# Evaluation of a Manual CD4+ Count Kit for Determination of Absolute CD4+ T-Lymphocyte Counts

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***Objective:*** *The aim of the present study was to evaluate a manual CD4+ count kit assay (CD4+: cytospheres) for CD4+ T-lymphocyte count compared with flow cytometric method in HIV infected patients.*
***Material and Method:*** *One hundred thirty three HIV infected patients were recruited from the out patient department of Khon Kaen Hospital. Blood samples were done by a manual CD4+ count kit assay (CD4+: cytospheres) and flow cytometry for CD4+ T-lymphocyte count. The data were analyzed for diagnostic test and correlation coefficient.*
***Results:*** *The data of cytospheres assay and flow cytomeric method showed good correlation (r = 0.88) for the total group. At the absolute CD4+ T-lymphocyte 200 cells/cu.mm, the cytospheres assay demonstrated sensitivity 83.10% (76.73-89.47%), specificity 93.55% (89.37-97.72%), PPV 93.65% (89.51-97.79%), NPV 82.86% (76.45-89.26%). In the case of CD4+ T-lymphocyte count were lower than 200 cells/cu.mm, the cytospheres assay displayed progressive decrease in sensitivity successive increase in specificity.*
***Conclusion:*** *The cytospheres technique is an alternative noncytofluorometric assay for CD4+ T-lymphocyte count. This test may be useful for screening in HIV infected adult patients in community hospitals where flow cytometry technique is not available. But the assay is limited in determination only absolute CD4+ T-lymphocyte count with higher than 30 cells/cu.mm. This technique is not benefit in pediatric HIV/AIDS patient due to percentage CD4+ value did not obtained. The quality control should be concern technical skill and proficiency testing for laboratory setting.*

***Keywords:*** *Flow cytometry, Cytospheres, CD4+ count*

***J Med Assoc Thai 2008; 91 (10): 1495-8***
***Full text. e-Journal:*** *http://www.medassocthai.org/journal*

Absolute CD4+ T-lymphocyte counts are the most widely used surrogate markers for determining disease progression and patient staging and for therapeutic monitoring of patients with human immunodeficiency virus (HIV) infection[1,2]. The gold standard for accurate determination of CD4+ cell count is flow cytometry, an expensive technique and requires sophisticated equipment as well as trained personnel to perform it. From the limitation of technical support and high cost, this technique is unavailable in most resource-limited countries[3,4]. On the other hand, a manual non-flow cytometric technique is the alternative method for counting CD4+ count. A manual bead assay, called the cytospheres assay (Counter Corporation), which can enumerate CD4+ T-lymphocyte, has been developed. This technique based on inert latex spheres coated with monoclonal antibody and manually enumerated by visible light microscope, which can be applied in any laboratory.

Several studies have demonstrated a good correlation between flow cytometry and non-flow

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cytometric technique[5-7]. The results suggested that the non-flow cytometric method is an alternative technique for the CD4+ T-lymphocyte count, especially in outlying areas where the technology is unavailable in developing countries. In the present study, the authors aimed to determine the reliability of the result of the cytospheres assay in determination absolute CD4+ T-lymphocyte count compared with flow cytometry.

## Material and Method

Blood samples of the present study were obtained from 133 patients at the out patient department, Khon Kaen Hospital. All specimens determined CD4+ T-lymphocyte count by flow cytometer (FACScan, Becton-Dickinson, San Jose, CA, USA) and a manual CD4+ count kit (Cytospheres, Coulter Corporation, USA) on the same day.

The data of CD4+ T-lymphocyte count from the flow cytometry technique and cytospheres assay were analyzed for correlation coefficient and diagnostic test by STATA statistical software. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) with 95% confidence interval (CI) of various cut-points of CD4+ T-lymphocyte between cytospheres assay compared with flow cytometry were calculated.

## Results

The overall correlation coefficients (r) between the cytospheres assay and flow cytometry of 133 samples was 0.88 for CD4+ T-lymphocytes count (Fig. 1) and statistically significantly different (t-test, t = 3.496 p-value = 0.001). At the absolute CD4+ T-lymphocyte 200 cells/cu.mm, the cytospheres assay demonstrated sensitivity 83.10% (76.73-89.47%), specificity 93.55% (89.37-97.72%), PPV 93.65% (89.51-97.79%), NPV 82.86% (76.45-89.26%). In the case of CD4+ T-lymphocytes were lower than 200 cells/cu.mm, the cytospheres assay displayed progressive decrease in sensitivity by successive increase in specificity. In addition, the number of CD4+ T-lymphocyte higher than 200 cells/cu.mm, by cytospheres assay demonstrated progressive decreased in specificity by successive increase in specificity (Table 1).

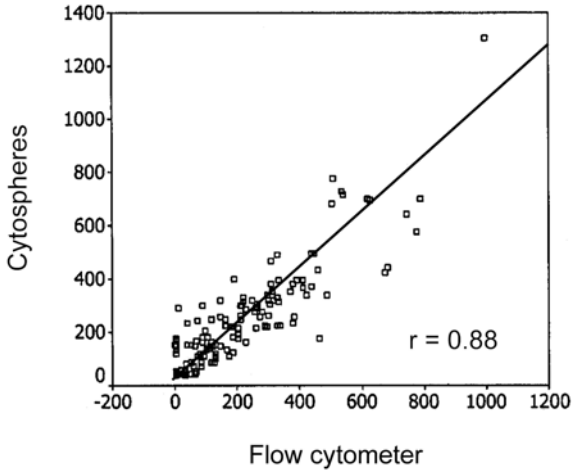


**Fig. 1** Overall correlation between the cytospheres assay and flow cytometry for absolute CD4+ T-lymphocyte counts (n = 133)

## Discussion

Although flow cytometry is the gold standard for the measurement of CD4+ T-lymphocyte count. This

**Table 1.** Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), of CD4+ T-lymphocyte count as determined by cytospheres assay compared with flow cytometry in various cut-points (95% CI = 95% confidence interval)

| Absolute CD4+ count by flow cytometry (cell/cu.mm) | Absolute CD4+ count by cytospheres assay | | | |
|---|---|---|---|---|
| | Sensitivity % (95% CI) | Specificity % (95% CI) | PPV % (95% CI) | NPV % (95% CI) |
| 350 | 98.59 (96.54-100.59) | 41.94 (33.55-50.32) | 66.04 (57.99-74.09) | 96.30 (93.09-99.51) |
| 300 | 97.18 (94.37-100.00) | 61.29 (53.01-69.59) | 74.19 (66.76-81.63) | 95.00 (91.30-98.70) |
| 250 | 92.96 (88.61-97.31) | 79.03 (72.11-85.95) | 83.54 (77.24-89.85) | 90.74 (85.81-95.67) |
| 200 | 83.10 (76.73-89.47) | 93.55 (89.37-97.72) | 93.65 (89.51-97.79) | 82.86 (76.45-89.26) |
| 150 | 66.20 (58.16-74.24) | 100.00 (100.00-100.00) | 100.00 (100.00-100.0) | 72.09 (64.47-79.72) |
| 100 | 36.68 (28.43-44.81) | 100.00 (100.00-100.00) | 100.00 (100.00-100.0) | 57.94 (49.55-66.33 |
| 50 | 19.72 (12.96-26.48) | 100.00 (100.00-100.00) | 100.00 (100.00-100.0) | 52.10 (43.61-60.59) |

technique requires highly trained personnel and expensive reagent, which makes it unavailable in many hospitals and laboratories. The cytosphere assay is the noncytofluorometric method for alternative technique to resource-limited countries because it is relatively inexpensive and requires no specialized equipment other than flow cytometry. In a cost analysis, the cytospheres assay is three times less expensive than flow cytometry[8-10].

In the present study, the overall result of the cytospheres assay with flow cytometry was good correlative for CD4+ T-lymphocyte count. The data demonstrated progressive decrease in sensitivity and had successive increase in specificity in patients with CD4+ T-lymphocyte less than 200 cells/cu.mm. The cytospheres assay showed a progressive increase in sensitivity and decrease in specificity in individuals with higher than 200 CD4+ T-lymphocyte per microlitre. However, the cytospheres assay was not as sensitive as flow cytometry. This reduced sensitivity may have resulted from the small population of cells that were counted by the cytospheres assay. At least 10,000 target cells were counted by flow cytometry while 250 cells were counted by the cytospheres assay. The limitation of cytospheres assay are the result of the test giving absolute CD4+ T-lymphocyte count, % CD4+ value is not obtained by this assay and this assay cannot report CD4+ T-lymphocyte lower than 30 cells/cu.mm.

In Thailand, the government has supported antiretroviral (ARV) therapy for HIV/AIDS patients since 2004. The community hospitals send blood samples to general or regional hospital for measurement of CD4+ T-lymphocyte count. The patient who has CD4+ T-lymphocyte count lower than 200 cells/cu.mm will receive ARV drugs. In some rural areas, the transportation between general or regional hospitals and community hospital network is not convenient. The cytospheres assay proved to be performed and setting for determination of CD4+ T-lymphocyte count. This test is beneficial in community hospital laboratories where equipment is at a minimum and cost effective for the areas limited by budgetary constraints because the instruments needed to perform the assay are a reliable pipette, a hemocytometer and a light microscope. Human skill and proficiency testing are important factors and should be of concern before laboratory setting. In the HIV infected pediatric group, they need percentage CD4+ more than absolute CD4+ because a pediatric patient has a high number of white cell count and percentage lymphocyte. From the limitation of the cytospheres assay, this technique is not useful in the pediatric group and HIV/AIDS patients who have a CD4+ count lower than 30 cells/cu.mm.. The application of this test is used as a screening test in HIV infected adult patients before ARV therapy in community hospitals where flow cytometry technique is not available.

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## *การประเมินชุดตรวจนับจำนวน CD4+ ด้วยวิธี manual เพื่อหาค่าสมบูรณ์ของเซลล์ลิมโฟซัยต์ชนิด CD4+*

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***วัตถุประสงค์****: ต้องการประเมินผลการตรวจวิเคราะห์จำนวนลิมโฟซัยต์ชนิด CD4+ T-lymphocyte ระหว่างวิธี flow cytometry และเทคนิคการนับจำนวน CD4+ ด้วยชุดตรวจ cytospheres*

***วัสดุและวิธีการ****: ผู้ป่วยและผู้ติดเชื้อ HIV/AIDS จำนวน 133 คน จากคลินิคผู้ป่วยนอกโรงพยาบาลขอนแก่น ถูกเก็บตัวอย่างเลือดและนำไปตรวจหาจำนวนลิมโฟซัยต์ CD4+ T-lymphocyte ด้วยวิธี flow cytometry และ ชุดตรวจ cytospheres*

***ผลการศึกษา****: จากผลการศึกษาพบว่าผลการตรวจวิเคราะห์ จำนวนลิมโฟซัยต์ CD4+ ด้วยวิธี flow cytometry และ cytospheres มีความสัมพันธ์กัน (r = 0.88) ในภาพรวม วิธี cytospheres มีความไวร้อยละ 83.10 (CI = 76.73-89.47) ความจำเพาะร้อยละ 93.55 (CI = 89.37-97.72) ค่าทำนายผลบวกร้อยละ 93.65 (CI = 89.51-97.79) ค่าทำนายผลลบร้อยละ 82.86 (CI = 76.41-89.26) ที่จำนวน CD4+ 200 เซลล์/ลบ.มม ในกลุ่มผู้ที่มีค่า CD4+ น้อยกว่า 200 เซลล์/ลบ.มม. วิธี cytospheres มีความไวลดลงและ มีความจำเพาะเพิ่มขึ้น*

***สรุป****: วิธี cytospheres เป็นวิธีทางเลือกที่สามารถทำได้ในห้องปฏิบัติการทั่วไป สำหรับตรวจหาจำนวน CD4+ ข้อจำกัดของการตรวจคือสามารถรายงานค่าสมบูรณ์ของลิมโฟซัยต์ CD4+ เท่านั้น ไม่สามารถรายงานจำนวนร้อยละ และไม่สามารถรายงานผลการตรวจที่มีค่าจำนวนลิมโฟซัยต์ CD4+ น้อยกว่า 30 เซลล์/ลบ.มม. ดังนั้นจึงไม่เหมาะกับการตรวจวิเคราะห์ในกลุ่มผู้ป่วยเด็กและผู้ที่ติดเชื้อหรือผู้ป่วยเอดส์ที่มีค่า CD4+ ในปริมาณน้อย ๆ แต่วิธีนี้สามารถใช้ในการตรวจกรองเบื้องต้นก่อนการขอรับยาต้านไวรัสในกลุ่มผู้ใหญ่ การตรวจวิเคราะห์ต้องอาศัยทักษะของผู้ตรวจ รวมทั้งการทดสอบความชำนาญระหว่างห้องปฏิบัติการในการควบคุมคุณภาพ*