



## The reference intervals for biochemistry analytes obtained through the indirect method indicate the metabolic shifts in the Thai adult population

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### ABSTRACT

**Background:** Reference intervals (RIs) of biochemistry analytes are required to make medical decisions. According to the standards, medical laboratories shall validate and/or verify RI for their own uses because there are biological and laboratory variations.

**Objective:** To determine RIs of 14 biochemistry analytes, including fasting blood glucose, total cholesterol, HDL cholesterol, triglyceride, total protein, albumin, BUN, creatinine, uric acid, AST, ALT, ALP, amylase, and lipase in Thai adults.

**Materials and methods:** RIs were determined by using the indirect method. Test results from 18 years old or older out-patient department patients were collected from the hospital laboratory database from January 2019 to December 2021. The results from subjects with a high likelihood of disease were excluded, outliers were removed, data distribution was tested, and RIs were determined as either mean $\pm$ 2SD or the 2.5<sup>th</sup> to 97.5<sup>th</sup> percentile for normal and non-normal distribution data, respectively.

**Results:** There were 197,897 test results available for RI determination. Most of the determined RIs were comparable to those from the manufacturer, except uric acid. All 14 RIs passed verification criteria in the population served. Interestingly, our RIs for blood total cholesterol, triglyceride, and uric acid were higher than those established in the previous reports in Thai population.

**Conclusion:** The RIs were established and verified in Thai adults; therefore, they could be implemented for the population. In addition, our RIs of total cholesterol, triglyceride, and uric acid reflect metabolic changes of the studied population.

### Introduction

Biochemistry analysis is laboratory tests used to assess metabolic abnormalities and organ pathology. Routine biochemical analytes include fasting blood glucose (FBG), total cholesterol (TC), HDL cholesterol (HDL-c), triglyceride, total protein, albumin, blood urea nitrogen (BUN), creatinine, uric acid, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), amylase, and lipase. Medical laboratories must report the results with reference intervals (RIs). Therefore, they are interpreted and provide information for medical decisions, including diagnosis and treatment plan. RI is the statistical intervals of analyte levels, representing the 95% central of the test result data from reference population,<sup>1,2</sup> which are determined as either mean $\pm$ 2SD or the 2.5<sup>th</sup> to 97.5<sup>th</sup> percentile ( $P_{2.5} - P_{97.5}$ ), depending on the data distribution.

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According to standards and regulations for medical laboratory quality, including the Clinical & Laboratory Standards Institute (CLSI) guideline C28-A3c, medical laboratories shall validate and/or verify RI for their own uses because there are biological and laboratory variations.<sup>2,3</sup> The biological variations include age, sex, ethnicity or genetic background, diets, lifestyle and environmental conditions,<sup>4</sup> while the laboratory variations include pre-analytical and analytical system, especially method principles being used.<sup>3,5</sup> Therefore, RIs may not be transferable if there is incompatibility of the measurement system or the subject population.<sup>3</sup> Consequently, determining RIs for the subject population served is crucial for interpretation of test results.

There are two methods for determination of RI, direct and indirect methods.<sup>5-8</sup> The former is done by collecting specimens of interest from at least 120 presumably healthy subjects, measuring the analytes of interest, gathering laboratory test results, and establish the RI. To select presumably healthy subjects, the inclusion and exclusion criteria must be cautiously designed. Altogether, the direct method is costly and time-consuming. Alternatively, medical laboratories can conduct the indirect method by using their own database.<sup>1,5,6</sup> With the present technology, medical laboratories with limited resources can achieve the method. Data collection and analysis can be done by designing selection criteria and analyzing the data for RI determination by using data processing software.

The indirect method is costly and time-consuming; therefore, it is not a practical task in most of medical laboratories in Thailand. Moreover, RIs provided by the manufacturers, which is usually done in foreign population, may not be transferable because of incompatibility in

population, and measurement system.<sup>3,6</sup> Therefore, this study aimed to determine RIs for analytes in routine blood tests, including FBG, TC, HDL-c, triglyceride, total protein, albumin, BUN, creatinine, uric acid, AST, ALT, ALP, amylase, and lipase, in Thai adults by using the indirect method.

### Materials and methods

This study was exempt from review by the Human Research Ethics Thammasat University (project no. 113/2565), and data collection was done under the approval by Saraburi Hospital Human Ethics committee (project no. SRBR65-033).

### Biochemical analytes

Fourteen routine biochemistry analytes, FBG, TC, HDL-c, triglyceride, total protein, albumin, BUN, creatinine, uric acid, AST, ALT, ALP, amylase, and lipase, were studied. All test results were done by using the chemical analyzer, model DxC700AU (Beckman Colter, Inc., California, USA), based on method principles shown in Table 1. The calibrators were purchased from Beckman coulter Ireland Inc. (Co. Clare, Ireland). The MAS ChemTRAK Controls were derived from Thermos Fisher Scientific Inc. (California, USA) and used as internal quality control (IQC) materials. Three levels of IQC materials were done twice a day. External quality assurance (EQA) was done monthly, and laboratory performance was reported at either good ( $1 < |z| \leq 2$ ) or excellent ( $|z| < 1$ ) levels from two EQA providers, the External Quality Assessment Schemes in Clinical Laboratory, Faculty of Medical Technology, Mahidol University (EQAS MUMT) and Division of Proficiency Testing Department of Medical Sciences, Ministry of Public Health (DMSc PT).

**Table 1.** Method principles used for analysis of biochemical substances in blood.

Analytes	Principle of analysis	Method principle
Glucose	Enzymatic UV	Hexokinase / glucose-6-phosphate dehydrogenase
Total cholesterol	Enzymatic colorimetric	Cholesterol oxidase/peroxidase
Triglyceride	Enzymatic colorimetric	Lipase/ glycerol kinase/glycerol phosphate oxidase/peroxidase
HDL cholesterol	Enzymatic colorimetric	Anti human- $\beta$ -lipoprotein antibody/cholesterol esterase/cholesterol oxidase/peroxidase
Total protein	Chemical colorimetric	Biuret reaction
Albumin	Chemical colorimetric	Bromocresol green
BUN	Enzymatic UV	Urease/glutamate dehydrogenase
Creatinine	Enzymatic colorimetric	Creatininase/creatinase/sarcosine oxidase/ peroxidase
Uric acid	Enzymatic	Uricase/peroxidase
AST	Kinetic UV	IFCC without pyridoxal phosphate activation
ALT	Kinetic UV	IFCC without pyridoxal phosphate activation
ALP	Kinetic colorimetric	DGKC optimized standard method
Amylase	Kinetic colorimetric	2-chloro-4-nitrophenol
Lipase	Kinetic colorimetric	monoglyceride lipase/glycerol kinase/ glycerol phosphate oxidase/ peroxidase.

### **Data collection and Data selection**

Laboratory results and baseline characteristics were collected from the laboratory database of Saraburi Hospital from January 2019 to December 2021. Data selection was done by using the inclusion and exclusion criteria. The inclusion criteria were test results collected from check-ups and out-patient department (OPD) patients who were 18 years old or older, and the results with a high probability of disease were excluded by using the exclusion criteria. The following data were excluded: the FBG data from individuals with abnormal amylase and lipase results; the lipid profile (TC, HDL-c, and triglyceride), AST, ALT, ALP, BUN, total protein, and albumin data from individuals with positive hepatitis B screening test (chemiluminescent immunoassay, the ARCHITECT i2000SR immunoassay analyzer, Abbott, USA) and abnormal liver function tests (total protein, albumin, total and direct bilirubin, AST, ALT, and ALP); the BUN, creatinine, and uric acid data from individuals with abnormal non-protein nitrogenous (NPN) compounds (BUN, creatinine, and uric acid) and UACR; the total protein and albumin data requested from oncology or nephrology clinics; the amylase and lipase data from individual with abnormalities of either amylase or lipase; and the ALP data requested from antenatal care and orthopedics clinics.

### **Determination of reference intervals and statistical analysis**

The selected data were analyzed by Microsoft Excel, Version 2408 Build 16.0.17928.20114. Determination of reference intervals was done as previously described.<sup>5,9</sup> First, the outliers were removed by using Turkey method.<sup>8</sup> The outliers were identified by interquartile range (IQR), quartile (Q)1, and Q3. The lower limit and the upper limit were calculated as  $Q1-1.5IQR$  and  $Q3+1.5IQR$ , respectively. The data value outside the limit were specified as outliers and removed from the dataset. Next, data distribution was assessed by using skewness, kurtosis, and normal distribution was tested by the Jarque-Bera

(JB) and chi square tests. After that, RIs were determined based on their distribution. If the dataset is normally distributed, mean and SD were used for RI determination as  $mean \pm 2SD$ . However, if the dataset is not normally distributed, data transformation was performed, based on Tukeys' ladder of power.<sup>10</sup> The datasets with positive skewness were transformed by using log<sub>10</sub> or square root, and the negative skewness datasets were transformed by using square or cube. After data transformation, RIs were determined, according to data distribution,  $mean \pm 2SD$  for normal distribution data and  $P_{2.5} - P_{97.5}$  for non-normal distribution. In addition, difference between male and female data was tested by the student's *t* test, and if there is significant difference between sex ( $p < 0.05$ ), RIs for males and females were done separately.

### **Data verification**

The determined RIs were verified as previously described.<sup>2,11</sup> Briefly, twenty laboratory results were collected from assumed normal individuals and compared with the corresponding RIs. The percentage of the result value in the interval was calculated. If ninety percents of the laboratory results (N=18) are in the interval, the RI is acceptable for clinical use.

## **Results**

### **Baseline characteristics of biochemical analytes**

RIs were determined for 14 routine blood tests, including FBG, TC, HDL-c, triglyceride, total protein, albumin, BUN, creatinine, uric acid, AST, ALT, ALP, amylase, and lipase, by the indirect method. There were 205,485 test results collected by using the inclusion and exclusion criteria. Amounts of males and females in the datasets were shown in Table 2. The data with a high likelihood of disease were further removed as outliers, resulting in 197,897 data for determination of RIs. The average age in the datasets were between 47 and 64 years old, and the average age for males and females for each dataset was comparable (Table 2).

**Table 2.** Characteristics of the datasets used for determination of reference intervals.

Tests	Total		Males		Females	
	N	Age*	N (%)	Age*	N (%)	Age*
FBG	4,112	47±9	1,108 (26.9)	47±10	3,004 (73.1)	47±8
Total cholesterol	4,470	47±9	1,207 (27.0)	47 ± 11	3,263 (73.0)	47±9
Triglyceride	3,817	48±9	881 (23.1)	48 ± 11	2,936 (76.9)	47±9
HDL cholesterol	3,365	47±9	715 (21.2)	44 ± 11	2,650 (78.8)	47±9
Total protein	32,716	61±19	17,108 (52.3)	59 ± 19	15,608 (47.7)	63±18
Albumin	44,680	62±17	22,930 (51.3)	60 ± 17	21,750 (48.7)	64±18
BUN	2,402	51±14	961 (40.0)	52±14	1,441 (60.00)	51±13
Creatinine	5,115	48±12	1,398 (27.3)	49 ± 13	3,717 (72.7)	48±11
Uric acid	1,741	49±10	724 (41.6)	49 ± 10	1,071 (58.4)	48±9
AST	4,604	49±12	1,184 (25.7)	51 ± 12	3,420 (74.3)	49±11
ALT	4,499	49±12	1,197 (26.6)	52 ± 13	3,302 (73.4)	49±11
ALP	74,889	58±17	34,701 (46.3)	57 ± 17	40,188 (53.7)	58±17
Amylase	5,949	50±18	3,476 (58.4)	49 ± 18	2,473 (41.6)	52±19
Lipase	5,538	50±18	3,182 (57.5)	49± 18	2,356 (42.5)	52±19

**Note:** \*data are presented as mean±SD, FBG: fasting blood glucose.

#### **RIs of biochemical analytes derived by the indirect method**

There were six datasets becoming normal distributions after outlier removal, including uric acid (males and females), AST (males and females) and ALT (male and females) datasets, and RIs could be then determined as mean±2SD; 4.1-9.9, 3.1-7.4 mg/dL, 14-39, 12-31, 4-47, and 4-29 IU/L, respectively (Table 3). For other test results with non-normal distributions, the datasets were transformed to adjust the values to become normal distributions. After data transformation, two

datasets become normally distributed, FBG and BUN, and the RIs were established as mean±2SD: 75-111 and 7.1-19.7 mg/dL, respectively (Table 3). For the rest of the datasets, TC, HDL-c, triglyceride, total protein, albumin, creatinine (males and females), ALP, amylase, and lipase, the distributions were still non-normal after data transformation and their RIs were determined as  $P_{2.5} - P_{97.5}$ ; 135-287, 41-216, 34-87 mg/dL, 5.3-8.6, 2.2-4.6 g/dL, 0.65-1.25, 0.50-0.90 mg/dL, 38-127, 35-121, 22-128, and 5-59 IU/L, respectively (Table 3).

**Table 3.** Comparison of RIs derived from the present study, the manufacturer, and previous studies in Thai population.

Analytes <sup>#</sup>	The present study	Manufacturer	Viriyayudhakorn <sup>13</sup>	Chamnanpai <sup>14</sup>	Yotsakullert <sup>15</sup>	Chatchawal <sup>16</sup>
Method	Indirect	Direct	Direct	Direct	Direct	Indirect
FBG	75-111	74-106	69-100	74-106	NA	71-103
Total cholesterol	135-287	<200*	120-246	150-220	NA	145-299
Triglyceride	41-216	<150*	NA	26-143	NA	37-212
HDL cholesterol	34-87	>40*	NA	41-91	41-85	32-77
Total protein	5.3-8.6	6.0-8.0	6.7-8.3	NA	NA	NA
Albumin	2.2-4.6	3.5-5.0	NA	NA	NA	NA
BUN	7.1-19.7	6-20	NA	6-19	NA	8-19
Creatinine						
Males	0.65-1.25	0.70-1.20	0.8-1.4		0.64-1.29	
Females	0.50-0.90	0.60-1.00	0.6-1.1	0.5-1.1	0.51-0.91	0.5-1.3
Uric acid						
Males	4.1-9.9	3.5-7.2	2.5-7.7			
Females	3.1-7.4	2.6-6.0	2.0-5.6	2.5-7.0	NA	NA
AST						
Males	14-39	0-50	NA		16-35	
Females	12-31	0-35	NA	13-30	15-33	16-35
ALT						
Males	4-47	0-50	NA		12-32	
Females	4-29	0-35	NA	10-38	12-31	9-36
ALP						
Males	38-127	30-120				
Females	35-121	30-120	NA	28-93	40-113	30-86
Amylase	22-128	25-160	NA	NA	NA	NA
Lipase	5-59	0-60	NA	NA	NA	NA

**Note:** FBG: fasting blood glucose, <sup>#</sup>concentration units: mg/dL for FBG, total cholesterol, triglyceride, HDL cholesterol, BUN, creatinine, and uric acid, mg/dL for total protein and albumin (g/dL), IU/L for AST, ALT, ALP, amylase, and lipase, NA: not available, \*based on NCEP-ATPIII.<sup>12</sup>

#### Data verification of RIs

After RIs have been established, they were verified by using laboratory results analyzed in the laboratory. It was shown that over 90 percent of the results were in the

RI of FBG, TC, HDL-c, triglyceride, total protein, albumin, BUN, creatinine, uric acid, AST, ALT, ALP, amylase, and lipase (Table 4).

**Table 4.** Verification results of reference intervals (RI) determined by using the indirect method.

Analytes	Results within RI* (%)	Analytes	Results within RI* (%)
Glucose	95	Albumin	90
TC	95	AST (males)	90
Triglyceride	100	AST (females)	90
HDL-c	100	ALT (males)	90
BUN	100	ALT (females)	90
Creatinine (males)	100	Amylase	95
Creatinine (females)	100	Lipase	95
Uric acid (males)	100	ALP (males)	95
Uric acid (females)	100	ALP (females)	100
Total protein	90		

**Note:** \*percent of test results from 20 assumed normal individuals lining within the corresponding RI.

## Discussion

This study established and verified RIs for 14 routine tests, FBG, TC, HDL-c, triglyceride, total protein, albumin, BUN, creatinine, uric acid, AST, ALT, ALP, amylase, and lipase, by using the indirect method (Table 3). The protocol is not costly and less complicated than the direct method, but it requires skills for data analysis and data processing software. In this study, Microsoft Excel was chosen as a data processing software for data analysis because of its availability in medical laboratories in Thailand. In method prospective, the RI determination process could be divided into three patterns. The first pattern was done for uric acid, AST, and ALT. Their RIs were defined as mean $\pm$ 2SD by using the datasets which were normally distributed after outlier removal. The second pattern was FBG and BUN. The datasets were normally distributed after outlier removal and data transformation; therefore, their RIs were defined as mean $\pm$ 2SD. The third pattern was done for TC, HDL-c, triglyceride, creatinine, total protein, albumin, ALP, amylase, and lipase. Their RIs were determined as P<sub>2.5</sub> - P<sub>97.5</sub> since the datasets were not normally distributed after outlier removal and data transformation.

When compared to RIs provided by the manufacturer (using NCEP-ATPIII as RIs of TC, HDL-c and triglyceride),<sup>12</sup> the lower reference limits (LRLs) and the upper reference limits (URLs) of FBG, TC, HDL-c, triglyceride, BUN, creatinine (males), AST (females), ALT (males and females), ALP (males and females), amylase, and lipase determined in the present study were comparable. However, both LRL and URL of RI of uric acid for male and female were higher than those from the manufacturer with % difference of 17.1% (4.1 vs 3.5 mg/dL), 19.2% (3.1 vs 2.6 mg/dL), 37.5% (9.9 vs 7.2 mg/dL), and 23.3% (7.4 vs 6.0 mg/dL), respectively. Therefore, RI of uric acid from manufacturers might not be transferable. The explanations include variations of the measurement system and the subject population.<sup>3</sup> It is possible that Thai population have different genetic background, diets, lifestyle and environmental conditions, affecting non-diseased levels of the analytes.<sup>4</sup>

When we compared our RIs with previous studies in Thai population, it was found that the RIs for FBG, HDL-c, BUN, creatinine, AST, ALT, and ALT were comparable.<sup>1,13-16</sup> However, our RIs of TC, triglyceride and uric acid were increased when compared with previous studies published a few decades ago by Viriyayudhakorn, *et al.*,<sup>12</sup> and Chamnanpai, *et al.*<sup>14</sup> Conversely, our RIs for TC and triglyceride were comparable to more recent study by Chatchawal, *et al.*<sup>16</sup> Unfortunately, these two recent studies did not report RI of uric acid. Nonetheless, the increased trend of TC, triglyceride and uric acid RIs may reflect changes in diets and lifestyle, affecting metabolism and blood levels.<sup>17-19</sup> It has been shown that animal-based diet, red and processed meat is associated with hyperlipidemia and plant-based diet is conversely associated with hyperuricemia.<sup>20,21</sup> Nowadays, there are alterations in health behaviors among Thai people, including imbalance diets and less exercise. It is possible that foods rich in purine, such as bamboo shoot, vegetables with high purine

content, and organ meat, alcohol, fructose, and fructose-sweetened beverages are the reason for change in blood uric acid.<sup>22</sup> Moreover, hyperlipidemia is a part of metabolic syndrome, and hyperuricemia is now considered as a risk factor of metabolic syndrome.<sup>23-26</sup> Altogether, elevations of TC, triglyceride, and uric acid reflect metabolic change, leading to metabolic syndrome and other non-communicable diseases, including gout, diabetes, and cardiovascular diseases. Therefore, our study revealed not only the RIs of the routine tests, but also increasing trends in blood lipids and uric acid among Thai people.

## Limitations

The number of the data in the study was large enough for RI determination by the indirect method with the minimum of 724 for uric acid (males) and the maximum of 40,188 for ALP (males).<sup>27</sup> However, it is possible that the data were not represent Thai population because it is derived from a single database. Moreover, the datasets in the present study may contain sub-clinical diseased subjects, affecting validity of RIs, which is a limitation of RI determination by using the indirect method. Moreover, medical information in the database was limited; therefore, eliminations of test results from diseased subjects may not be completed, subsequently interfering with the LRL and URL of RIs. Therefore, further research in multicenter setting and complete set of medical information will make RIs transferable to other laboratories.

## Conclusion

In conclusion, the present study established and verified RIs of 14 routine tests, FBG, TC, HDL-c, triglyceride, total protein, albumin, BUN, creatinine, uric acid, AST, ALT, ALP, amylase, and lipase through the indirect method. They can be implemented in medical laboratories with verification. In addition, our results indicated increasing trends in blood lipids (TC, triglyceride) and uric acid among Thai adult population. This observation indicates metabolic change among Thai adult population and should be further investigated.

## Conflict of interest

The authors state no conflict of interest.

## Ethical approval

This study was exempt from review by the Human Research Ethics Thammasat University (project no. 113/2565), and data collection was done under the approval by Saraburi Hospital Human Ethics Committee (project no. SRBR65-033) during January 2019 to December 2021.

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