Accuracy and Precision Evaluation of Thai Plastic Microhematocrit Tubes: The First Product from Thailand

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Background: *In Thailand, the spun microhematocrit method is usually performed using glass microhematocrit tubes even though broken glass tube during use may result in a risk of injury and blood-borne infection. The main reason is that the safer product alternatives such as plastic microhematocrit tubes are more expensive. Now, plastic tubes for hematocrit determination can be produced in Thailand at a much cheaper price. However, precision and accuracy studies are necessary before being able to use them.*
Objective: *To compare the accuracy and precision of Thai plastic microhematocrit tubes against the routinely used glass microhematocrit tubes and imported plastic microhematocrit tubes using spun microhematocrit method.*
Materials and Method: *One hundred residual EDTA blood samples from the Department of Clinical Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand along with the three level hematology control materials were measured with spun microhematocrit values using three different types of plastic microhematocrit tubes. This was compared to the routinely used glass microhematocrit tubes as a gold standard.*
Results: *The repeated measures one-way ANOVA found no significant difference between the hematocrit values from each type of tubes with an $F(1,99) = 0.667$ and p-value = 0.574. Intraclass correlation coefficient(ICC) between four types of microhematocrit tubes ranged from 0.996-0.998 (p-value < 0.001). Correlation coefficients (r) between four types of microhematocrit tubes ranged from 0.996-0.998 (p-value < 0.05). Coefficient of variation (CV) for precision of both within run and between run of Thai plastic microhematocrit tubes ranged from 1.44 to 2.17% compared to 1.39 to 4.01% of the imported plastic microhematocrit tubes.*
Conclusion: *The hematocrit values determined by all plastic microhematocrit tubes can be considered relatively equivalent to those of glass microhematocrit tubes in terms of accuracy and precision. The Thai plastic microhematocrit tubes are economical and with the cost-benefit over other plastic tubes of about 12 to 16 times. Therefore, the Thai plastic microhematocrit tubes should be the choice for glass tube replacement.*

Keywords: *Plastic microhematocrit tube, Capillary tube, Spun hematocrit, Spun microhematocrit, Hematocrit*

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The objective of the present study was to evaluate the plastic microhematocrit tubes produced from Zigma Biotech, Co., Ltd., Thailand in order to decide to implement them in Siriraj Hospital. The personnel that performed the present study were from the Department of Clinical Pathology, Faculty of Medicine Siriraj Hospital. The plastic microhematocrit tubes used in the present study were provided by the manufacturer for the present study. Besides the supplies previously mentioned, the authors have not received any financial support from the manufacturer. None has any other conflict of interest involving the manufacturer.

Hematocrit, also known as packed cell volume (PCV), is a ratio of packed red blood cell volume to total blood volume. It is one of the important hematology parameters for diagnosing, assessing, and monitoring many conditions such as anemia, bleeding etc. Hematocrit can be derived from several techniques or principles. The four most commonly used methods are 1) spun microhematocrit method, 2) Coulter impedance principle on automatic cell count analyzers, 3) conductivity method on blood gas analyzers, and 4) calculation method of hematocrit

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from hemoglobin[1]. However, the most frequently used method for bedside management is the spun microhematocrit method because it is simple to perform, inexpensive, and require small sample volume. This method is also recommended by the Clinical and Laboratory Standards Institute (CLSI) to be a reference method[2]. Moreover, the Clinical Laboratory Improvement Amendments of 1988 (CLIA' 88) characterizes the spun microhematocrit as a waived test, which is defined as a simple laboratory procedure allowing non-laboratory personnel such as nurses or medical students to perform this procedure as to render the likelihood of erroneous results negligible; or pose no reasonable risk of harm to the patient if the test is performed incorrectly[3]. As the results obtained from those operators are acceptable for accuracy and precision.

Glass microhematocrit tubes have been used for performing the spun microhematocrit since the first time this test was done in Thailand[4]. However, they are fragile and occasionally cracked when forced into the sealing clay. It leads to unnecessary occupational risks such as exposure to blood and infectious micro-organism. Because they contain more blood than a needle and can produce a more complicated laceration, they are a potential to introduce larger inoculums of microbial particles into the wound. Not only have the operators taken more risks when inserting the tubes but also the health workers when cleaning the clay. All injuries from broken glass microhematocrit tubes are preventable by substituting it to a safer product alternative such as mylar wrapped and plastic[5-9].

In 1999, the joint safety advisories from three organizations: 1) the National Institute for Occupational Safety and Health (NIOSH) of the Centers for Disease Control and Prevention (CDC), 2) the Occupational Safety and Health Administration (OSHA), and 3) the Food and Drug Administration (FDA) recommended the use of plastic microhematocrit tubes or mylar wrapped microhematocrit tubes to minimize the risk of injury and infection from glass of broken microhematocrit tubes[3]. To follow the recommendation in Thailand, the plastic microhematocrit tubes have to be imported and they are 10 to 16 times more expensive than glass tubes.

Fortunately, in 2010, plastic microhematocrit tubes started to be produced in Thailand by Zigma Biotech, Co., Ltd., Thailand. Their price is equal to the price of glass microhematocrit tubes. Since a laboratory test is considered an important part in the diagnosis and monitoring of medical treatment, any new procedure or equipment must be validated before being introduced for patient testing to insure that the values reported will meet clinical expectations with a desired degree of reliability. Although the spun hematocrit is characterized as a waived test that has no recommendations for method validation[10], it is given equal attention as any other tests to give clinicians confidence. Therefore, evaluation of the Thai plastic microhematocrit tubes according to the international standard of good clinical and laboratory practice is essential before they are widely used.

The objective in the present study was to evaluate the accuracy and precision of the plastic microhematocrit tubes as compared to those of glass microhematocrit tubes as a gold standard.

Material and Method

The protocol and design were approved by the Siriraj Ethic Committee before the present study began. The protocol number was 127/2553 (EC4). To eliminate an inter-operator variation, all hematocrit values were measured by only one lab personnel throughout the present study.

Specimen

One hundred residual venous blood samples from in-patients and out-patients sent to the Central Laboratory of the Department of Clinical Pathology, Faculty of Medicine Siriraj Hospital were used. They were obtained after all ordered testings were completed. All specimens were collected into Vacutainer blood collection tubes anticoagulated with K_3EDTA (Becton Dickinson, Franklin Lakes, NJ, USA). No personal identifying information of any sample was recorded. The specimens were excluded from the study if there were hemolysed, lipemia, clotted, or if the volume was less than 1 ml.

Microhematocrit tube

Microhematocrit tubes from four manufacturers with a variety of materials were assessed in the present study. The characteristics of each tube were shown in the Table 1.

Accuracy

Accuracy of hematocrit values obtained from each type of plastic microhematocrit tube was evaluated by comparing those values with hematocrit resulted from glass microhematocrit tube. Duplicate microhematocrit tubes of each type were filled with each EDTA blood sample until two-thirds full.

Table 1. Microhematocrit tubes used in this study (all tubes were plain and no anticoagulation)

Tube code	Source	Item name	Specification		(mm)	Price (baht)
A	Zigma Biotech Co., Ltd., Thailand	Plastic micro-hematocrit tubes	Polystyrene	ID Length	0.90 ± 0.05 75	500
B	Globe Scientific Inc., USA	Plastic micro-hematocrit tubes	Polystyrene	ID Length	0.90 ± 0.05 75	8,000
C	Iris Sample Processing, USA	SafeCrit micro-hematocrit tubes (plastic)	Polycarbonate	ID Length	0.89 75	6,500
D	Vitrex Medical A/S, Denmark	BRIS micro-hematocrit tubes	Glass (Sodalime glass)	ID Length	1.15 ± 0.05 75	500

One end of each tube was sealed with Critoseal (Vitrex Medical A/S, Denmark). Tubes were placed in the microhematocrit centrifuge (Boeco M-24; Hettich, Germany) and centrifuged for five minutes at 12,000 rpms. The hematocrit values was read as the percentage of whole venous blood volume occupied by packed red blood cells (RBC) volume using a Damon/IEC Division Microcapillary Reader. In reading the hematocrit value, care was placed on excluding the buffy coat (platelets and leukocytes) and the measurement was performed within 10 minutes to avoid merging of the layers[1]. The readings of the two tubes were averaged.

Precision

Precision of three kinds of the plastic micro-hematocrit tubes were determined using three levels of quality control materials that have hematocrit values 17.5%, 36.5%, and 46.5%. To estimate the within-run imprecision of each plastic microhematocrit tube, each control material was measured for spun microhematocrit 20 times. For the between-run imprecision, each control material was measured for spun microhematocrit for 20 consecutive days. The coefficient of variation (CV) was used to analyze the imprecision of the plastic microhematocrit tubes.

Data analysis

All statistical analyses were performed in SPSS-PC+ version 13.0. For the accuracy study, scatterplots of hematocrit values obtained from each plastic microhematocrit tube were produced against the values from the glass microhematocrit tubes. Correlation coefficients(r) and intraclass correlation coefficients (ICC)[11-14] with 95% confidence interval were also calculated to determine their agreement. The ICC was obtained from mean squares (MS) of the two-way random effects model shown in the following equation.

$$ICC = \frac{MSr - MSe}{MSr + (N - 1)\,MSe}$$

MSr is the between target mean square, MSe is the within target mean square, and N is the number of type of microhematocrit tubes. A repeated one-way analysis of variance (ANOVA) was used to determine whether the hematocrit values from four types of microhematocrit tubes are statistically significantly difference.

All analyses in accuracy evaluation were evaluated for statistical significance with an alpha at 0.05.

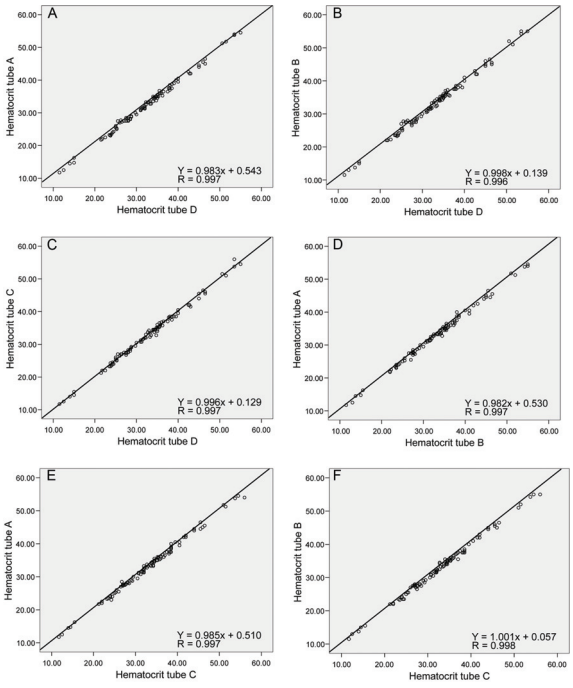
Results

From the 100 residual venous blood samples included in the present study, none was excluded. The specimens were collected from both newborns (n = 23) and adults (n = 77). The hematocrit values ranged from 11.0% to 55%.

Accuracy

Comparisons of hematocrit values from each type of plastic microhematocrit tubes to the glass microhematocrit tubes by scatterplots and correlation coefficients(r) are shown in Fig. 1. All scatterplots show only minimal differing of results among each plastic and glass tubes. Furthermore, the hematocrits of the four types of microhematocrit tubes were in close agreement. All Pearson correlation coefficients comparing four types of microhematocrit tubes were statistically significant agreement at p-value < 0.05.

Comparisons of tubes by intraclass correlation coefficients (ICC) are in Table 2. All comparisons have ICC close to 1. Agreements of hematocrit values



Tube A = Plastic Microhematocrit tube from Zigma Biotech Co., Ltd., Thailand
Tube B = Plastic microhematocrit tube from Globe scientific Inc., USA
Tube C = Plastic microhematocrit tube from Iris Sample Processing,USA
Tube D = Glass microhematocrit tube from Vitrex Medical A/S, Denmark

Fig. 1 Correlation of spun hematocrit using 4 different microhematocrit tubes of different material and dimension

obtained from four types of microhematocrit tubes by ICC were statistically significant (p-value < 0.01).

The repeated measures one-way ANOVA was found no statistically significant difference among the microhematocrit tubes with an $F(1,99) = 0.667$, p-value = 0.574.

Precision

Within-run precisions of Thai polystyrene tube (tube A) when measurement of level I (17.5%), level II (36.5%), and level III (46.5%) control materials were 2.17%,1.51%, and 1.44% respectively. For those of imported polystyrene tube (tube B) were 2.87%, 1.50%, and 1.59% while those of imported polycarbonate tube (tube C) were 1.83%,1.72%,and 1.48% respectively.

The between-run precisions of tube A when measurement levels I, II, and III of control materials were 2.17%,1.78%, and 1.39%. For the between-run precisions of tube B were 3.55%, 1.60%, and 1.58% and for those of tube C were 4.01%, 2.34%, and 1.57% respectively Within-run and between-run precisions for the plastic microhematocrit tubes A, B, and C when tested with 3 levels of hematology control materials are summarized in Table 3.

Discussion

This is the first pre-market study for evaluation of accuracy and precision of plastic microhematocrit tube first produced in Thailand. An accuracy of a test is performed to estimate inaccuracy or systematic error while the precision is the reproducibility or the agreement of the measurements of replicate runs of the same sample. It is the process of determining the range of random error[10]. For accuracy evaluation, the hematocrit values of 100 patients samples measured in all plastic microhematocrit tubes were close to the reference method and to each other. Agreement of all plastic tubes with the glass tubes was confirmed by the two statistical evaluations: the ICC and the Pearson correlation coefficients. Both showed excellent correlations and statistically significant agreement with the p-value < 0.05. Additionally, based on repeated measures one-way ANOVA, the p-value obtained from spun microhematocrit test was consistent with no significant difference between the plastic microhematocrit tubes and the glass tubes when testing the EDTA blood with hematocrit between 11% and 55%. Since the hematocrit values from four types of microhematocrit tubes can be considered relatively equivalent with the ultimate agreement, the results from all plastic microhematocrit tubes are accurate and precise enough for hematocrit measurement in clinical specimen. This indicated similarity of the different products[11-14].

From the%CV value for precision study among three kinds of plastic microhematocrit tubes, the Thai plastic microhematocrit tubes ranked in the second place of the within run precision but it was the first rank of between run precision. In addition, almost all of its %CV are within 2% which are in allowable variation range except %CV of level 1 control material, which were slightly higher than 2[15]. The authors may conclude that the Thai plastic microhematocrit tube possesses the best of all precisions in hematocrit values for all control materials.

Table 2. Intraclass correlation coefficients of spun hematocrit using 4 different microhematocrit tubes for accuracy evaluation

Microhematocrit tubes	Intraclass correlation coefficients (ICC)	95% confidence interval (95% CI)	p-value
Tube A-Tube D	0.997	0.996-0.998	<0.001
Tube B-Tube D	0.996	0.994-0.997	<0.001
Tube C-Tube D	0.997	0.996-0.998	<0.001
Tube A-Tube B	0.997	0.996-0.998	<0.001
Tube A-Tube C	0.997	0.996-0.998	<0.001
Tube B-Tube C	0.998	0.997-0.998	<0.001

Tube A = Plastic microhematocrit tube from Zigma Biotech Co., Ltd., Thailand
Tube B = Plastic microhematocrit tube from Globe scientific Inc., USA
Tube C = Plastic microhematocrit tube from Iris Sample Processing, USA
Tube D = Glass microhematocrit tube from Vitrex Medical A/S, Denmark

Table 3. Within-run and between-run precision summary of the plastic tube A, B, C for spun microhematocrit

Hematology control material	Within run precision (%CV)			Between run precision (%CV)		
	Tube A	Tube B	Tube C	Tube A	Tube B	Tube C
level 1 (17.5%)	2.17	2.87	1.83	2.17	3.55	4.01
level 2 (36.5%)	1.51	1.50	1.72	1.78	1.60	2.34
level 3 (46.5%)	1.44	1.59	1.48	1.39	1.58	1.57

Tube A = Plastic microhematocrit tube from Zigma Biotech Co., Ltd., Thailand
Tube B = Plastic microhematocrit tube from Globe scientific Inc., USA
Tube C = Plastic microhematocrit tube from Iris Sample Processing, USA

There are two drawbacks of the plastic microhematocrit tubes. First, it is more difficult to fill blood into the plastic microhematocrit tubes than into the glass tubes especially when filling with the high hematocrit level blood. The reason may be that the plastic microhematocrit tubes have less capillary effect than the glass microhematocrit tubes[16,17]. This problem should be gradually decreased and overcome eventually if the operators keep on practicing and get used to the plastic tubes.

Another disadvantage of the plastic microhematocrit tubes is that the frequencies of blood lost during centrifugation are much more than the glass tube. The authors found that there were 7.5% of Thai plastic microhematocrit tube (tube A) lost blood during centrifugation compared to 10.6% of imported polystyrene tube (tube B), 5.3% of imported polycarbonate tube (tube C), and 3.5% of glass microhematocrit tube (tube D) (Table 4). Interestingly, 91.1% of all blood lost from microhematocrit tubes during centrifugation were from the low level hematocrit value (hematocrit value < 35%). The reason for the second problem may be from the Critoseal. As the Critoseal is supposed to be specific for the glass microhematocrit tubes only, an incomplete sealing function can therefore happen when used with other materials. If the blood-lost from glass microhematocrit tubes is considered as 1 time, the blood lost from the

Table 4. The problems of using the microhematocrit tubes

Tube code	Degree of difficulty for filling blood	Blood lostduring centrifugation
A	Moderate	7.5%
B	Moderate	10.6%
C	Severe	5.3%
D	Mild	3.5%

Tube A = Plastic microhematocrit tube from Zigma Biotech Co., Ltd., Thailand
Tube B = Plastic microhematocrit tube from Globe scientific Inc., USA
Tube C = Plastic microhematocrit tube from Iris Sample Processing, USA
Tube D = Glass microhematocrit tube from Vitrex Medical A/S, Denmark

plastic microhematocrit tubes A, B and C will be 2 times, 3 times, and 1.5 times respectively. It would be interesting to study further whether or not the percentage of blood lost would be reduced if the proper sealant for the plastic microhematocrit tubes were used.

Limitation of the present study was that the study design included only EDTA venous blood specimens. Capillary blood was not investigated. Additional research of Thai plastic microhematocrit tubes should be done at the point of care by non-laboratory personnel to demonstrate whether the similar results are obtained. Furthermore, the specific sealant for plastic microhematocrit tubes should be applied along with this additional work, therefore the reason of blood lost during centrifugation of the plastic microhematocrit tubes can more clearly be summarized.

In conclusion, accuracy and precision of Thai plastic microhematocrit tubes are comparable to the glass microhematocrit tubes and virtually equivalent to the imported microhematocrit plastic tubes. Although Thai plastic microhematocrit tubes possess more minor difficulties when using them than the glass tubes, they offer a safer working standard with a lower cost than imported tubes. Therefore, Thai plastic microhematocrit tubes are considered the most cost-effectives. As they are not inferior to the gold standard, they are appropriate to be put in practice. However, user training before glass tube replacement is recommended.

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Potential conflicts of interest

None.

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การประเมินประสิทธิภาพการทำงานของหลอดไมโครฮีมาโตคริตพลาสติกที่ผลิตในประเทศไทย

ปรีชญา วงษ์กระจ่าง, นิศารัตน์ โอภาสเกียรติกุล, วิมล ชินสว่างวัฒนกุล, สมศักดิ์ อารีวัฒนา

ภูมิหลัง: *ในประเทศไทยการวัดระดับฮีมาโตคริตโดยวิธีการปั่นใช้เป็นหลอดไมโครฮีมาโตคริตแก้วมาตลอด แม้ว่าหลอดไมโครฮีมาโตคริตแก้วจะแตกได้ง่ายและก่อให้เกิดความเสี่ยงในการได้รับบาดเจ็บและติดเชื้อได้ เนื่องจากหลอดไมโครฮีมาโตคริตพลาสติกซึ่งปลอดภัยกว่ามีราคาแพง อย่างไรก็ตามในขณะนี้ประเทศไทยสามารถผลิตหลอดไมโครฮีมาโตคริตพลาสติกได้เองซึ่งมีราคาถูกกว่าหลอดไมโครฮีมาโตคริตพลาสติกที่นำเข้าจากต่างประเทศ จึงมีความจำเป็นที่จะต้องประเมินความถูกต้องและความแม่นยำก่อนนำมาใช้*

วัตถุประสงค์: *เพื่อประเมินประสิทธิภาพของหลอดไมโครฮีมาโตคริตพลาสติกในการวัดระดับฮีมาโตคริตโดยวิธีการปั่น ในเรื่องความถูกต้องและแม่นยำของหลอดไมโครฮีมาโตคริตพลาสติกที่ผลิตในประเทศไทยเทียบกับหลอดไมโครฮีมาโตคริตแก้ว และหลอดไมโครฮีมาโตคริตพลาสติกที่นำเข้าจากต่างประเทศ*

วัสดุและวิธีการ: *นำเลือดที่มีสารกันเลือดแข็ง EDTA ที่เหลือจากการเจาะเลือดจากหลอดเลือดดำของผู้ป่วยที่ส่งมา ตรวจ ณ ห้องปฏิบัติการกลาง ภาควิชาพยาธิวิทยาคลินิก คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล จำนวน 100 ตัวอย่าง และสารควบคุมคุณภาพทางโลหิตวิทยา 3 ระดับ มาวัดระดับฮีมาโตคริตโดยวิธีการปั่นด้วยหลอดไมโครฮีมาโตคริตพลาสติก 3 ชนิด และหลอดไมโครฮีมาโตคริตแก้วซึ่งถือว่าเป็น gold standard ในการวัดระดับฮีมาโตคริต*

ผลการศึกษา: *ในเรื่องความถูกต้อง ผลจากการวิเคราะห์ความแปรปรวนพบว่าระดับฮีมาโตคริตที่ได้จากหลอดไมโครฮีมาโตคริตทั้ง 4 ชนิด ที่ได้ไม่แตกต่างอย่างมีนัยสำคัญ, $p = 0.574$, สัมประสิทธิ์สหสัมพันธ์ภายในกลุ่มของหลอดไมโครฮีมาโตคริตทั้ง 4 ชนิด อยู่ในช่วง 0.996-0.998 ($p < 0.001$), สัมประสิทธิ์สหสัมพันธ์ของหลอดไมโครฮีมาโตคริตทั้ง 4 ชนิด อยู่ในช่วง 0.996-0.998 ($p < 0.05$) ส่วนเรื่องความแม่นยำพบว่าสัมประสิทธิ์ของความแปรปรวนทั้ง within run และ between run ของหลอดไมโครฮีมาโตคริตพลาสติกที่ผลิตในประเทศไทยอยู่ในช่วง 1.44-2.17% ในขณะที่หลอดพลาสติกที่นำเข้าจากต่างประเทศมีค่าอยู่ในช่วง 1.39-4.01%*

สรุป: *ระดับฮีมาโตคริตที่ได้จากหลอดไมโครฮีมาโตคริตพลาสติกทั้ง 3 ชนิด มีความถูกต้องและความแม่นยำเทียบเท่าหลอดแก้ว เนื่องจากหลอดไมโครฮีมาโตคริตพลาสติกมีราคาถูกกว่าหลอดไมโครฮีมาโตคริตพลาสติกจากต่างประเทศ 12-16 เท่า ดังนั้นจึงเป็นหลอดไมโครฮีมาโตคริตพลาสติกที่น่าจะนำมาใช้แทนหลอดไมโครฮีมาโตคริตแก้วมากที่สุด*