

Proficiency testing results of CD4+ T cell determination in Thailand

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ABSTRACT

Background: CD4+ T cell number is an important marker for assessing immune status, differentiating the severity of infections, and monitoring treatment in patients infected with human immunodeficiency virus (HIV). The determination of the percentage and absolute count of CD4+ T cells plays an essential role in treatment decisions and in ongoing patient care. Consequently, this analysis must be highly effective, with proper quality control processes. Participation in proficiency testing is a key component of the quality system.

Objectives: This study aimed to develop a proficiency testing program for CD4+ T cell analysis in Thailand.

Materials and methods: Two proficiency testing materials were prepared and assigned the numbers CD4-01, which was low level, and CD4-02, which was normal level. PT materials were tested for the four following parameters: %CD3+ cells, CD3+ cell count, %CD4+ T cells, and CD4+ T cell count in 39 participating laboratories. Performance assessment of the laboratories was shown as a z score according to the ISO13528:2022 standard.

Results: Proficiency testing materials met the acceptable criteria for homogeneity and stability according to ISO13528:2022. As due to uncertainty of assigned values are smaller than $0.3\sigma_{pt}$, the performance of participating laboratories was assessed using a z score. More than 89 percent of participated laboratories demonstrated acceptable performance across all four parameters, while less than six percent were classified as within the warning signal range, and less than eight percent were deemed unacceptable. The causes of unacceptable performance included inappropriate instrument settings, inadequate compensation, and issues with gating techniques.

Conclusion: Proficiency testing materials for CD4+ T cell determination were satisfactorily prepared and met the criteria for homogeneity and stability according to ISO13528:2022. The results of the pilot study conducted in 39 laboratories showed that more than 87 percent of the results were within acceptable range.

Introduction

CD4+ T cell enumeration is important for assessing immune status, differentiating the severity of infections, and monitoring treatment in patients infected with human immunodeficiency virus (HIV). This test plays an essential role in treatment decisions and ongoing patient care.¹ Thus, this analysis must be highly effective, with proper quality control processes. Moreover, it is essential to conduct proficiency testing to compare the results of CD4+ T cell enumeration across different instruments to ensure consistency. Participation in proficiency testing is a key component of the quality system according to the international

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standard for medical laboratory quality management, ISO 15189:2022, clause 7.3.7.3, which states that laboratories should engage in external quality assessment programs that comply with the ISO/IEC 17043 standard.²

CD4+ T cells are reported as both percentage and absolute count. The percentage of CD4+ T cells is analyzed using flow cytometry. The absolute count of CD4+ T cells can be estimated using either the dual-platform or single-platform technique. In the dual-platform technique, the absolute count is derived by multiplying the lymphocyte count (obtained from a blood cell counter) with the percentage of CD4+ T cells which is determined by flow cytometry. In contrast, the single-platform technique uses only a flow cytometer to calculate the absolute CD4+ T cell count. This method involves adding a known concentration of polystyrene beads to the stained blood sample for analysis.

The Associated Medical Sciences, Chiang Mai University External Quality Assessment (AMS CMU EQA) unit is dedicated to developing proficiency testing (PT) materials for laboratory tests that meet user needs, enabling participating laboratories to receive evaluation results that reflect the accuracy and precision of their laboratory test results. This ensures that results are reported effectively and uniformly, serving as an indicator for quality development and the ongoing maintenance of laboratory standards.

This study aimed to develop PT materials for CD4+ T cell enumeration to facilitate proficiency testing services and subsequently apply for ISO/IEC 17043 accreditation. The processes included the development of quality control materials, testing appropriate storage conditions, assessing the homogeneity and stability of the PT materials, and conducting practical trials in at least 30 medical laboratories across Thailand. This research was expected to lead to the development of PT materials for CD4+ T cell enumeration, enabling proficiency testing services in the future and providing knowledge for developing PT materials for other tests.

Materials and methods

Proficiency testing material preparation

Proficiency testing materials were prepared from rejected donor blood bags that did not meet volume standards from the Blood Bank Section, Maharaj Nakorn Chiang Mai Hospital. The study was reviewed by the ethics committee, Faculty of Associated Medical Sciences, Chiang Mai University (AMSEC-67EM-005). Fixed and preserved whole blood samples were prepared as two proficiency testing materials, which were then stored in a refrigerator.³⁻⁸ The proficiency testing materials were assigned the numbers CD4-01, for low level, and CD4-02, for normal level of CD4+ T cells. In this proficiency testing program, PT materials were tested for the four following parameters: %CD3+ cells, CD3+ cell count, %CD4+ T cells, and CD4+ T cell count.

Assessment for homogeneity and stability of PT materials

For each lot of proficiency testing materials, ten tubes were randomly chosen and tested for %CD3+ cells, CD3+ cell count, %CD4+ T cells, and CD4+ T cell count in duplicate to assess the between-sample standard deviation

(Ss).^{9,10} The acceptance criteria for homogeneity, as outlined in ISO 13528:2022, involve comparing Ss to the σ_{pt} values used for laboratory assessment. The proficiency testing material was considered adequately homogeneous when $Ss \leq 0.3\sigma_{pt}$. Additionally, for each lot of proficiency testing materials, two tubes were randomly selected and tested in duplicate to evaluate stability during transport, and another two tubes were tested for stability at the end of the program. To evaluate stability during transportation, two tubes of each PT material were sent to the participating laboratory in Yala province, which was requested to return the materials to the PT provider. For stability at the end of the program, two tubes of each PT material were stored in a refrigerator until reporting deadline. These stability results (y_2) were compared to those of the homogeneity test (y_1). The σ_{pt} values used for laboratory assessment served as the acceptance criteria for the stability test. Proficiency materials were considered adequately stable when $|\bar{y}_1 - \bar{y}_2| \leq 0.3\sigma_{pt}$, and sufficiently stable when $|\bar{y}_1 - \bar{y}_2| \leq 0.3\sigma_{pt} + 2\sqrt{u^2_{(y_1)} + u^2_{(y_2)}}$

Operation of the pilot study

The proficiency testing materials were packed in a box with ice packs and sent to 43 medical laboratories in Thailand that participated voluntarily. The laboratories were required to return their results to the provider within 15 days. The %CD3+ cells and %CD4+ T cells parameters were designated to be reported with one decimal place, while the CD3+ cell count and CD4+ T cell count were required to be reported as absolute numbers. The provider then assessed laboratory performance using z scores, which compared the analytical results to the consensus values from all participating laboratories. Assigned values (X_{pt}) and standard deviations (σ_{pt}) from the consensus values of results submitted by the laboratories were calculated using Algorithm A.¹⁰ Subsequently, the uncertainties of the assigned values ($u_{(x_{pt})}$) were proved to be less than $0.3\sigma_{pt}$ thus validating the use of z scores. The z score was calculated using the equation: (participant value - X_{pt}) / σ_{pt} according to ISO 13528:2022 standard. For interpretation, the absolute value of the z score was considered as follows: |z score| ≤ 2.0 indicated that the results were within acceptable limits, $2.0 < |z \text{ score}| < 3.0$ indicated that the results were in a warning signal range, and $|z \text{ score}| \geq 3.0$ indicated that the results were unacceptable.

Results

Assessment of homogeneity and stability of PT materials

The homogeneity test results of both PT materials in all parameters met the acceptance criteria for adequately homogeneous according to ISO 13528:2022, confirming their suitability for proficiency testing. The results of the homogeneity test for PT materials number CD4-01 and CD4-02 are shown in Table 1 and 2, respectively.

The stability test results indicated that PT materials number CD4-01 and CD4-02 remained stable throughout the transportation and at the end of the program. The results of the stability test for the PT materials were shown in Table 3-4.

Table 1. Results of the homogeneity test for PT material number CD4-01.

Test	Homogeneity means	Between-samples deviation (S_s)	$0.3\sigma_{pt}$	$S_s \leq 0.3\sigma_{pt}$	Result
%CD3+ cell	64.28%	0.29	0.93	Pass	Adequately homogeneous
%CD4+ T cell	1.48%	0.10	0.13	Pass	Adequately homogeneous
CD3+ cell (cells/ μ L)	382.4	0.0	19.5	Pass	Adequately homogeneous
CD4+ T cell (cells/ μ L)	8.8	0.6	0.9	Pass	Adequately homogeneous

Table 2. Results of the homogeneity test for PT material number CD4-02.

Test	Homogeneity means	Between-samples deviation (S_s)	$0.3\sigma_{pt}$	$S_s \leq 0.3\sigma_{pt}$	Result
%CD3+ cell	70.31%	0.47	0.68	Pass	Adequately homogeneous
%CD4+ T cell	40.03%	0.42	0.45	Pass	Adequately homogeneous
CD3+ cell (cells/ μ L)	1,116.8	14.2	39.2	Pass	Adequately homogeneous
CD4+ T cell (cells/ μ L)	635.7	0.0	22.4	Pass	Adequately homogeneous

Table 3. Results of the Stability Test for PT material number CD4-01.

Test	y_1	y_2	$ \bar{y}_1 - \bar{y}_2 $	$0.3\sigma_{pt}$	Criteria*	Expanded criteria ⁺	Result
%CD3+ cell	64.28%	64.53% ^T	0.25	0.93	Pass	Not done	Adequately stable
		65.95% ^E	1.68		Not pass	Pass	Sufficiently stable
%CD4+ T cell	1.48%	1.55% ^T	0.07	0.13	Pass	Not done	Adequately stable
		1.30% ^E	0.18		Not pass	Pass	Sufficiently stable
CD3+ cell (cells/ μ L)	382.4	380.5 ^T	1.9	19.5	Pass	Not done	Adequately stable
		403.3 ^E	20.9		Not pass	Pass	Sufficiently stable
CD4+ T cell (cells/ μ L)	8.8	9.0 ^T	0.3	0.9	Pass	Not done	Adequately stable
		7.8 ^E	1.0		Not pass	Pass	Sufficiently stable

Note: y_1 : mean of homogeneity test, y_2 : mean of stability test, ^Tstability of transportation, ^Estability at the end of round, * $|\bar{y}_1 - \bar{y}_2| \leq 0.3\sigma_{pt}$, ⁺ $|\bar{y}_1 - \bar{y}_2| \leq 0.3\sigma_{pt} + 2\sqrt{u^2_{(y_1)} + u^2_{(y_2)}}$

Table 4. Results of the Stability Test for PT material number CD4-02.

Test	y_1	y_2	$ \bar{y}_1 - \bar{y}_2 $	$0.3\sigma_{pt}$	Criteria*	Expanded criteria ⁺	Result
%CD3+ cell	70.31%	69.05 % ^T	1.25	0.68	Not pass	Pass	Sufficiently stable
		69.63 % ^E	0.68		Pass	Not done	Adequately stable
%CD4+ T cell	40.03%	38.50 % ^T	1.53	0.45	Not pass	Pass	Sufficiently stable
		38.28 % ^E	1.76		Not pass	Pass	Sufficiently stable
CD3+ cell (cells/ μ L)	1,116.8	1,103.5 ^T	13.3	39.2	Pass	Not done	Adequately stable
		1,108.3 ^E	8.5		Pass	Not done	Adequately stable
CD4+ T cell (cells/ μ L)	635.7	615.8 ^T	20.0	22.4	Pass	Not done	Adequately stable
		609.5 ^E	26.2		Not pass	Pass	Sufficiently stable

Note: y_1 : mean of homogeneity test, y_2 : mean of stability test, ^Tstability of transportation, ^Estability at the end of round, * $|\bar{y}_1 - \bar{y}_2| \leq 0.3\sigma_{pt}$, ⁺ $|\bar{y}_1 - \bar{y}_2| \leq 0.3\sigma_{pt} + 2\sqrt{u^2_{(y_1)} + u^2_{(y_2)}}$

Participating laboratories

Proficiency testing materials were sent to 43 medical laboratories, and results from 39 of those laboratories were returned to the proficiency testing provider. The techniques and analytical instruments used in the 39 laboratories are shown in Table 5.

Statistical data analysis of the participating laboratories

The data reported by a total of 39 laboratories included 39 data points for %CD3+ cells and %CD4+ T cells, but only 35 and 36 data points for CD3+ cell count and CD4+ T cell count for PT materials number CD4-01 and CD4-02 respectively. Four laboratories using the dual-platform technique did not report CD3+ cell count and CD4+ T cell count. Additionally, one laboratory reported

CD3+ cell count and CD4+ T cell count only for PT material number CD4-01 because the differential WBC count for PT material number CD4-02 could not be analyzed by blood cell counter.

The distribution of all data was first analyzed using a kernel density plot as recommended by ISO13528:2022 and all plots showed only one high peak that revealed no large difference was enough to warrant a separate group of method assessment.^{9,10} Subsequently, the assessment of laboratory performance was presented using z scores, which compared the analytical results with the consensus values from all laboratories. The number of laboratories in each performance score range for the pilot study is shown in Table 6.

Table 5. The techniques and analytical instruments used in participating laboratories.

No	Instrument	Number of laboratories (%)		
		Dual-platform technique	Single-platform technique	Total
1	Beckman Coulter FC500	10	9	19 (48.7)
2	Beckman Coulter DxFLX	1	7	8 (20.5)
3	BD FACSCalibur	2	4	6 (15.4)
4	Beckman Coulter Navios	1	2	3 (7.7)
5	BD FACSLytic	2	0	2 (5.1)
6	BD FACSVia	0	1	1 (2.6)
Total		16 (41)	23 (59)	39 (100)

Table 6. Number of participating laboratories in each performance level.

Test	PT material number	N	Laboratory performance Number of laboratories (%)		
			Acceptable	Warning signal	Unacceptable
%CD3+ cell	CD4-01	39	37 (94.9)	0 (0.0)	2 (5.1)
	CD4-02	39	35 (89.7)	1 (2.6)	3 (7.7)
%CD4+ T cell	CD4-01	39	35 (89.8)	2 (5.1)	2 (5.1)
	CD4-02	39	34 (87.2)	2 (5.1)	3 (7.7)
CD3+ cell count	CD4-01	35	32 (91.4)	2 (5.7)	1 (2.9)
	CD4-02	36	34 (94.4)	2 (5.6)	0 (0.0)
CD4+ T cell count	CD4-01	35	32 (91.4)	1 (2.9)	2 (5.7)
	CD4-02	36	34 (94.4)	2 (5.6)	0 (0.0)

Discussion

As stated in ISO 15189:2022, section 7.3.7.3, to monitor the performance of the examination methods, laboratories should participate in External Quality Assessment (EQA) programs that are appropriate to the examinations performed. This section also emphasizes the importance of selecting EQA programs that are both suitable and competent. Where possible, EQA programs that meet the requirements of ISO/IEC 17043 should be selected. In alignment with this requirement, this study was developed in compliance with ISO/IEC 17043:2023. Furthermore, the statistical analysis of this study was conducted in accordance with ISO 13528:2022, as

recommended by ISO/IEC 17043:2023 for ensuring the validity and reliability of performance evaluation.

Both PT materials were adequately homogeneous ($S_s \leq 0.3\sigma_{pt}$) in all four parameters. The difference in the average values of %CD3+ cells and %CD4+ T cells between the homogeneity mean (when prepared) compared to the stability mean of transportation and stability mean at the end of the program did not exceed 2%, and the maximum difference for absolute CD3+ cells and CD4+ T cells count was only 26.2 cells/ μ L. Some parameters were not classified as adequately stable (as shown in Table 3 and Table 4). However, when the uncertainty of measurement was considered to apply expanded criteria,

the results were still considered acceptable and classified as “sufficiently stable” for the assessment. These data shown that both PT materials for all parameters were suitable for this proficiency program.

Although around 5-10 percent of the participating laboratories assessed z score evaluation results outside the acceptable range (absolute z score ≤ 2.0).

Potential causes of analytical errors that laboratories with z score evaluation results outside the acceptable range should investigate were as follows.¹¹⁻¹⁴

First, the voltage settings of the detector and compensation settings were inappropriate; this might result in the lymphocyte populations not being clearly distinguished. Additionally, the placement of the quadrant boundaries may not be suitable.

Secondly, the gating of lymphocyte populations was inadequate. When analyzing CD3 and CD4 in lymphocyte populations, the largest group consisted of T cells (CD3+) at approximately 61-85%. These cells had a higher CD45 expression as compared to B and NK cells (CD3-, CD4-), with NK cells exhibiting higher SSC properties than T cells. If the gating was not comprehensive, it might lead to inaccuracies in analyzing %CD3+ cells and %CD4+ T cells. Additionally, care should be taken to ensure that the proportion of monocytes (CD3-, CD4+) does not exceed 5%.

In some cases, the number of lymphocytes analyzed in the sub-population was less than 2,500 cells. If using an auto-stop setting, it should be set at more than 2,500 cells (typically set to 3,000 lymphocytes). It was found that some laboratories using the single-platform technique which set the auto stop at 3,000 for the bead population. However, in blood samples with a low cell count, the bead population may reach 3,000 events before the lymphocyte count reaches 2,500 cells.

Moreover, the selection of beads for absolute cell count calculation was inappropriate. Beads located at the top may represent clusters of more than one bead, while those at the bottom may be broken beads or non-bead particles.

The last cause was the PT materials may not be suitable for analyzing differential cell counts using certain blood cell analyzers, as there may be discrepancies in results across different analytical principles. Additionally, some analyzers may not be capable of performing differential cell counts. When analyzing the absolute count data from all laboratories using a Kernel density plot, no distinct peak separation was observed, leading to the analysis of z scores from the overall data. This may result in some laboratories using the Dual-platform technique having z scores for Absolute CD3+/CD4+ T cell counts that fall outside the acceptable range. However, if a laboratory's z score for %CD3+/%CD4+ T cell counts is within the acceptable range, it indicates that the laboratory's performance in the analysis is considered acceptable.

Conclusion

PT materials for CD4+ T cell determination were satisfactorily been prepared and passed the criteria for

homogeneity and stability according to ISO13528:2022. The results of the pilot study in 39 laboratories were within acceptable range more than 87 percent. This study may offer valuable insights for evaluating the performance of CD4+ T cell determination in clinical laboratories and could serve as a framework for developing future proficiency testing programs according to ISO17043.

Ethical approval

The study was reviewed by the ethics committee, Faculty of Associated Medical Sciences, Chiang Mai University (AMSEC-67EM-005).

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Conflict of interest

The authors declare no conflicts of interest regarding this manuscript.

CRediT authorship contribution statement

Saowanit Chairatanapiwong: conceptualization, methodology, formal analysis, writing - original draft; **Panida Kulawong:** investigation; **Puwadon Lawapakul:** investigation; **Panida Pongpunyayuen:** resources; **Phennapha Klangsinsirikul:** supervision, writing - review and editing.

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