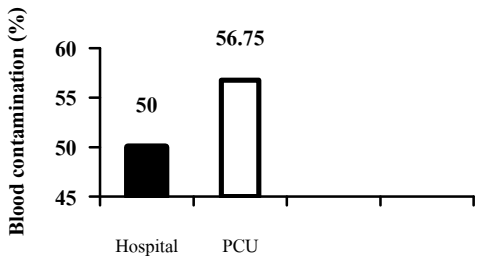

Figure 3.The comparison of blood contaminate on glucose meter during hospital with PCU

In total, 108 glucose meters were tested for blood contamination using reduced phenolphthalein as the point-of-care test. 60 of these glucose meters exhibited blood contamination 55.56%. Most of blood contamination was found on the side of the glucose meters 30.0% (Figure2) There were 50.0% of glucose meters from hospital and 56.75% of glucose meters from PCU that were contaminated (Figure 3).

Discussion

The sensitivity of the pre-prepared reduced phenolphthalein reagent for both dry and wet samples use of between $1:10^3$ to $1:10^4$ dilutions. This result is comparable with previous studies (Forensic Analysis 2009; Prry SM, Monaghan WP. 2001). The reproducibility of the reduced phenolphthalein method was high with whole blood positive controls testing positive and negative controls testing negative in all cases. This test has some limitations. Namely, the enzymes in some vegetable can make a false positive test. Other non-blood substances which give positive result are some fruit extracts or any other peroxidase-like substances. (Glaister J. 1996; Shanan S. Tobe, Msc,Nigel Watson, et al. 2007). But vegetables juice has will no a few chances to contamination on the medical devices.

The survey of blood contamination on glucose meters showed significant frequency of contaminated on glucose meters. High contamination percentage in our study was found on the side surface of the meters. The results are comparable with the previous study that found overall, meters were contaminated on outside surfaces.

The presence of hemoglobin does not indicate infectivity. However, the presence of blood indicates a potential risk for exposure to infectious agents. (Louise, M.E., Andrew, R. 2009). Many factors are involved in blood contamination, such as user knowledge, glucose meter cleaning, and the cleaning method used. However, 24.1 % of users did not clean glucose meters after use. Wearing protective attire, such as gloves, is also important for user protection from infectious agents. The survey found that only 29.53% of operators put on gloves prior to handling blood samples for bedside glucose measurement.

Conclusions

We conclude that point-of-care reduced phenolphthalein testing has high sensitivity and precision for the detection of blood contamination on glucose meters. Point-of-care reduced phenolphthalein testing can be used conveniently during surveys of blood contamination on glucose meters in hospitals and primary care sites. The method can be used to detect
both fresh and degraded blood. The results, which showed significant levels of contamination, are clinically important because blood potentially could spread infectious agents that may lead to nosocomial infections.

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