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Original Research Article

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Performance of a Hematocrit Determination in Health Promotion Hospitals When Using Different Assigned Values

Poonnapatch Sakaew,[1] Kunchit Kongros,[2, 3] Phanthira Wongsri,[4] Napaporn Apiratmateekul,[2, 3] and Wanvisa Treebuphachatsakul[2, 3*]

[1] *Medical Technology Laboratory, Khuntan Hospital, Chiang Rai, Thailand.*

[2] *Department of Medical Technology, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok, Thailand.*

[3] *Reference Material and Innovation Research Unit, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok, Thailand.*

[4] *Medical Technology Laboratory, Saingam Hospital, Kamphaeng Phet, Thailand.*

Abstract: Hematocrit (Hct) measurement using a centrifuge method is a common laboratory testing procedure at primary care units (PCU). This study evaluated the performance of Hct measurement by a centrifuge method in health promotion hospitals (HPH) using two assigned values from expert laboratories and participant consensus. Two processed blood materials were tested for homogeneity and stability and then used to evaluate the performance of Hct measurement by a centrifuge method. All the materials were distributed to 132 participants, including 24 hospitals and 108 HPHs through the Proficiency Testing (PT) Scheme, following ISO/IEC 17043:2010 guidelines. Assigned robust means and standard deviations (SDs) were calculated from three expert laboratories and consensus from all participants using algorithm A according to ISO 13528: 2015. A paired t-test was used to compare robust means between the expert laboratories and participant consensus. The performance of the Hct measurement using a centrifuge method was evaluated using z scores. The Hct in processed blood materials were homogenous and stable for use as PT materials for three weeks. The means of Hct assigned from the expert laboratories were significantly different (p<0.05) from those assigned from participant consensus. Over 80% of HPHs had a satisfactory performance with z scores = 2.00 when using both assigned means from the expert laboratories and participant consensus, with the exception of low levels of Hct when using the assigned mean from the expert laboratories. The Hct measurement performance at a low level of Hct, , was significantly lower compared to using participant consensus (Chi-square =134.89, p<0.001). This study shows significant differences in the performance of Hct measurement by a centrifuge method when using assigned means from expert laboratories and from all participants. Most of the HPHs had a satisfactory performance for Hct measurement by a centrifuge method when using participant consensus. However, half of HPHs require performance improvement where there are questionable and unsatisfactory evaluations when assigned means from expert laboratories are used.

Keywords: Performance evaluations, microhematocrit, packed red cell volume, ISO/IEC 17043, whole blood material, external quality assurance

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***Corresponding Author**

Wanvisa Treebuphachatsakul , Department of Medical Technology, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok, Thailand.

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1. INTRODUCTION

The centrifuge method is recommended by the World Health Organization (WHO) to determine packed cell volume or hematocrit (Hct)[1]. Hct is a useful laboratory test to diagnose, monitor, and screen for anaemia[2]. In Thailand there are approximately 9,800 primary care units (PCU)—also referred to as "health promotion hospitals (HPHs)"—and they all determine hematocrit (Hct) using a centrifuge method performed by either a nurse or public health staff as a form of point-of-care testing (POCT). In the Thai Ministry of Public Health's (MOPH) 2011 'Strategic Plan for Good Health' was announced to determine Hct in elderly and chronic disease patients to screen and monitor anaemia[3]. The quality of Hct results depends on the measurement skills of HPH staff and the accuracy of the Hct centrifuge. The Thai MOPH provided the national guidelines for laboratory testing as POCT in 2015[4] and the proficiency testing (PT) scheme for Hct determination using a centrifuge method by the Regional Science Centres to assess and improve the quality of Hct results. However, few HPHs participated in the Hct PT scheme due to limited amounts and quality of blood material such as homogeneity and stability and the regulation quality of laboratory testing at PCUs. The present study assesses the quality of Hct determination by a centrifuge method in HPHs in Chiang Rai Province, Thailand using processed blood materials that were investigated for the homogeneity and stability of the Hct in blood materials.

2. MATERIALS AND METHODS

The study was approved by the Human Research Ethics Committee of Naresuan University (IRB No.359/2016). Processed blood materials[5] with two levels of hematocrit, a lot.#61801, and a lot.#61802, was produced by We Med Lab Center Co., Ltd. using a production process which was approved by ISO 13485:2016 & EN ISO 13485: 2016 (certification number MD 703611) and which also followed standard guidelines for reference material producers, ISO 17034[6]. The homogeneity and stability of the Hct in the blood materials were investigated by the technical request of ISO 17034 and by following statistical methods in ISO Guide 35[7]. The stability of Hct in processed materials was within 6 months and 30 days after opening.

2.1 Homogeneity and stability of Hct in PT samples

Before using the processed blood materials with the PT samples for Hct measurement, the homogeneity and stability of Hct in the two blood materials were investigated by following ISO 13528[8] to perform statistical analysis with the PT. Ten vials of each material were randomly selected and measured in duplications for Hct by a centrifuge method at an expert laboratory for both within-sample and between-sample variations. Cochran's rank statistical analysis was used for a within-sample variation and deemed acceptable when the Cochran values did not exceed 95% of the critical values of Cochran at 10 samples. Between-sample standard deviations (S_s) were calculated according to ISO 13528. Between-sample variations were acceptable when S_s was within 0.3 fold of sigma pt (σpt). Five vials of each material were randomly selected and measured in triplicate for Hct by a centrifuge method at the baseline date before use in the Hct PT Scheme and 3 weeks after the baseline date. Mean differences of Hct at 3 weeks from the baseline were accepted when they did not exceed 0.3-fold of σpt.

2.2 Performance evaluations of Hct determination through the PT Scheme

A total of 132 participants, including 24 hospitals and 108 HPHs in Chiang Rai Province, Thailand were enrolled in the PT Scheme for Hct determination by a centrifuge method. Two blood materials were used as PT samples and shipped to all participants. The assigned values including robust mean and SD of hematocrit were calculated from the consensus of all the participants and the three expert laboratories using robust algorithm A and by following the ANNEX B worksheets of ISO/IEC 13528: 2015[8]. Three expert laboratories were selected based on the following criteria: Certification of ISO 15189[10]; CV ≤ 2.0%; and accepted performance for six previous rounds of Hct determination by a centrifuge method. Performance evaluation was performed by following ISO/IEC 17043 standard guidelines for PT[9]. Instructions were provided to the participants in Hct PT Scheme and the transportation of PT materials was processed by NU MLC Proficiency Testing Center[11], which collaborated in this study, and its PT process is certified by ISO/IEC 17043. The performances of Hct was investigated through the PT Scheme and using z scores calculated using the following formula:

$$z = \frac{(x_i - X_{pt})}{\sigma_{pt}}$$

The x_i is the meaning of an individual laboratory. The X_{pt} is an assigned value consensus from all the participants or calculated from the expert laboratories. The σ_{pt} was calculated from the relative standard deviation (RSD) of 15 previous rounds of the PT Scheme of Hct by a centrifuge method which was 4.95%. The formula to calculate σ_{pt} is from the mean of each material multiplied by RSD%, in which RSD% is 4.95. The performance of the Hct determinations was divided into three categories: Satisfactory ($0 \le z \le 2$); questionable ($2<z\le3$); and unsatisfactory ($z>3$).

3. STATISTICAL ANALYSIS

The data obtained were analyzed by following ISO 13528. Student's (paired) "t" test was used for analysis comparison between assigned mean from expert laboratories and those from all participants. Probability value (P) of less than 0.05 was considered statistically significant.

4. RESULTS

4.1 HCT homogeneity and stability

The homogeneity and stability of the three levels of Hct in the processed blood materials are shown in Tables 1 and 2. Within-sample variations of Hct in the two processed blood materials were accepted with Cochran values which were less than 95% critical value of Cochran statistic at 10 samples. Between-sample variations (Ss) were within $0.3\sigma_{pt}$ for two PT materials and were accepted according to ISO 13528 for homogeneity. The mean differences of Hct in the third week were less than $0.3\sigma_{pt}$ as shown in Table 2, therefore the Hct

in the two materials was accepted according to ISO 13528 for stability in the third week.

4.2 *Assigned values of Hct determinations*

The assigned values, including the robust mean and SD of the Hct determinations by a centrifuge method obtained from the expert laboratories and consensus from all the participants, are shown in Table 3. The assigned values obtained from the expert laboratories were significantly different (p<0.05) compared to those from the participant consensus, by 1.56% for blood material #61801 and by 1.08% for blood material #61802.

4.3 *Performance of the Hct determination by a centrifuge method*

Performance evaluations using z scores are shown in Table 4, Fig.1, and Fig.2. 80% of HPHs had a satisfactory performance with a z score of 2.00 when using the assigned means from the participant's consensus. However, only 54% accepted performance when using the assigned mean from the expert laboratories instead of using the assigned mean from the participant consensus (Table 5). Performance of Hct measurement at a low level of Hct, when classified by z scores using the assigned means from expert laboratories,ories was significantly lower than those using the participant consensus (Chi-square =134.89, p<0.001).

Table 1. Homogeneity of hematocrit (n=20) in blood materials according to ISO 13528

Detail	61801	61802
Average	23.70	43.30
Standard deviation of sample averages	0.22	0.46
Relative standard deviation (RSD), %CV	1.93	1.06
Cochran value	0.50	0.50
Between-sample variation (*Ss*)	0.40	0.40
95% critical value of Cochran statistic	0.60	0.60
σ_{pt}	1.17	2.14
$0.3\sigma_{pt}$	0.40	0.70

Table 2. Stability of Haematocrit in processed blood materials

Parameters	61801		61802	
	0 wk	3 wk	0 wk	3 wk
Overall average	23.70	23.60	43.80	44.00
Standard deviation	0.44	0.22	0.40	0.00
Mean difference	-	0.10	-	0.22
σ_{pt}	-	1.22	-	2.20
$0.3\ \sigma_{pt}$		0.40		0.70

Table 3. Assigned values from three expert laboratories and 132 participants

Assigned value	61801		61802	
	Expert laboratories	Participant consensus	Expert laboratories	Participant consensus
Robust mean	23.70	25.16*	43.40	42.32*
Robust SD	0.55	1.71*	0.55	2.98*
Uncertainty (u_x	0.32	0.19	0.32	0.32

**** Significant differences using paired t-test with p <0.05.***

Table 4. Performance evaluations of haematocrit determination using z scores and two assigned values from the reference laboratories and all-participant consensus.

Performance	60801		60802	
	Expert laboratories	Participant consensus	Expert laboratories	Participant consensus
Satisfactory (z≤2.00)	71 (54)	107 (81)	110 (83)	112 (85)
Questionable (z>2.00≤3.00)	51 (39)	22 (16)	20 (15)	16 (12)
Unsatisfactory (z>3.00)	9 (7)	4 (3)	3 (2)	4 (3)
Total	132 (100%)	132 (100%)	132 (100%)	132 (100%)

Table 5. Acceptable performance of hematocrit determination in two blood materials

Performance	Expert laboratories			Participant consensus		
	60801	60802	Acceptable	60801	60802	Acceptable
z≤2.00	71 (54)	110 (83)	71 (54)	107 (81)	112 (85)	107 (81)
z>2.00	60 (46)	23 (17)	-	26 (19)	20 (15)	-
Total	132 (100%)	132 (100%)	-	132 (100%)	132 (100%)	-

Table 6 . The details of Hct determination and quality control at three expert laboratories, 24 hospital laboratories, and 108 HPHs.

Items	Expert laboratories (n=3)	Participant (N= 132)	
		Non expert-laboratories (n=24)	HPHs (n=108)
Hct calibration (once/year)	100%	88%	53%
Appropriated Hct reader	100%	100%	40%
Performed internal quality control (IQC) for Hct	100% (CV ≤2.0%, 100%)	100% (CV ≤2.0%, 91.7%)	0%
Participation in Hct PT scheme/ Inter-lab comparisons	100%	100%	52%
Certification of laboratory testing	ISO 15189	• ISO 15189, 8.3% • LA, Medical Technology Council, 62.5% • MOPH, 70.8%	0%

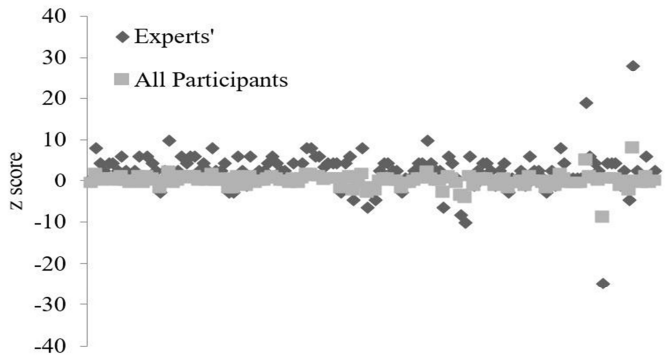

Fig 1. The performance assessment of hematocrit by centrifuge method plotted by z score in the Laboratory Networking of Chiang Rai (Blood material#61801).

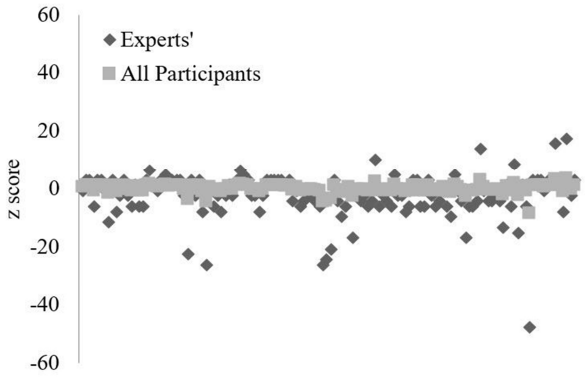

Fig 2 The performance assessment of hematocrit by centrifuge method plotted by z score in the Laboratory Networking of Chiang Rai (Blood material#61802).

5. DISCUSSION

The Thai Ministry of Public Health provided the PT scheme for Hct determination by a centrifuge method through the Regional Science Centres to assess and improve the quality of Hct results. Yet only a few HPHs participated in the Hct PT scheme due to limited blood materials and the high number of HPHs in Thailand. The MOPH provided National Guidelines for laboratory testing in the form of POCT at PCUs[4,12] pitch to improve medical services and laboratory

test quality, including Hct. This study used processed blood materials manufactured by We Med Lab Center Co., Ltd. and the production processes were certified by ISO 13485. Homogeneity and stability were investigated by the manufacturer and also before used as the PT samples for Hct determination by a centrifuge method. The processed blood materials were homogeneous and stable until the closing date of the PT Hct Scheme which ensured the quality of the processed blood material samples during transportation and the PT process. The homogeneity of Hct in the processed materials #61801 shows a between-sample variation (s_s) not greater than $0.3\sigma_{pt}$. In theory, the homogeneity of Hct was considered to be adequately homogeneous since the s_s were less than $0.3\sigma_{pt}$[8]. The stability of Hct in the two materials was accepted at least 3 weeks from the baseline and also at the closing date of the PT Scheme with biases from the baseline Hct in the third week were less than $0.3\sigma_{pt}$. Hct in both processed blood materials were stable and appropriated for use as PT materials according to ISO/IEC 17043 (9). A previous study[13] of CDC used dried blood specimens for infectious markers in a PT Scheme, but materials for Hct by a centrifuge method must consist of cells and plasma portions, and variations in a matrix of material are broader that dried and lyophilized forms. The SD of the Hct measurements from all participants was greater than the variations (data not shown) obtained from the 15 rounds of Hct PT. This study used σ_{pt}. for Hct determination derived from RSD (%) at 4.95% of the previous 15 rounds of the PT Scheme of Hct. RSD technically removed the outliers and the data was provided by NU MLC Proficiency Testing Center which is certified by ISO/IEC 17043 and PT Scheme ID# 499362 on EPTIS website[11]. It is thought that assigned values can be generated from the general consensus of participants or from a group of expert laboratories. However, the differences between these assigned values can be an influential factor for the quality of the Hct results, such as their measurement skills, the accuracy of Hct centrifuge, and the accuracy of the Hct reading scale. This study therefore selected three expert laboratories certified by ISO 15189[10] and with certified calibrations for Hct centrifuge and where professional medical technologists performed the Hct measurements. A disadvantage of using assigned means from the participant consensus value is that it can lead to an inadequate attitude to improve performance and achieve high-quality Hct using the centrifugation method if most participants have systemic errors in their Hct measurement. Poor analytical techniques, uncalibrated equipment, and other errors can lead to unacceptable performance evaluations. The details of Hct determination and quality control at three expert laboratories, 24 hospital laboratories, and 108 HPHs are shown in Table 6. HPHs have used inputs such as Hct centrifuge calibration and staff training instead of performing internal quality control[12], according to a low source of blood material for Hct determination by a centrifuge method. Only a few HPHs had appropriated Hct centrifuge and Hct reader. CLIA 2019[14] recommends the use of bias ≤4% for criteria to accept the performance of Hct determinations in proficiency testing, and this criteria can be used for further investigation by using assigned means from expert laboratories as target values and use cutoff at the bias that does not exceed 4% of the target values to be considered as acceptable performance.

6. CONCLUSION

The performance of Hct determination by a centrifuge method derived from assigned means that obtained from an expert group and all participants was a significant difference. Most of the HPHs were satisfied with the performance of the Hct measurement by a centrifuge method when using participant consensus. However, approximately half of the HPHs must improve their performance as they had questionable and unsatisfactory evaluations when using assigned means from the expert laboratories. Proficiency testing based on assigned values that were used to assess the quality laboratory results for Hct. Therefore, PT providers of the Hct measurement should consider using an assigned mean and criteria for acceptable performance.

7. ACKNOWLEDGMENT

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8. AUTHORS CONTRIBUTION STATEMENT

Dr. Wanvisa Treebuphachatsakul and Ms. Poonnapatch Sakeaw conceptualized and gathered the data with regard to this work. Ms. Poonnapatch Sakaew and Dr. Kunchit Kongros analyzed these data. Dr. Napaporn Apiratmateekul and Ms. Phatthira Wongsri analyzed necessary inputs given towards the designing of the manuscript. All authors discussed the methodology and results and contributed to the final manuscript.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

10. REFERENCES

1. The recommended method for the determination of packed cell volume by centrifugation. In:. World Health Organization; 2000. Diagnostic imaging and laboratory technology [internet] [cited Jun 9 2020]. Available from: https://apps.who.int/iris/handle/10665/66240.
2. Lee GR. Anemia: A diagnostic strategy. In: Lee GR, Foerster J, Lukens J, editors. Wintrobe's clinical hematology. 10th ed. Baltimore: Williams & Wilkins; 2004. p. 908-40.
3. Strategic plan for good health, Thai way of life 2011-2020 . Thailand: Ministry of Public Health; 2011 [cited Jun 12 2020]. Available from: https://www.ryt9.com/s/cabt/1104256.
4. National Guidelines of Point-of-Care Testing for Thailand. Boon ya wongwiroj J Ministry of Public Health, editor Thailand Press; 2015.
5. Whole blood QC Material Instruction for Use [internet]. We Med Lab center; 2016 [cited Jun 9 2020]. Available from: https://www.wemedlab.com/th/pblood/.

6. ISO 17034:. General requirements for the competence and consistent operation of reference material producers. International Organization for Standardization; 2016.
7. Guide ISO. Reference materials - Guidance for characterization and assessment of homogeneity and stability. Vol. 35. International Organization for Standardization; 2017.
8. ISO 13528:2015. Statistical methods for use in proficiency testing by interlaboratory comparison. International Organization for Standardization; 2015.
9. ISO/IEC 17043:2010. Conformity assessment – general requirements for proficiency testing. International Organization for Standardization; 2010.
10. ISO 15189:2013. Medical laboratories – requirements for quality and competence. International Organization for Standardization; 2013.
11. Proficiency testing scheme; 2018. EPTIS Database [cited Jun 9 2020]. Available from: https://www.eptis.bam.de/eptis/WebSearch/main?lastSearchTerm=&searchTerm=&facetCountry=th&facetFieldClassifications=medical+analysis&facetButton=true.
12. Manual guidelines for development of starred Hospitals for Health Promotion. Ministry of Public Health; 2019 [cited Jun 8 2020]. Available from: http://thlp.ops.moph.go.th/pdf/รพ.สต/คู่มือ%20รพ.สต.ติดดาว%202563%2016.01.63_FN.pdf.
13. Nguyen S, Ramos A, Chang J, Li B, Shanmugam V, Boeras D, Nkengasong JN, Yang C, Ellenberger D. Monitoring The quality of HIV-1 viral load testing through a proficiency testing program using dried tube specimens in resource-limited settings. J Clin Microbiol. 2015 Apr;53(4):1129-36. doi: 10.1128/JCM.02780-14, PMID 25609733.
14. New CLIA proposed rules for acceptance limits for proficiency testing [internet]; 2019. Westgard QC [cited Jun 9 2020]. Available from: https://www.westgard.com/2019-clia-changes.htm.