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Reference intervals of leukocyte parameters in adults by automated blood cell analyzer at King Chulalongkorn Memorial Hospital (KCMH)

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Objective : *To establish reference intervals of leukocyte parameters for clinical use at Central Laboratory of King Chulalongkorn Memorial Hospital (KCMH).*

Study Design : *A descriptive study.*

Materials and Methods : *The study was done in 167 subjects, 61 males and 106 females, age between 18 to 70 years old. All subjects were found healthy by annual health check-up program at KCMH. The leukocyte parameters were performed on Advia120. The study method was performed regarding to recommendation of the International Committee for Standardization in Haematology (ICSH) and the National Committee for Clinical Laboratory Standards (NCCLS).*

Results : *Reference interval of white blood cell count (WBC) was 3.92-9.40 x $10^3/\mu L$. Reference intervals of neutrophil, lymphocyte, monocyte, eosinophil, basophil, and large unstained cell (LUC) in percent were 39.26-71.64 %, 20.92-47.48 %, 2.72-8.40 %, 0.60 -7.27 %, 0.10 -1.00 %, and 0-4.17%, respectively; their absolute numbers were 1.88-6.00 x $10^3/\mu L$, 1.19-3.38 x $10^3/\mu L$, 0.15-0.57 x $10^3/\mu L$, 0.04-0.50 x $10^3/\mu L$, 0.01-0.07 x $10^3/\mu L$, and 0-0.26 x $10^3/\mu L$, respectively.*

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Conclusion : *Our results agree with previous studies and are also with in the recommended limit level of reference of the manufacturer. Therefore our reference level could be used as reference intervals for population at KCMH to support patients' care.*

Keywords : *Leukocyte parameters, Reference intervals.*

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นวพรรณ จารุรักษ์, สร้อยสุดา คชสวัสดิ์, วีรยา หัวยหงษ์ทอง. ค่าอ้างอิงของพารามิเตอร์เม็ดเลือดขาวในผู้ใหญ่ ที่ได้จากเครื่องตรวจวิเคราะห์เม็ดเลือดอัตโนมัติ ณ โรงพยาบาลจุฬาลงกรณ์. จุฬาลงกรณ์เวชสาร 2548 ก.ค; 49(7): 377 - 86

วัตถุประสงค์ : *เพื่อหาค่าอ้างอิงของพารามิเตอร์ของเม็ดเลือดขาวในผู้ใหญ่ สำหรับการใช้ทางคลินิก ที่ห้องปฏิบัติการกลาง โรงพยาบาลจุฬาลงกรณ์*

วิธีการศึกษา : *การศึกษาเชิงพรรณนา*

วัสดุและวิธีการ : *การศึกษานี้ทำในกลุ่มตัวอย่างจำนวน 167 ราย แบ่งเป็นชาย 61 คน และหญิง 106 คน อายุระหว่าง 18-70 ปี กลุ่มตัวอย่างทั้งหมดเป็นผู้ที่มีผลการตรวจสุขภาพประจำปีโดยโรงพยาบาลจุฬาลงกรณ์เป็นปกติ การศึกษานี้ทำการตรวจวิเคราะห์ด้วยเครื่อง Advia 120 โดยทำตามคำแนะนำของ International Committee for Standardization in Haematology (ICSH) และ National Committee for Clinical Laboratory Standards (NCCLS)*

ผลการศึกษา : *ค่าอ้างอิงของ WBC เท่ากับ 3.92-9.40 x $10^3/\mu L$, neutrophil, lymphocyte, monocyte, eosinophil, basophil, และ large unstained cell (LUC) เท่ากับ 39.26-71.64 %, 20.92-47.48 %, 2.72-8.40 %, 0.60-7.27 %, 0.10-1.00 %, และ 0-4.17 %, ตามลำดับ ส่วนค่าสัมบูรณ์อ้างอิง เท่ากับ 1.88-6.00 x $10^3/\mu L$, 1.19-3.38 x $10^3/\mu L$, 0.15-0.57 x $10^3/\mu L$, 0.04-0.50 x $10^3/\mu L$, 0.01-0.07 x $10^3/\mu L$, และ 0-0.26 x $10^3/\mu L$*

สรุป : *ผลการศึกษานี้สอดคล้องกับผลการศึกษาที่ผ่านมา และตามค่าอ้างอิงของบริษัท ดังนั้นค่าอ้างอิงนี้สามารถนำมาใช้กับกลุ่มประชากรของโรงพยาบาลจุฬาลงกรณ์ สำหรับการดูแลรักษาผู้ป่วย*

คำสำคัญ : *พารามิเตอร์ของเม็ดเลือดขาว, ค่าอ้างอิง*

Differential cell counts have provided extensive data for more than a century of laboratory hematology. Routine bench morphology count is being replaced by automated blood cell analyzers that provide greater precision and consistency. Nowadays, there is no doubt that automated blood cell analyzers are used worldwide to perform complete blood count and leukocyte differential count. In order to permit adequate discrimination between health and disease using the analyzers the reference intervals or values of healthy population have to be determined. In general reference intervals are derived from textbooks or manufacturers' instructions. However due to biological and physiological variability, individual laboratories are recommended to establish their own reference intervals.[1] To establish proper reference intervals for a laboratory tests, three important criteria need to be considered. First, the subject populations must be evaluated for their health conditions. In another word, ostensible subjects could not be included into the study without adequate evaluation for their health status. Second, the size of the population must be adequate for statistical calculation. Third, suitable statistical methods should be selected. In order to establish proper reference intervals of laboratory tests worldwide, the International Committee for Standardization in Haematology (ICSH) and the National Committee for Clinical Laboratory Standards (NCCLS) have provided a standard method to determine reference intervals.[2-6]

The objective of this study is to establish the reference intervals for leukocyte parameters on routine automated blood cell analyzer, Advia 120,based on a recommended standard of ICSH and NCCLS.[2-6] The leukocyte parameters provided by Advia 120 are white blood cell count (WBC), relative (%) and absolute numbers (#) of differential leukocyte counts such as neutrophil, lymphocyte, monocyte, eosinophil, basophil, and large unstained cell (LUC). In general, only 5 normally WBC (neutrophil, lymphocyte, monocyte, eosinophil, and basophil) are appeared and determined routinely. However, the background principle of Advia 120 could provide extended differential count by adding another cluster of cells, LUC. LUC are peroxidase-negative and those white cells larger than most lymphocyte. In healthy subjects, LUC are mainly large lymphocytes.[7] The reference intervals of leukocyte parameters would be useful for helping the physician to differentiate between the healthy and the ill at the Central Laboratory of King Chulalongkorn Memorial Hospital (KCMH).

Materials and Methods

Four hundred and eighty volunteers were randomly selected: 240 males and 240 females, their age was between 16 to 70 years old. All subjects were employees of private or governmental organizations enrolling for their annual checkup, at KCMH, during July to November 2003. Some were seeking for their own routine annual checkup. All are interviewed, then checked for vital signs, blood pressure, and inspected by doctors. Individuals with abnormal vital signs or abnormal blood pressure or abnormal doctoral inspection were not included in further chest X-ray (CXR) and laboratory investigation. After written informed consent was obtained, blood sample was collected by venipuncture from each subject. All volunteers were filmed CXR, and investigated according to the same laboratory test

menu for complete blood count (CBC), fasting blood glucose (FBG), blood urea nitrogen (BUN), creatinine (Cr), uric acid, cholesterol (Chol), triglyceride (Tg), high-density lipoprotein cholesterol (HDLc), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urinalysis (UA), and stool examination. In addition, CBC was analyzed by Advia 120 which analyze specimens by using light scattering and cytochemistry methods, and FBG, BUN, Cr, uric acid, Chol, Tg, HDLc, AST, ALT, and ALP were determined by Hitachi 912.[8] Only those who had no anemia based on the WHO criteria[9], received no medication, and all of them had normal results of physical examinations, urinalysis, stool examinations for parasites and chest X-ray were recruited in to the study. Subjects who had abnormal CXR and/or laboratory results were excluded. After careful investigations, only 167 (34.8 %) healthy subjects were recruited: 61 males and 106 females, age between 18 to 70 years old.

To cover for 95 % of population (95 % reference intervals or ranges) the reference intervals were calculated using mean $\pm$ 2 standard deviation (mean $\pm$ 2SD) for those laboratory parameters that have Gaussian distribution. For those laboratory parameters that have skewed distributions, the 95 % reference intervals are calculated from 2.5 and 97.5 percentiles.[10]

Results

The reference means and intervals are shown in Table 1. The distributable pattern of WBC is demonstrated in Figure 1. The distributable patterns of each parameter of relative (%) and absolute numbers (#) of differential leukocyte counts are shown in Figure 2 (a-f) and 3 (a-f). Regarding the distributable

Table 1. Comparison of our reference intervals and others reference intervals.

Laboratory parameters	Reference intervals (our study)	Reference intervals (manufacturer)[13]	Reference intervals (Krailadsiri P, et al)[14]
WBC ($x10^3/\mu L$)	3.92-9.40	4.8-10.8	3.6-9.8
Neutrophil (%)	39.26-71.64	40.0-74.0	36.4-68.9
Lymphocyte (%)	20.92-47.48	19.0-48.0	20.4-49.0
Monocyte (%)	2.72-8.40	3.4-9.0	2.5-8.1
Eosinophil (%)	0.60-7.27	0-7.0	0-8.4
Basophil (%)	0.10-1.00	0-1.5	0-2.5
LUC (%)	0-4.17	0-4.0	0-9.5
Neutrophil (#,$x10^3/\mu L$)	1.88-6.00	1.90-8.00	-
Lymphocyte (#,$x10^3/\mu L$)	1.19-3.38	0.90-5.20	-
Monocyte (#,$x10^3/\mu L$)	0.15-0.57	0.16-1.00	-
Eosinophil (#,$x10^3/\mu L$)	0.04-0.50	0-0.80	-
Basophil (#,$x10^3/\mu L$)	0.01-0.07	0-0.20	-
LUC (#,$x10^3/\mu L$)	0-0.26	0-0.40	-

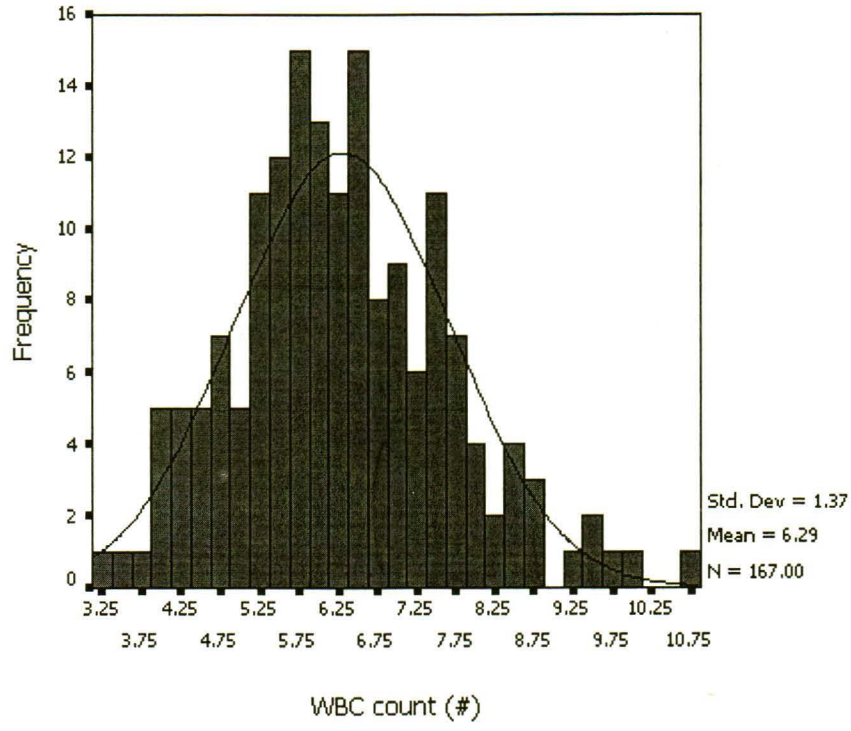

Figure 1. The distributable pattern of WBC in 167 healthy subjects is almost a Gaussian distribution the mean of which is 6.29 $x10^3/\mu L$ and ranges 3.92-9.40 $x10^3/\mu L$.

patterns of each parameter, the reference intervals were calculated. The reference intervals of WBC, neutrophil, lymphocyte, and monocyte were close to Gaussian distribution, while the reference intervals of eosinophil, basophil, and LUC were leftward skewed pattern. To cover for 95 % of population (95 % reference intervals or ranges), we decide to calculated all the reference intervals from 2.5 and 97.5 percentiles.[10-12]

Discussion

Our reference intervals of white blood cell count (WBC), relative (%) and absolute numbers (#) of differential leukocyte counts such as neutrophil, lymphocyte, monocyte, eosinophil, basophil, and large unstained cell (LUC) are close to reference intervals recommended by the manufacturer[13], including reference intervals of a previous study[14], (Table 1). So far, we notice that reference interval of absolute numbers of WBC in Thai people is slightly lower than that of the manufacturer, especially in lymphocyte, monocyte, eosinophil, basophil, and LUC.[13-14] The difference may be contributed by race, geography, and life style.

The distribution pattern of reference intervals of WBC, neutrophil, lymphocyte, and monocyte were close to Gaussian distribution. However, the distribution of reference intervals of levels of eosinophil, basophil, and LUC were leftward skewed pattern. These suggested that the level of eosinophil, basophil, and LUC in the majority of healthy population trend to shift toward the lower levels, few have higher levels. In this study, all ostensive subjects took physical examination but they were also received CXR,

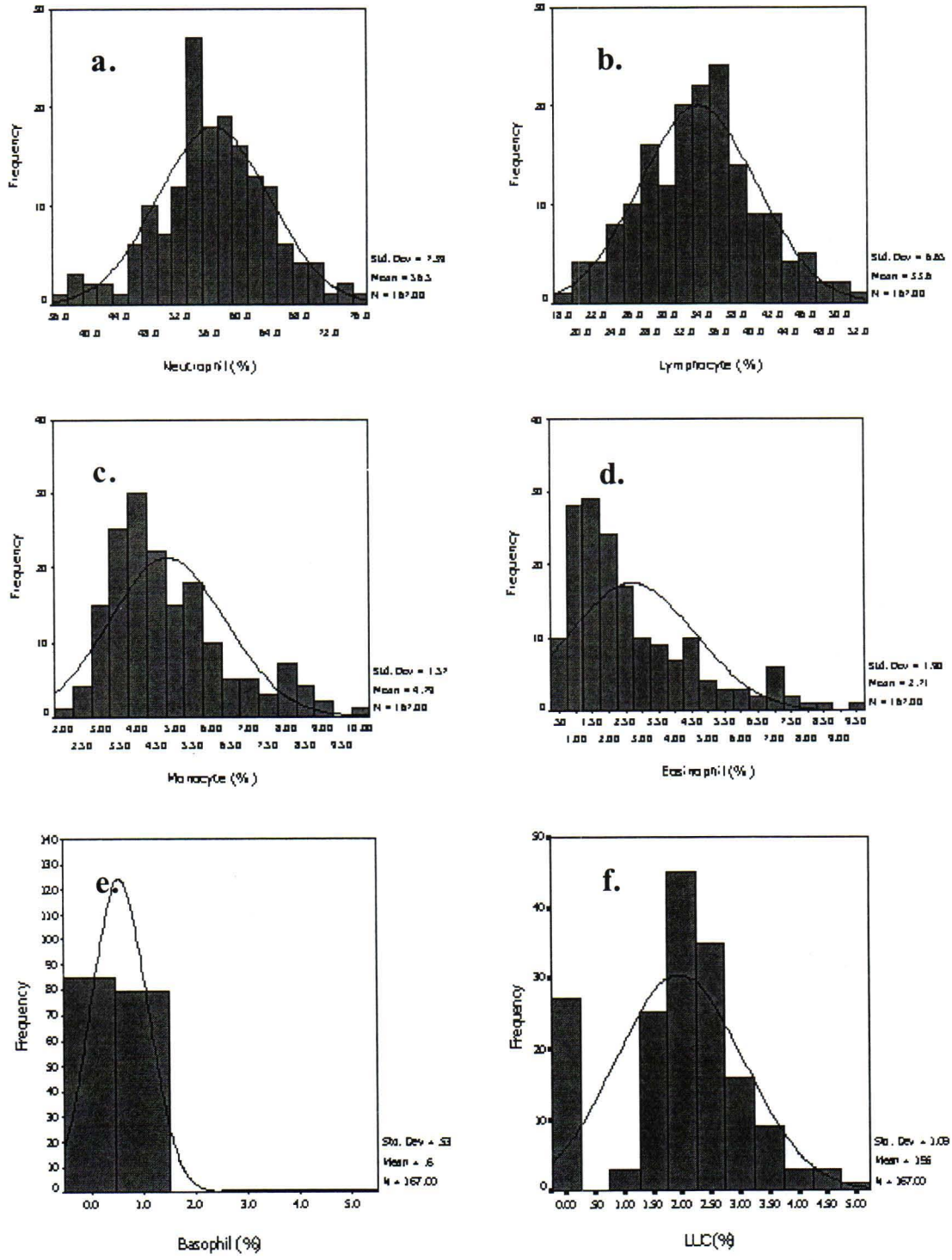

Figure 2. The distributable patterns of each parameter (a-f) in relative (%) of differential leukocyte counts: a) neutrophil, b) lymphocyte, c) monocyte, d) eosinophil, e) basophil, and f) LUC, with the relative means of 56.50 %, 33.60 %, 4.79 %, 2.71 %, 6.00 %, and 2.31 %, respectively.

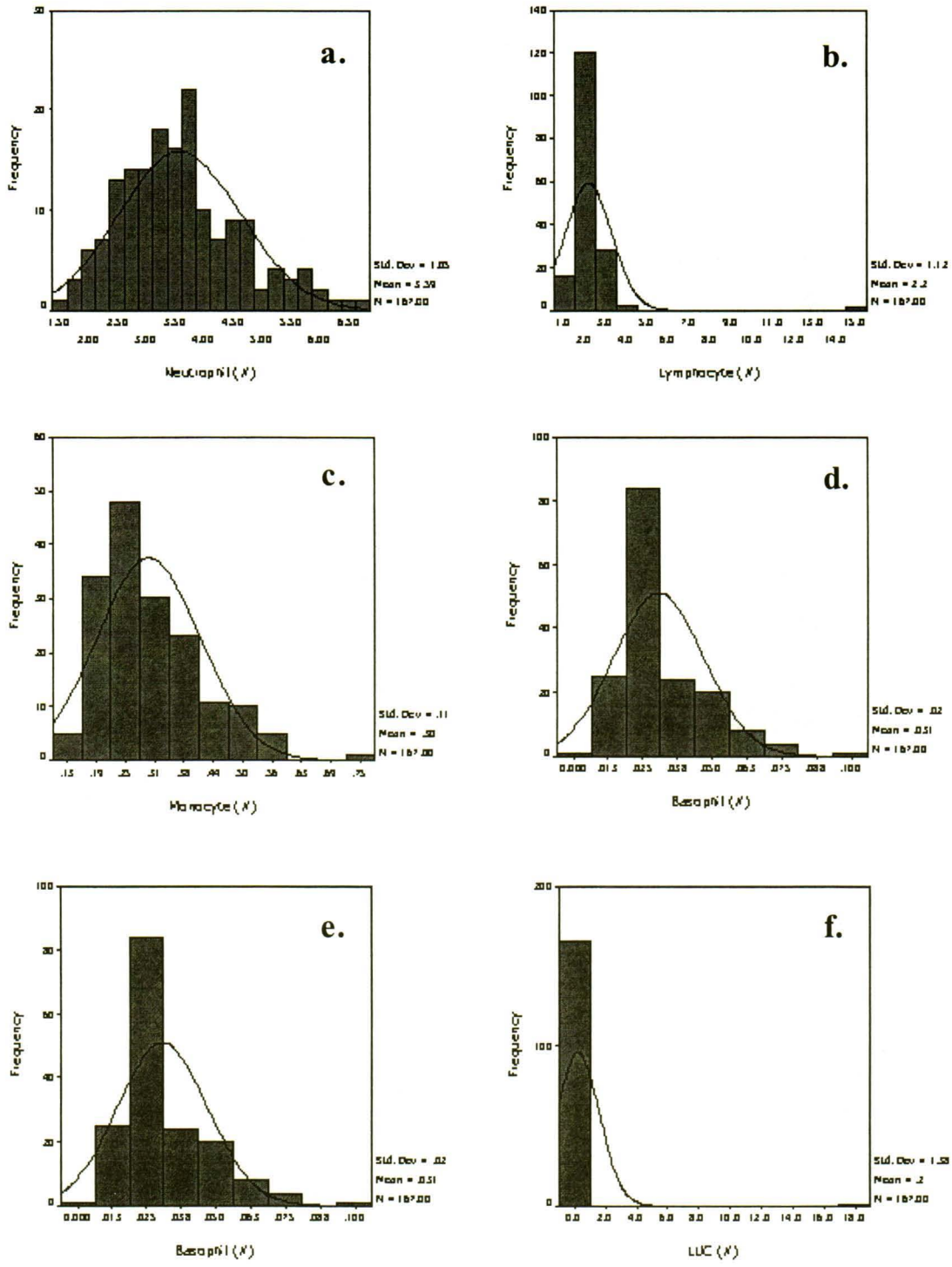

Figure 3. The distributable patterns of each parameter (a-f) in absolute numbers (#) of differential leukocyte counts: a) neutrophil, b) lymphocyte, c) monocyte, d) eosinophil, e) basophil, and f) LUC, with the means of absolute numbers: 3.59, 2.20, 0.30, 0.17, 0.03, and 0.20 $x10^3/\mu L$, respectively.

and laboratory tests, such as; CBC, FBG, BUN, Cr, uric acid, Chol, Tg, HDLc, AST, ALT, ALP, UA, and stool examination. Only those who had normal physical examination result, CXR, and laboratory tests were included in this study. We also compare our results with reference level recommended by the manufacturer [13] and Krailadsiri P, et al.[14] (Table 1). We have found that our results agree with previous studies. All our reference levels are also with in the limits of recommended reference levels of the studies. The important factor contributed to the study results is that it was conducted under strict criteria of subject inclusion and exclusion according the recommendation of ICSH and NCCLS.[2-6]

Hematological parameters are affected not only by age, sex, ethnic origin, and altitude, but also by a number of other biological factors and extraneous influences. Therefore the international organizations such as; ICSH[2-3], NCCLS [4-6], etc. recommend every laboratory to establish their own reference intervals. In addition, the reference intervals are systemic-specific and they should be established by each laboratory.[1] We conclude that our reference level could be used as reference intervals for population at KCMH. In addition, the reference intervals of leukocyte parameters in this study will hopefully be useed not only as baseline data for reason of requirement of standard services, but also as an efficient tool for supporting the patients' care. We agree with the recommendation of ICSH[1-2], NCCLS[4-6], etc, that every laboratory needs to establish its own reference intervals. In order to achieve the standard requirement and need of patients' care for laboratory services in developing countries such as Thailand, reference intervals should be considered a basic requirement. Supporting funds to establish reference intervals from associated or research organizations should be made available to every laboratory.

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