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The Asian project for collaborative derivation of reference intervals: (1) strategy and major results of standardized analytes

Abstract

Background: A multicenter study conducted in Southeast Asia to derive reference intervals (RIs) for 72 commonly measured analytes (general chemistry, inflammatory markers, hormones, etc.) featured centralized measurement to clearly detect regionality in test results. The results of 31 standardized analytes are reported, with the remaining analytes presented in the next report.

Method: The study included 63 clinical laboratories from South Korea, China, Vietnam, Malaysia, Indonesia, and seven areas in Japan. A total of 3541 healthy individuals aged 20–65 years (Japan 2082, others 1459) were recruited mostly from hospital workers using a well-defined common protocol. All serum specimens were transported to Tokyo at −80°C and collectively measured using reagents from four manufacturers. Three-level nested ANOVA was used to quantitate variation (SD) of test results due to region, sex, and age. A ratio of SD for a given factor over residual SD (representing net between-individual variations) (SDR) exceeding 0.3 was considered significant. Traceability of RIs was ensured by recalibration using value-assigned reference materials. RIs were derived parametrically.

Results: SDRs for sex and age were significant for 19 and 16 analytes, respectively. Regional difference was significant for 11 analytes, including high density lipoprotein (HDL)-cholesterol and inflammatory markers. However, when the data were limited to those from Japan, regionality was not observed in any of the analytes. Accordingly, RIs were derived with or without partition by sex and region.

Conclusions: RIs applicable to a wide area in Asia were established for the majority of analytes with traceability to reference measuring systems, whereas regional partitioning was required for RIs of the other analytes.

Keywords: common reference interval; multicenter study; nested ANOVA; regionality; standardization.

Non-standard abbreviations

Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMY, amylase; AST, aspartate aminotransferase; BC, Beckman Coulter; BMI, body mass index; C3, complement component 3; C4, complement component 4; Ca, total calcium; CK, creatine kinase; Cl, chloride; CRE, creatinine; CRM470, certified reference material 470; CRP, C-reactive protein; E2, estradiol; GGT, γ-glutamyltransferase; Glu, glucose; Hb, hemoglobin; HDL-C, high density lipoprotein-cholesterol; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; JCTLM, Joint Committee on Traceability of Laboratory Medicine; JSCC, Japan Society of Clinical Chemistry; K, potassium; LD, lactate dehydrogenase; LDL-C, low density lipoprotein-cholesterol; MCV, mean corpuscular volume; Na, sodium; RI, reference interval; TCho, total cholesterol; Tf, transferrin; TG, triglycerides; TTR, transthyretin (prealbumin); UA, uric acid.

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Introduction

The global standardization of laboratory tests for major analytes has been achieved by the efforts of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and its national member organizations [1]. However, reference intervals (RIs) still frequently differ from one laboratory to another. According to the directive on in vitro diagnostic medical devices of the European Union [2], diagnostic manufacturers are now requested to supply their clients with appropriate RIs attached to their reagents, and the ISO 15189 standard for clinical laboratory accreditation claims that the laboratory should periodically re-evaluate its RIs [3]. However, despite these requirements, there have been only a limited number of attempts to conduct appropriate multicenter studies to achieve this goal [4–6]. Most studies to date were conducted by gathering test results of healthy donors' specimens measured at each participating laboratory. This relies on two main assumptions: comparability of assay results across different laboratories and lack of regionality in test results. Validation of these assumptions, however, is not easy and thus, the interpretation of RIs is not straightforward.

One multicenter study was conducted in six Asian cites by the Committee on Plasma Proteins (C-PP) of the IFCC Scientific Division (IFCC-SD) in 2000 to derive common reference intervals for 14 major serum proteins [7] whose standardization had almost been achieved by the availability of certified reference material 470 (CRM470) produced under the direction of the C-PP [8]. This study adopted a different approach, that of centralized measurement, by sending all of the specimens to one center in Japan and measuring them collectively in a single laboratory. Unexpectedly, it revealed large interregion variation for many analytes belonging to the class of inflammatory markers, such as IgG, C3, and CRP [7]. To confirm these findings, another study was conducted in 2006 with the participation of six other centers belonging to the Asia Pacific Federation of Clinical Biochemistry (APFCB) [9]. A total of 560 healthy individuals, mainly those working in clinical laboratories, donated blood to allow measurement of 32 common analytes (22 biochemical analytes and 10 proteins). This second survey confirmed significant regionality in about one third of the analytes examined.

Prompted by these observations, this present third study was planned jointly by the C-PP, the Committee on Reference Intervals and Decision Limits (C-RIDL) of the IFCC-SD, and by the Scientific Committee of the APFCB. The primary goal was to verify the possibility of establishing common RIs (i.e., RIs that can be applied to the entire population, independently from the analytical method in use [10]) for the standardized analytes by ensuring traceability of test results. To do this, we collected samples from several relatively homogenous groups of individuals in Japan and in Southeast Asia together with a considerable amount of information allowing us to put in evidence regarding possible effects due to environment and/or lifestyle. Thus, we were able to investigate biological sources of variations in each analyte, including the presence of differences in RIs related to geographical areas (regionality). Our work was not limited to well-standardized analytes but also included non-standardized analytes mostly measured by immunoassays, such as tumor markers, and hormones. To achieve these goals, we again adopted the centralized measurement approach to eliminate assay-related variations. For clarity of presentation, we report herein the results limited to 31 standardized analytes and leave results of other analytes to the next report.

Materials and methods

Participating centers

Invitations to the study together with its protocol were sent to clinical laboratories of major hospitals and commercial laboratories in countries belonging to the APFCB. Fifteen laboratories located in Seoul, Beijing, Taipei, Tainan, Ho Chi Minh City, Kuala Lumpur, Hong Kong, Macau, and Jakarta accepted the invitation. In Japan, collaboration was obtained nationwide from 47 laboratories. All laboratories are listed in the Acknowledgments.

Target population and sample size

We limited the population mainly to those working in clinical laboratories or hospitals to eliminate the potential influence of differences in job profiles on the test results. A minimum sample size from each local area was set at 120 samples to gain adequate statistical power to detect differences among areas. Usually two or more laboratories from each city collaborated to achieve the target number. The age range of donors was set at 20–65 years. The utmost effort was made to ensure an even distribution of age and an equal male to female ratio.

The following exclusion criteria were applied at the time of recruitment: 1) body mass index (BMI) ≥28 kg/m^2; 2) average daily consumption of ethanol ≥75 g [this amount of alcohol intake is rather high, but it was decided to leave it as a general criterion to evaluate the effect of alcohol assumption and to adopt specific exclusion criteria for analytes known to be influenced by alcohol intake (e.g., ALT, GGT, etc.)], 3) cigarette smoking >20/day, 4) regular medication for chronic disease (diabetes mellitus, hypertension, hyperlipidemia, allergic disorders, etc.), 5) recent (≤2 weeks) recovery from acute illness or surgery requiring hospitalization, 6) known hepatitis B or C virus carrier, and 7) pregnancy or within 1 year after childbirth.

For analysis of sources of variation for each analyte, a health-status survey was conducted via questionnaire to obtain information on BMI, ABO blood type, eating patterns, recent episodes of infection or allergy, menstrual status, and other factors. Written informed consent was obtained from each volunteer. The specimens, the test results, and questionnaires were processed anonymously by assigning sequential numbers on arrival to each individual. After completion of measurement for major analytes, the test results together with newly derived sex-specific RIs were reported confidentially.

The study was approved by the Ethical Committee of Yamaguchi University Graduate School of Medical Sciences in December, 2008. The other collaborative laboratories followed their own rules in taking part in the study.

Collection of blood samples

Prior to blood sampling, each volunteer was requested to avoid strenuous physical exertion/exercise during the preceding 4 days, excessive eating or drinking the night before, and to fast overnight at least for 10 h.

Venipuncture was done between 7 am and 10 am after the donor sat quietly for at least 15 min to avoid variations of test results due to physical activity and postural influence. Blood in the amount of 17 mL was drawn from each donor. All participating laboratories received the same blood drawing set containing the vacuum sampling system, provided by Becton-Dickinson Corp. (BD), (Franklin Lakes, NJ, USA), including BD Vacutainer SST tubes (capacity: 8.5 mL) containing a clot-activator and CryoTube cryovials (capacity: 4.8 mL) (Nunc, Roskilde, Denmark). After blood collection, the tubes were rocked 180° at least five times, and centrifugation for serum separation was done within 2 h of collection. The separated serum was transferred and aliquoted immediately to the freezing vials for storage at −80°C. As an adjunct test, a peripheral blood count (CBC) in EDTA-2K blood was performed locally. The results were used as criteria to exclude those who were anemic. The CBC test results were obtained from 1978 individuals in laboratories that used the Beckman-Coulter hematology analyzer [Beckman Coulter Inc. (BC), Brea, CA, USA] for their routine assays.

Target analytes and measurements

Seventy-two analytes (lipids, electrolytes, enzymes, tumor markers, hormones, vitamins, etc.) were targeted for collective measurements. Assay methods, reference materials, and their precisions for the 31 standardized analytes included in this report are listed in Table 1. The auto-analyzer used in the centralized assays was the UniCel DxC (BC). Enzyme activities except for amylase (AMY) were measured in duplicate, one by the IFCC- and the other by JSCC (Japan Society for Clinical Chemistry)-recommended method. The assays were done at BC Japan's Central Laboratory located in Ariake, Tokyo, using the manufacturer's reagents, calibrators, and controls, unless otherwise indicated.

For quality control monitoring, a set of four fresh-frozen pooled sera from healthy individuals, stored aliquoted at −80°C, were used to evaluate imprecision of assays. The average results of imprecision using these samples are listed in Table 1.

Specimen transportation

Serum specimens were packed in a styrene foam box container filled with dry ice and transported to the central laboratory by a domestic express courier service within Japan. Outside Japan, the sample containers were picked up from each laboratory by the coordinator of this study and transported to Japan by airplane, carried as check-in baggage.

Verification of traceability

Reference methods and/or materials for the 31 analytes are available in the database of the Joint Committee on Traceability of Laboratory Medicine (JCTLM). We checked traceability to the corresponding reference system using the materials or methods listed in Table 1. When reference laboratory services were involved (enzymes and hormones), a set of three fresh-frozen (−80°C) pooled sera for each enzyme (AST, ALT, LD, GGT, and CK), or five to nine levels of lyophilized specimens (for the four steroid hormones), were first value-assigned by the reference laboratories (see Acknowledgments) and then used to check alignment of the analytical systems used for obtaining reference

Table 1 List of assay methods and assay imprecision near the mid-normal ranges.

Abbr	Analytes	Method	Reagent	Traceability	Within-day CV	Between-day CV
Alb	Albumin	Timed endpoint bromcresol purple method	Beckman Coulter	CRM470	1.53	1.37
Urea	Urea	Enzymatic rate method	Beckman Coulter	JCCRM521	1.54	2.46
UA	Uric acid	Timed endpoint uricase POD method	Beckman Coulter	JCCRM521, 21	0.68	1.11
CRE	Creatinine	Enzymatic method	Beckman Coulter	JCCRM521	2.41	2.97
Na	Sodium	Indirect potentiometry	Beckman Coulter	JCCRM111, 321	0.22	0.91
K	Potassium	Indirect potentiometry	Beckman Coulter	JCCRM111, 321	0.46	0.89
Cl	Chloride	Indirect potentiometry	Beckman Coulter	JCCRM111, 321	0.25	0.72
Ca	Total serum calcium	Indirect potentiometry	Beckman Coulter	JCCRM321	0.57	1.26
Glu[d]	Glucose	Timed endpoint hexokinase method	Beckman Coulter	JCCRM521	0.77	1.26
TCho	Total cholesterol	Timed endpoint enzyme colorimetry	Beckman Coulter	JCCRM223	1.20	0.74
TG	Triglyceride	Timed endpoint enzyme colorimetry	Beckman Coulter	JCCRM223, 224	1.21	0.80
HDL-C	HDL-cholesterol	Timed endpoint direct method	Beckman Coulter	JCCRM223, 224	0.99	1.61
LDL-C	LDL-cholesterol	Timed endpoint direct method	Beckman Coulter	JCCRM224	1.17	1.31
AST	Aspartate aminotransferase	IFCC recommended method	Beckman Coulter	JCTLM ref lab[b]	2.22	0.73
AST-JSCC		JSCC recommended method	Beckman Coulter	JCCLS CRM-001b	3.40	0.37
ALT	Alanine aminotransferase	IFCC recommended method	Beckman Coulter	JCTLM ref lab[b]	2.88	1.27
ALT-JSCC		JSCC recommended method	Beckman Coulter	JCCLS CRM-001b	3.44	0.44
LD	Lactate dehydrogenase	IFCC recommended method	Beckman Coulter	JCTLM ref lab[b]	1.42	0.29
LD-JSCC		JSCC recommended method	Beckman Coulter	JCCLS CRM-001b	1.93	0.80
ALP	Alkaline phosphatase	IFCC reccommended method	Beckman Coulter	–	1.91	2.60
ALP-JSCC		JSCC recommended method[a]	Beckman Coulter	JCCLS CRM-001b	1.45	0.83
GGT	γ-glutamyltransferase	IFCC recommended method	Beckman Coulter	JCTLM ref lab[b]	2.14	4.63
GGT-JSCC		JSCC recommended method	Beckman Coulter	JCCLS CRM-001b	1.60	1.86
CK	Creatine kinase	IFCC recommended method	Beckman Coulter	JCTLM ref lab[b]	0.86	0.88
CK-JSCC		JSCC recommended method	Beckman Coulter	JCCLS CRM-001b	1.22	0.91
AMY	Amylase	JSCC recommended method	Beckman Coulter	JCCLS CRM-001b	0.89	0.61
IgG	Immunoglobulin G	Turbidimetric method	Nittobo Medical	CRM470	0.63	1.76
IgA	Immunoglobulin A	Turbidimetric method	Nittobo Medical	CRM470	1.76	3.40
IgM	Immunoglobulin M	Turbidimetric method	Nittobo Medical	CRM470	1.09	1.46
C3	Complement component 3	Turbidimetric method	Beckman Coulter	CRM470	0.81	1.77
C4	Complement component 4	Turbidimetric method	Beckman Coulter	CRM470	2.04	2.82
CRP	C-reactive protein	Latex immunoturbidimetric method	Nittobo Medical	CRM470	0.68	2.29
TTR	Transthyretin(prealbumin)	Turbidimetric method	Beckman Coulter	CRM470	1.72	2.98
Tf	Transferrin	Turbidimetric method	Beckman Coulter	CRM470	2.65	3.61
Testo	Testosterone	Chemiluminescent enzyme immunoassay	Beckman Coulter	JCTLM ref lab[c]	2.70	2.38
E2	Estradiol	Chemiluminescent enzyme immunoassay	Beckman Coulter	JCTLM ref lab[c]	7.43	6.40
Prog	Progesterone	Chemiluminescent enzyme immunoassay	Beckman Coulter	JCTLM ref lab[c]	5.98	6.35
Cortisol	Cortisol	Chemiluminescent enzyme immunoassay	Beckman Coulter	JCTLM ref lab[c]	2.17	1.94

[a]Kinetic rate method (using AMP buffer); [b]used for recalibration were pooled sera of three concentrations, which were prepared for each enzyme, and values were assigned by the reference measurement system (RMS); [c]used for recalibration were lyophilized specimens of five to eight concentrations that were value-assigned by RMS; [d]The analyte was measured in specimens collected without addition of sodium fluoride. The result was used as one of the exclusion criteria.

values. Particularly, recalibration of test results was made based on the experimental relation between the assigned values and the test results (measured on three separate days, each in triplicate) obtained at BC's laboratory in Tokyo. When there were more than two levels of reference materials, the regression line to be used for recalibration was computed by use of the reduced major-axis regression method [11, 12]. For reference materials that have just a single level, the ratio of the assigned value to average measured values was used for recalibration.

Statistical analyses

Analyses for sources of variations and partitioning criterion

The test results were evaluated by use of the same statistical procedures employed in the previous study [9]. In brief, sources (factors) of variations were analyzed by three-level nested ANOVA (3N-ANOVA).

The factors considered were sex, region, age, and BMI. Primarily, the first three factors were analyzed; then, after separating data by sex, the analyses using the last three factors were performed. The magnitude of variation due to each factor is expressed as a standard deviation (SD): i.e., between-region SD (SD_{reg}), between-sex SD (SD_{sex}), and between-age SD (SD_{age}). In the analysis, regions were categorized into 14 areas (7 within Japan, 7 outside Japan), age was stratified into four groups (20–29, 30–39, 40–49, and 50–65 years), and BMI was separated into six categories by setting the following boundary values: 18, 20, 22, 24, and 26 kg/m^2.

The relative magnitude of each variable to that of the residual SD representing a net between-individual SD ($SD_{net\text{-}btw\text{-}indiv}$), which roughly corresponds to one quarter of the width of the RI obtained after adjusting for the influence of age and sex, was computed as the SD ratio (SDR) by the following formula:

$$SDR_{factor}=SD_{factor}/SD_{net\text{-}btw\text{-}indiv}$$

An SDR of ≥0.3 was regarded as high, requiring partition of reference values by the factor [13]. Meanwhile, an SDR between 0.25 and 0.29 was considered moderate in degree. In the above computation, when the distribution of the test results was skewed and had a long upper tail, the values were logarithmically transformed before 3N-ANOVA was applied (as indicated in Table 2). In these cases, the back-transformed SD described elsewhere [9] was used in the above equation. In the analysis of regionality, when computing SDR for region (SDR_{reg}), test results obtained within Japan were all combined, as were those from the two cities in Taiwan, and those from Hong Kong and Macau, respectively, as single regions. Meanwhile, test results from Seoul, Beijing, Ho Chi Minh City, Kuala Lumpur, and Jakarta were treated as separate regions; thus, the number of regions served for the analysis of SDR_{reg} was eight in all.

Derivation of reference intervals

As a preliminary step to select appropriate reference individuals, those who had extreme values in analytes related to obesity, anemia, inflammation, and thyroid dysfunction as follows were excluded before the derivation of RIs: Hb ≤96 or ≥180 g/L, MCV ≤70 fL, UA ≤95 or ≥536 μmol/L (≤1.6 or ≥9.0 mg/dL), TG >3.96 mmol/L (350 mg/dL), Glu >6.95 mmol/L (125 mg/dL), TCho >8.04 mmol/L (311 mg/dL), ALT >101 U/L, GGT >140 U/L, AST >80 U/L, CK >800 U/L, CRP >20 mg/L, FT4 >23.17 pmol/L (1.8 ng/dL), and TSH >14 mU/L. As for BMI, 65 (1.8%) individuals exceeded the upper limit of 28 kg/m^2 set at the time of recruitment. In consideration of the imprecision of BMI and the availability of related information indicating obesity, the final exclusion criterion was set as a BMI of ≤14 or ≥30 kg/m^2. Thus, 21 individuals were excluded by these BMI extremes.

The RIs were derived parametrically by use of modified Box-Cox transformation [14], which invariably succeeds in transforming reference values into those of Gaussian distribution as long as there are not many results that are below the detectable limits [13]. As a tertiary exclusion step to further refine the reference values, an iterative approach called the 'latent abnormal values exclusion' (LAVE) method was applied for the actual derivation of the RIs [7, 13, 14]. As exclusion criteria, the RIs of the following 13 analytes were used: Alb, Glb, UA, Glu, AST, ALT, LD, GGT, CK, TG, HDL-C, LDL-C, and CRP. In short, the initial RIs were derived analyte by analyte independently of the results of the others. From the second iteration, the RIs for the above exclusion criteria analytes obtained at the previous cycle of iteration were used to exclude individuals who had abnormal results in analytes other than the one being evaluated. In applying the exclusion criteria, the RIs were extended on both ends by 5% of the interval, or (upper limit–lower limit) ×0.05. Therefore, when the test results are normally distributed, results outside of the mean±2.16 SD (total of 3% on two tails) are regarded as abnormal. This adjustment was made to avoid excluding too many individuals unnecessarily. The computation was continued until the RIs of all the analytes became stable.

Statistical analyses and 3N-ANOVA was performed with general purpose statistical software StatFlex for Windows Ver. 6.0 (Artech, Osaka, Japan). Original software named 'Reference Master' developed by the first author was employed to derive RIs based on the LAVE principle and parametric methods.

Results

Profile of the subjects

The demographic profile of the participants from each city is summarized in Table 3. The tabulation was made after deleting those with extreme values based on the criteria described in the Methods. As a whole, there were more females than males (1959 vs. 1582). The information on ethnicity was not obtained in Japan, Korea, and China with known homogeneity of the target population in the medical facilities involved. The ethnic composition in other countries was as follows: in Kuala Lumpur: Chinese 36%, Malay 45%, Indian 13%; in Ho Chi Minh City: Vietnamese (Kinh) 98%; in Jakarta: Malay 86%, Chinese 13%.

The between-region differences in age, BMI, and levels of smoking and alcohol use were analyzed by one-way ANOVA separately for each sex. The ratios of pure component of between-region SD divided by residual SD representing between-individual SD in males (females) were 0.10 (0.10) for age, 0.21 (0.22) for BMI, 0.18 (0.17) for smoking, and 0.63 (0.55) for alcohol, respectively. The ratio has the same implication as the SDR described above as a means of computing the magnitude of a given variation source. If we set 0.30 as the practically significant effect size of each factor, only the level of alcohol use was apparently different across the Asian cities, reflecting differences in religion and culture.

The job profile of the reference individuals (n=3314) after the selection process is shown in Supplementary Table 1 which accompanies the article at http://www.degruyter.com/view/j/cclm.2013.51.issue-7/issue-files/cclm.2013.51.issue-7.xml. Those individuals who belonged to the medical institutions and those who did not accounted for 83.4% (62.9%–91.5%) and 9.9% (2.7%–27.3%), respectively,

Table 2 The results of three-level nested ANOVA.

Analytes, Units	Trans	Male (M)			Female (F)			Harris-Boyd method[a]			Nested ANOVA[b]		
		n	mean	SD	n	mean	SD	z	k	az=k×z	SDR-sex	SDR-reg (M, F)	SDR-age (M, F)
Alb, g/L		1437	43.2	2.6	1871	41.8	2.5	15.97	0.269	4.3	0.40	0.00 (0.00, 0.00)	0.45 (0.51, 0.42)
Urea, mmol/L		1437	4.8	1.1	1871	4.3	1.1	14.35	0.269	3.9	0.33	0.24 (0.30, 0.18)	0.35 (0.24, 0.42)
UA, µmol/L		1437	349	65	1871	246	50	50.04	0.269	13.5	1.30	0.19 (0.16, 0.23)	0.13 (0.00, 0.19)
CRE, µmol/L	log	1437	77	10	1871	56	7	69.98	0.269	18.9	1.83	0.24 (0.25, 0.22)	0.07 (0.08, 0.00)
Na, mmol/L		1436	142.9	1.5	1871	141.9	1.6	17.78	0.269	4.8	0.44	0.00 (0.08, 0.00)	0.30 (0.10, 0.38)
K, mmol/L		1436	4.21	0.29	1871	4.14	0.29	6.98	0.269	1.9	0.08	0.23 (0.18, 0.27)	0.20 (0.18, 0.22)
Cl, mmol/L		1436	103.9	1.8	1871	104.5	1.7	9.33	0.269	2.5	0.23	0.00 (0.00, 0.00)	0.18 (0.22, 0.12)
Ca, mmol/L		1436	2.36	0.07	1871	2.32	0.07	14.15	0.269	3.8	0.35	0.00 (0.00, 0.00)	0.34 (0.33, 0.34)
TCho, mmol/L		1437	4.94	0.85	1871	4.96	0.89	0.36	0.269	0.1	0.00	0.00 (0.00, 0.00)	0.51 (0.45, 0.55)
TG, mmol/L	log	1437	1.23	0.66	1871	0.85	0.44	18.93	0.269	5.1	0.56	0.27 (0.25, 0.31)	0.36 (0.30, 0.36)
HDL-C, mmol/L		1437	1.38	0.33	1870	1.69	0.39	24.73	0.269	6.7	0.55	0.57 (0.64, 0.56)	0.00 (0.00, 0.00)
LDL-C, mmol/L		1437	3.06	0.76	1871	2.84	0.76	7.88	0.269	2.1	0.16	0.00 (0.00, 0.00)	0.46 (0.37, 0.51)
AST, U/L	log	1437	24.2	6.0	1871	21.2	5.4	15.02	0.269	4.0	0.41	0.12 (0.18, 0.00)	0.33 (0.19, 0.42)
ALT, U/L	log	1437	28.6	12.5	1871	20.1	8.2	22.32	0.269	6.0	0.68	0.12 (0.08, 0.14)	0.30 (0.08, 0.38)
LD, U/L	log	1437	188	27	1871	182	28	6.18	0.269	1.7	0.12	0.08 (0.16, 0.00)	0.34 (0.11, 0.43)
ALP, U/L	log	1437	64	16	1871	55	15	16.48	0.269	4.4	0.43	0.15 (0.21, 0.00)	0.35 (0.07, 0.50)
GGT, U/L	log	1435	36.0	20.6	1857	23.4	12.1	20.71	0.270	5.6	0.69	0.01 (0.10, 0.00)	0.34 (0.36, 0.30)
CK, U/L	log	1437	136	78	1871	83	38	23.81	0.269	6.4	0.82	0.11 (0.14, 0.04)	0.18 (0.00, 0.26)
AMY, U/L	log	1437	81	26	1871	86	27	6.18	0.269	1.7	0.15	0.11 (0.11, 0.12)	0.12 (0.15, 0.00)
IgG, g/L		1437	12.1	2.3	1870	12.9	2.3	11.00	0.269	3.0	0.15	0.38 (0.39, 0.37)	0.06 (0.04, 0.06)
IgA, g/L		1437	2.46	0.72	1871	2.54	0.73	3.15	0.269	0.8	0.00	0.27 (0.15, 0.32)	0.07 (0.11, 0.00)
IgM, g/L	log	1436	1.05	0.40	1871	1.54	0.64	26.48	0.269	7.1	0.71	0.17 (0.30, 0.00)	0.34 (0.26, 0.39)
C3, mg/L		1435	1061	181	1871	1020	180	6.48	0.269	1.7	0.00	0.47 (0.45, 0.56)	0.22 (0.00, 0.00)
C4, mg/L		1436	216	60	1871	207	62	4.49	0.269	1.2	0.00	0.41 (0.36, 0.46)	0.25 (0.17, 0.23)
CRP, mg/L	log	1411	0.97	1.77	1831	0.77	1.56	3.22	0.272	0.9	0.00	0.42 (0.46, 0.56)	0.15 (0.00, 0.07)
TTR, mg/L		1436	311	45	1871	252	39	40.00	0.269	10.8	1.05	0.24 (0.30, 0.19)	0.28 (0.28, 0.26)
Tf, g/L		1436	24	3.3	1871	27	4.1	16.09	0.269	4.3	0.36	0.26 (0.35, 0.21)	0.13 (0.00, 0.16)
Testosterone, nmol/L	log	1436	51.0	14.4	1869	5.8	2.2	118.07	0.269	31.8	7.57	0.00 (0.00, 0.00)	0.59 (0.27, 0.73)
Estradiol, pmol/L	log	1436	26.7	5.5	1869	69.8	61.6	30.14	0.269	8.1	0.89	0.00 (0.23, 0.00)	0.65 (0.05, 0.66)
Progesterone, nmol/L	log	1436	0.60	0.36	1869	3.12	5.06	21.40	0.269	5.8	0.42	0.21 (0.22, 0.00)	0.40 (0.29, 0.39)
Cortisol, nmol/L	log	1436	112	37	1869	98	39	10.83	0.269	2.9	0.24	0.00 (0.24, 0.15)	0.19 (0.11, 0.21)

Trans, transformation. SDR represents the ratio of the standard deviation for a given factor to that for a net between-individual variation. SDR-sex, -reg, and -age denote SDR representing between-sex, -region, and -age differences, respectively.

[a]Harris-Boyd method [15] was used to test for the need of partitioning by sex. The z score representing differences in two means is adjusted to az by multiplying a coefficient (k), which depends on the sample sizes of the two groups. For partitioning of RI, a $z \geq 3.0$ is usually considered as significant. [b]Three-level nested ANOVA was applied in two stages, first for between-sex, -region, and -age differences, and second for between-region, -age, and -BMI after partitioning by sex. SDR ≥0.3 was regarded as a significant between-sex, -city, or -age difference.

Table 3 Demography of the healthy individuals recruited.

Male	N_0	N	Age		BMI		Tobacco		Alcohol	
			Me	95%CI	Me	95%CI	No	Yes	Rare	Yes
North Japan	157	146	38	23–60	22.3	17.7–27.8	97	49	51	95
							(66%)	(34%)	(35%)	(65%)
Tokyo	130	119	36	22–59	22.4	17.3–27.5	87	31	46	72
							(74%)	(26%)	(39%)	(61%)
Central Japan	127	109	41	23–58	22.3	17.6–27.8	81	28	45	64
							(74%)	(26%)	(41%)	(59%)
Osaka	116	110	40	21–61	22.4	17.9–27.8	85	25	33	77
							(77%)	(23%)	(30%)	(70%)
West Japan	185	176	39	23–60	22.0	18.4–26.8	129	46	59	116
							(74%)	(26%)	(34%)	(66%)
South Japan	136	127	36	20–60	22.0	17.7–27.9	76	47	48	76
							(62%)	(38%)	(39%)	(61%)
Okinawa	96	92	35	21–59	22.8	17.9–27.7	69	23	26	66
							(75%)	(25%)	(28%)	(72%)
Seoul	60	56	38	23–58	22.8	19.2–27.5	36	20	24	32
							(64%)	(36%)	(43%)	(57%)
Beijing	59	51	36	23–58	24.4	17.3–28.2	38	13	41	10
							(75%)	(25%)	(80%)	(20%)
Taiwan	145	137	36	22–60	22.8	17.4–27.3	118	19	121	16
							(86%)	(14%)	(88%)	(12%)
Hong Kong	118	105	41	23–59	23.7	18.2–27.5	90	15	90	15
							(86%)	(14%)	(86%)	(14%)
HCMC	131	107	33	22–58	22.1	17.6–27.7	79	28	70	36
							(74%)	(26%)	(66%)	(34%)
Kuala Lumpur	63	52	31	20–60	23.2	17.8–28.4	37	15	49	0
							(71%)	(29%)	(100%)	(0%)
Jakarta	59	51	39	24–63	23.8	18.4–28.7	37	14	51	0
							(73%)	(27%)	(100%)	(0%)
Sum	1582	1438	37	21–59	22.6	17.7–27.8	1059	373	754	675
							(74%)	(26%)	(53%)	(47%)

Female	N_0	N	Age		BMI		Tobacco		Alcohol		Menopause		
			Me	95%CI	Me	95%CI	No	Yes	Rare	Yes	No	Yes	?
North Japan	186	184	37	22–59	19.8	16.4–26.1	160	23	107	77	144	37	3
							(87%)	(13%)	(58%)	(42%)	(80%)	(20%)	
Tokyo	183	177	35	22–58	20.9	17.4–28.1	161	16	109	68	150	23	4
							(91%)	(9%)	(62%)	(38%)	(87%)	(13%)	
Central Japan	188	186	36	22–57	19.8	16.2–24.9	176	9	116	69	150	29	7
							(95%)	(5%)	(63%)	(37%)	(84%)	(16%)	
Osaka	136	133	38	21–60	20.8	16.4–26.7	119	5	84	42	100	24	9
							(96%)	(4%)	(67%)	(33%)	(81%)	(19%)	
West Japan	232	224	37	23–60	19.8	16.6–24.6	217	7	144	79	172	45	7
							(97%)	(3%)	(65%)	(35%)	(79%)	(21%)	
South Japan	113	107	37	20–58	20.1	17–25.9	98	9	78	29	89	16	2
							(92%)	(8%)	(73%)	(27%)	(85%)	(15%)	
Okinawa	97	96	35	21–57	20.7	17.6–26.6	94	1	58	37	86	9	1
							(99%)	(1%)	(61%)	(39%)	(91%)	(9%)	
Seoul	73	71	38	22–61	20.9	17.7–27.6	69	2	54	16	57	11	3
							(97%)	(3%)	(77%)	(23%)	(84%)	(16%)	
Beijing	83	78	39	22–58	22.0	17.6–26.9	76	2	77	1	59	18	1
							(97%)	(3%)	(99%)	(1%)	(77%)	(23%)	
Taiwan	190	175	36	23–59	20.7	16.8–27.3	174	0	174	1	145	30	0
							(100%)	(0%)	(99%)	(1%)	(83%)	(17%)	

(**Table 3** Continued)

Female	N_0	N	Age		BMI		Tobacco		Alcohol		Menopause		
			Me	95%CI	Me	95%CI	No	Yes	Rare	Yes	No	Yes	?
Hong Kong	132	121	39	21–58	21.1	17.5–28	117	4	118	3	106	15	0
							(97%)	(3%)	(98%)	(2%)	(88%)	(12%)	
HCMC	170	166	33	22–53	20.1	17–25.9	165	1	162	3	152	13	1
							(99%)	(1%)	(98%)	(2%)	(92%)	(8%)	
Kuala Lumpur	104	88	31	21–59	21.1	16.8–27.7	87	1	84	1	71	11	6
							(99%)	(1%)	(99%)	(1%)	(87%)	(13%)	
Jakarta	72	70	39	21–60	21.7	17.4–26.9	69	1	70	0	55	15	0
							(99%)	(1%)	(100%)	(0%)	(79%)	(21%)	
Sum	1959	1876	36	21–59	20.4	16.8–26.7	1782	81	1435	426	1536	296	44
							(96%)	(4%)	(77%)	(23%)	(84%)	(16%)	
	3541												

CI, confidence intervals; Me, median; N_0, the number of subjects before the secondary exclusion; N, the number of subjects after the secondary exclusion.

of the reference individuals, whereas the remainder did not provide information on their job.

Standardization of test results

Excellent linearity between the test results and the values assigned to the reference materials with correlation coefficients very close to 1.0 were obtained for all the analytes that were measured in three or more levels (Supplementary Figure 1). The major axis regression was used to compute the regression line for recalibration. The results of reference materials measured at only a single level were used for recalibration by taking their ratio to the assigned values (Supplementary Table 2).

Sources of variations and regionality of results

The result of the 3N-ANOVA for all the analytes are summarized in Table 2. The three factors analyzed were sex, region, and age. Those analytes indicated as 'log' in the 3rd column were transformed logarithmically before applying 3N-ANOVA. The level of SDR for sex (SDR_{sex}) was high (≥0.3) for 19 analytes. In the table, the magnitude of between-sex difference quantified by 3N-ANOVA is compared with that by the Harris-Boyd method [12, 13]. The two approaches agreed quite well in almost all analytes. The SDRs for region (SDR_{reg}) were high in five analytes: HDL-C, CRP, IgG, C3, and C4. SDRs for age (SDR_{age}) were high in 16 analytes.

Additionally, we noted unmatched regionality between the two sexes when we applied the 3N-ANOVA separately for males and females using the entire dataset, with inclusion of BMI as the third factor. The SDR_{reg} computed for six analytes (urea, TG, IgA, IgM, Tf, and TTR) exceeded 0.3 only in either of the sexes (also shown in Supplementary Table 3A). As a whole, SDR_{reg} of 11 analytes exceeded 0.3 in either or both of the sexes. This finding implies the importance of separate analysis for both sexes to clearly identify regionality by use of 3N-ANOVA. When data was limited to those obtained from Japan (Supplementary Table 3B), there were no analytes that showed an SDR of ≥0.3. Examples of regional differences for eight analytes with a high SDR_{reg} score (urea, HDL-C, IgG, IgA, C3, C4, and CRP, and Tf) are illustrated in Figure 1. Similar figures for the entire group of results including the items from questionnaires are also available on-line as Supplementary Figure 2.

Reference intervals

RIs were derived from the reference individuals after the selection process in three ways: male plus female, male only, and female only, and by use of the modified Box-Cox formula. On the basis of the above results on regionality, when SDR_{reg} was ≥0.25, RIs were computed in four ways: all Asians, Japanese, Chinese (Beijing+Taiwan+Hong Kong+Macau), and Southeast Asians (Ho Chi Minh City+Kuala Lumpur+Jakarta); otherwise, RIs were computed from the entire dataset (all Asians), as listed in Table 4.

In the derivation of RIs, we applied the tertiary exclusion procedure based on the LAVE method. Approximately 13% of the original data were excluded (3314→ roughly 2880 individuals) by the iterative optimization process. The effect of this tertiary exclusion was apparent only for analytes

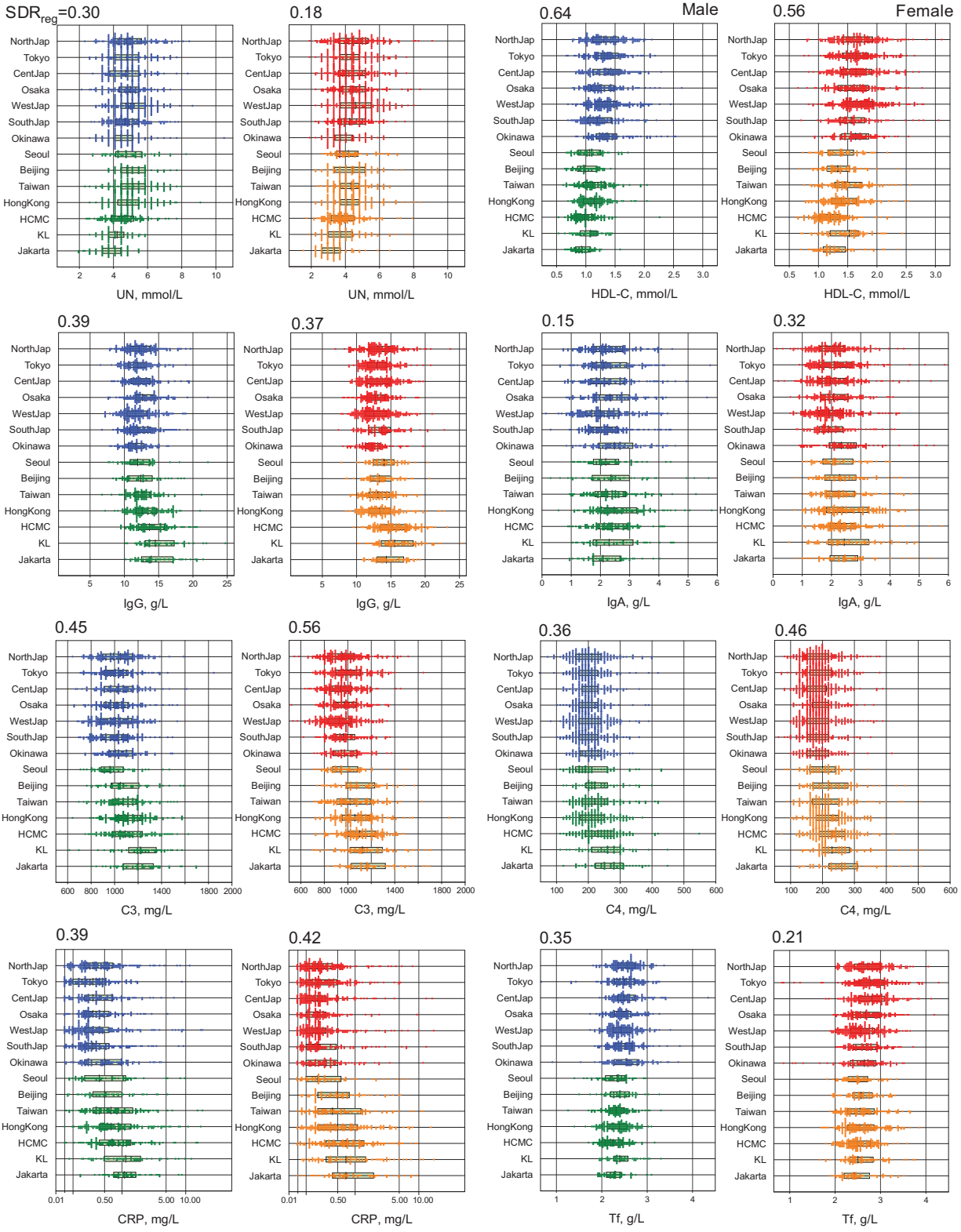

Figure 1 Examples of regional differences in test results observed in eight analytes.
Each panel consists of two figures, one for males (left) and one for females (right). Test results were sub-grouped into 14 areas: seven from Japan (blue, male; red, female) and another seven from East and Southeast Asia (green, male; orange, female). The regions were aligned from north to south. The box within each scattergram represents the central 50% range, and the vertical line in the middle of the box indicates the median. SDR$_{reg}$ displayed on top of each panel represents SD ratio of between-region variations to the pure between-individual variation. SDR>0.3 was considered as practically significant.

Table 4 List of RIs derived.

International Unit	SDR-reg	Area		Male + Female				Male				Female			
Analytes, Units			n	LL	Me	UL	n	LL	Me	UL	n	LL	Me	UL
Alb, g/L	0.00	Asia All	2920	41	45	51	1298	41	46	51	1626	40	45	50
Urea, mmol/L	0.24	Asia All	2888	2.7	4.4	7.1	1277	2.9	4.7	7.3	1608	2.6	4.1	6.8
UA, µmol/L	0.19	Asia All					1293	223	347	471	1629	153	240	344
CRE, µmol/L	0.24	Asia All					1276	61.0	76.7	97.0	1611	41.9	55.8	70.6
Na, mmol/L	0.00	Asia All	2886	139	142	146	1277	140	143	146	1606	139	142	145
K, mmol/L	0.23	Asia All	2872	3.7	4.2	4.7	1270	3.7	4.2	4.7	1604	3.6	4.1	4.7
Cl, mmol/L	0.00	Asia All	2889	101	104	108	1277	100	104	108	1611	101	104	108
Ca, mmol/L	0.00	Asia All	2887	2.19	2.33	2.47	1276	2.21	2.35	2.49	1611	2.18	2.31	2.45
TCho, mmol/L	0.00	Asia All	2889	3.47	4.85	6.73	1278	3.49	4.85	6.74	1611	3.48	4.83	6.80
TG, mmol/L	0.27	Asia All	1551	0.41	0.81	2.20	588	0.49	1.00	2.77	961	0.39	0.73	1.67
		Japanese	1307	0.41	0.73	1.72	474	0.47	0.90	2.18	836	0.39	0.66	1.40
		Chinese	538	0.41	0.84	2.12	216	0.55	1.02	2.80	312	0.43	0.77	1.91
		SE Asia	422	0.47	0.99	3.05	159	0.53	1.24	3.38	252	0.50	0.87	2.50
HDL-C, mmol/L	0.57	Asia All	2915	0.94	1.52	2.43	1283	0.89	1.34	2.11	1628	1.07	1.67	2.55
		Japanese	1748	1.04	1.63	2.54	790	0.99	1.44	2.27	955	1.19	1.78	2.64
		Chinese	596	0.95	1.40	2.25	268	0.85	1.24	1.81	336	1.02	1.56	2.32
		SE Asia	483	0.85	1.28	2.10	197	0.81	1.11	1.61	285	0.98	1.43	2.24
LDL-C, mmol/L	0.00	Asia All	1561	1.65	2.83	4.55	594	1.83	2.94	4.85	969	1.62	2.75	4.46
AST, U/L	0.12	Asia All	1872	14.4	21.0	32.3	803	15.8	22.8	34.8	1064	14.3	20.0	29.4
AST-JSCC, U/L	–	Japanese	1715	15.2	21.3	30.8	769	16.1	22.9	32.9	953	14.7	20.3	28.0
ALT, U/L	0.12	Asia All	1562	11.5	19.5	43.6	591	13.7	23.9	54.0	973	10.6	17.6	31.0
ALT-JSCC, U/L	–	Japanese	1280	10.5	16.8	32.6	467	12.0	20.5	40.7	826	10.2	15.4	26.5
LD, U/L	0.08	Asia All	2924	138	181	235	1291	142	184	240	1634	136	178	233
LD-JSCC, U/L	–	Japanese	1730	124	165	215	783	127	168	220	945	123	162	209
ALP-JSCC, U/L	0.15	Asia All	2873	34	56	90	1272	39	61	96	1604	32	52	84
GGT, U/L	0.01	Asia All	1526	14	21	49	588	15	27	68	959	15	19	43
GGT-JSCC, U/L	–	Japanese	1283	14	20	54	470	15	25	66	819	15	19	44
CK, U/L	0.11	Asia All	2912	43	89	226	1280	58	116	261	1619	40	74	152
CK-JSCC, U/L	–	Japanese	1712	42	86	192	781	60	108	263	938	40	71	135
AMY, U/L	0.11	Asia All	2876	47	80	136	1273	45	77	131	1604	51	82	148
IgG, g/L	0. 38	Asia All	2885	9.2	12.9	18.8	1274	8.8	12.4	18.0	1610	9.5	13.3	19.1
		Japanese	1728	9.0	12.5	17.8	769	9.0	12.1	17.2	948	9.4	12.9	18.3
		Chinese	595	9.8	13.0	18.8	264	9.5	12.6	18.2	332	9.8	13.4	18.6
		SE Asia	478	10.8	14.7	21.7	190	10.8	14.1	21.5	283	11.4	15.1	21.8
IgA, g/L	0. 27	Asia All	1270	1.06	2.22	4.33	561	0.93	2.22	4.19	709	1.02	2.21	4.26
		Japanese	1077	0.94	2.12	3.96	491	0.99	2.23	4.13	583	0.95	2.04	3.80
		Chinese	390	1.02	2.34	4.51	171	1.01	2.34	4.39	219	1.10	2.38	4.59
		SE Asia	354	1.09	2.32	4.29	158	1.24	2.27	4.31	195	1.18	2.40	4.65
IgM, g/L	0.17	Asia All	2869	0.37	1.06	2.38	1267	0.33	0.84	1.78	1603	0.49	1.26	2.65
C3, mg/L	0.47	Asia All	2875	729	1007	1389	1286	757	1036	1427	1586	716	983	1352
		Japanese	1727	715	971	1301	771	738	1000	1345	954	708	946	1261
		Chinese	593	773	1064	1414	264	802	1086	1438	326	786	1040	1431
		SE Asia	470	798	1128	1526	198	819	1158	1569	270	796	1107	1475
C4, mg/L	0.41	Asia All	2857	122	201	340	1271	122	205	339	1601	112	192	339
		Japanese	1725	113	188	308	780	120	198	313	942	109	180	293
		Chinese	598	129	208	383	267	132	214	368	331	123	207	371
		SE Asia	478	132	237	394	196	137	244	408	281	141	232	404
CRP, mg/L	0.42	Asia All	2849	0.01	0.38	2.81	1247	0.04	0.43	3.74	1590	0.01	0.30	2.57
		Japanese	1223	0.06	0.25	2.50	458	0.09	0.28	2.44	820	0.02	0.29	2.59
		Chinese	522	0.11	0.46	5.87	214	0.12	0.57	5.86	308	0.10	0.42	4.74
		SE Asia	417	0.11	0.71	5.71	156	0.12	0.85	4.92	260	0.11	0.61	6.66
TTR, mg/L	0.24	Asia All	2889	193	271	391	1278	230	308	401	1612	186	246	331
Tf, g/L	0. 26	Asia All	2880	1.91	2.52	3.47	1271	1.88	2.42	3.10	1604	2.00	2.62	3.60
		Japanese	1717	2.01	2.58	3.52	775	1.99	2.48	3.18	941	2.04	2.68	3.66
		Chinese	600	1.84	2.45	3.33	267	1.76	2.36	2.95	330	1.97	2.56	3.50

(Table 4 Continued)

International Unit	SDR-reg	Area	Male + Female				Male				Female			
Analytes, Units			n	LL	Me	UL	n	LL	Me	UL	n	LL	Me	UL
		SE Asia	478	1.90	2.41	3.23	195	1.86	2.28	2.94	283	1.99	2.50	3.40
Testo, nmol/L	0.00	Asia All					1269	10.1	17.4	28.4	1609	0.9	2.0	3.5
Estradiol, pmol/L	0.00	Asia All					1266	66	96	140	1609	50	198	840
Progest, nmol/L	0.00	Asia All					1265	0.37	1.69	4.48	1610	0.1	3.7	66.5
Cortisol, nmol/L	0.21	Asia All	2876	45	100	193	1274	51	109	197	1605	41	92	190

known to be related to the metabolic syndrome (TG, AST, ALT, GGT, and CRP) (Supplementary Table 4). For these five analytes, individuals consuming ethanol >40 g/day and those with BMI >26 kg/m^2 were excluded before deriving RIs. In addition, in derivation of RIs for IgA, individuals who reported presence of allergic conditions (allergic rhinitis, atopic dermatitis, etc.) in the questionnaire were excluded before the computation. Therefore, the number of reference individuals for those analytes was smaller.

As supplementary information, RIs were computed in total of seven ways (additionally for Taiwan, Hong Kong/Macau, and Ho Chi Minh City), irrespective of SDR_{reg} values, in both international and conventional units as listed in Supplementary Table 5. Age-related reference intervals for every 10 years of age for each sex were derived both from data of the entire regions and from data limited to Japan, and were provided as Supplementary Table 6A and B.

Discussion

To avoid the potential influence of differences in occupation on test results, we recruited candidate reference individuals mostly from healthy hospital workers (84%), with the remainder from those who worked indoors. This recruitment policy may give an impression of bias in the population. However, there are a variety of jobs in medical facilities, and the level of physical activity differs widely from one individual to another. Therefore, we believe that the target population actually represents a majority of the general population working mostly indoors. However, if we had sampled more broadly, we could not have distinguished true regional differences from those attributable to occupational environment and degree of labor. To assess the practicality of the RIs derived, further investigation may be required in each geographical area to compare the RIs with those derived based on data obtained in a setting of community health screening.

In defining reference individuals, it is also important to clarify a policy regarding how to exclude those with abnormal results due to latent diseases. Although there are many pathological conditions that may affect the test results of each analyte, we assumed that as long as the prevalence was low, inclusion of results from such cases would not have any influence on the determination of RIs targeting the central 95% interval. In contrast, careful consideration must be given to the exclusion of those individuals with latent but highly prevalent disorders like metabolic syndrome and diabetes. For excluding abnormal results, it is not appropriate to apply clinical decision limits (CDL), generally set by consensus among clinical experts as a guide for prevention of those diseases. Such a univariate approach of excluding abnormal values results in truncation of the reference distribution.

To exclude individuals with abnormal results due to latent diseases, we applied a multivariate approach called the LAVE method [7, 9, 13, 14], which is an iterative method for optimized selection of reference individuals. In the initial iteration, it determines RIs analyte by analyte in a univariate manner without consideration of other test results. From the second iteration on, a RI for any given analyte is computed from datasets after excluding the values of individuals who have abnormal results in other analytes. By repeating this process, the RIs are progressively optimized so long as there are associations among the abnormal results. This method applies a principle similar to the one presented by Grossi et al. [16] based on the correlation among variables. In the present study, we used the RIs of 13 'basic' analytes as criteria in judging the need for exclusion: Alb, Glb, UA, Glu, AST, ALT, LD, GGT, CK, TG, HDL-C, LDL-C, and CRP. The LAVE method effectively narrows the RI when there are associations with test results of the 'basic' analytes set for exclusion criteria (see Supplementary Table 4), whereas RIs of analytes that lack such association are not affected. The LAVE method also does not cause any change in RIs of analytes for which abnormal results are rare among healthy individuals,

such as Alb, CRE, urea, and Na. As expected, for the analytes in the lipid profile, the obtained results were different from the CDL, which have a different origin and are usually aimed at a specific group of diseases [17].

The most conspicuous finding of this study was the detection of no regionality in test results for any of the 31 analytes examined within the entire area of Japan. This implies that it is possible to share RIs nationwide in Japan for the standardized analytes that were made traceable to a reference measurement system. In contrast, when all Asian areas were included in the nested ANOVA, we observed apparent regional differences in five analytes: HDL-C, IgG, C3, C4, and CRP. In regard to inflammatory markers, we reproduced and confirmed the findings of the past two Asian studies [7, 9]: the closer the regional area is to the equator, the higher the serum concentrations of positive inflammatory markers (IgG, C3, CRP). This same tendency was also found for C4. It is assumed that exposure to infectious agents is higher in regions closer to the equator.

We were not able to identify the reason for the prominent difference in the level of HDL-C between Japan and the rest of the regions by use of 3N-ANOVA in the second Asian study. However, when we re-analyzed this separately for both sexes, we detected apparent regionality ($SDR_{reg} \geq 0.3$) with the same tendency for both sexes: higher HDL-C levels in two cities in Japan compared with four other cities. However, the regionality detected in electrolytes (Na, K, Cl, and Ca) and LD in the second study was not clearly observed this time, although there was a slight tendency for local differences in K and LD from the graph. The much smaller data size in the second study may have been responsible for the inconsistent results. This result poses a question as to the appropriateness of the cut-off value for SDR set at 0.3. In fact, when the SDR_{reg} was computed after separating the dataset by sex, regionality with a SDR_{reg} of ≥ 0.3 was observed in either or both of the sexes in 11 analytes, an increase of six analytes after the separation. Those analytes showing regionality only in males were urea, IgM, TTR, and Tf, and those with regionality only in females were TG and IgA. Furthermore, when we looked at the graphs (Supplementary Figure 2) showing the regional distributions, some of the test results showed a bias limited to just one or two areas. For example, test results for LD are lower in Kuala Lumpur and Jakarta, and those for ALP are higher in these two cities than elsewhere. Therefore, even when SDR_{reg} is well below 0.3, presence of localized bias in test results cannot be denied completely. We must also note that the number of volunteers recruited within Japan was much larger than that outside Japan, and there was no regionality within Japan. Therefore, this imbalance inevitably caused dilution of the effect of regional differences.

To adjust for regional differences in BMI and smoking and drinking habits, we applied multiple regression analyses and confirmed that none of the regionality observed in test results was influenced by the covariates. These results will be reported in the companion article to be published in this series.

Although we emphasized the presence of regionality in the above discussion, we could derive 'common' RIs valid at least in Southeast Asian countries for the majority of the analytes. As we made the test results traceable to the reference measurement procedures through careful recalibration using certified reference materials, the RIs are in a sense 'universal' as long as the constitution of the target population and the statistical methodologies do not change.

From the standpoint of traceability, we could make a valid comparison of our results with RIs for AST, ALT, and GGT made traceable to a reference measurement system that was recently derived by the IFCC C-RIDL targeting European and Asian population [6].

In conclusion, RIs which can be applicable to a wide geographical area in Asia were established for the majority of analytes with traceability to reference measuring systems, whereas regional partitioning was required for RIs of HDL-C, CRP, IgG, C3, and C4 in both sexes and for urea, TG, IgA, IgM, Tf, and TT in either of the sexes. However, no regional differences were observed in any analyte when data were limited to those from Japan.

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Conflict of interest statement

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