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The Asian project for collaborative derivation of reference intervals: (2) results of non-standardized analytes and transference of reference intervals to the participating laboratories on the basis of cross-comparison of test results

Abstract

Background: The 2009 Asian multicenter study for derivation of reference intervals (RIs) featured: 1) centralized measurements to exclude reagent-dependent variations; 2) inclusion of non-standardized analytes (hormones, tumor makers, etc.) in the target; and 3) cross-check of test results between the central and local laboratories. Transferability of centrally derived RIs for non-standardized analytes based on the cross-check was examined.

Methods: Forty non-standardized analytes were centrally measured in sera from 3541 reference individuals recruited by 63 laboratories. Forty-four laboratories collaborated in the cross-check study by locally measuring aliquots of sera from 9 to 73 volunteers (average 22.2). Linear relationships were obtained by the reduced major-axis regression. Error in converting RIs using the regression line was expressed by the coefficient of variation of slope b [CV(b)]. CV(b) <10% was set as the cut-off value allowing the conversion. The significance of factors for partitioning RIs was determined similarly as in the first report.

Results: Significant sex-, age-, and region-related changes in test results were observed in 17, 15, and 11 of the 40 analytes, respectively. In the cross-comparison study, test results were not harmonized in the majority of immunologically measured analytes, but their average CV(b)s were <10% except for total protein, cystatin C, CA19-9, free thyroxine, and triiodothyronine. After conversion, 74% of centrally derived RIs were transferred to each local laboratory.

Conclusions: Our results point to the feasibility of: 1) harmonizing test results across different laboratories; and 2) sharing centrally derived RIs of non-standardized analytes by means of comparative measurement of a set of commutable specimens.

Keywords: harmonization; immunoassays; multicenter study; standardization.

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Non-standard abbreviations

ADP, adiponectin; AFP, α-fetoprotein; ApoA1, apolipoprotein A-I; ApoB, apolipoprotein B; ApoE, apolipoprotein E; ASO, anti-streptolysin O; BAP, bone alkaline phosphatase; BC, Beckman Coulter; CA15-3, carbohydrate antigen 15-3; CA19-9, carbohydrate antigen 19-9; CA125, carbohydrate antigen 125; CEA, carcinoembryonic antigen; CV(b), coefficient of variation of slope 'b'; CysC, cystatin C; DHEA-S, dehydroepiandrosterone sulfate; EPO, erythropoietin; Fe, iron; Ferritin, ferritin; Folate, folic acid; FSH, follicle-stimulating hormone; FT3, free triiodothyronine; FT4, free thyroxine; IgE, immunoglobulin E; IP, inorganic phosphate; JSCC, Japan Society of Clinical Chemistry; LH, luteinizing hormone; LIP, lipase; LL, lower limit; Lp(a), lipoprotein (a); PG1, pepsinogen I; PG2, pepsinogen II; PRL, prolactin; PSA, prostate-specific antigen; PTH, intact parathyroid hormone; RBP, retinol-binding protein; RI, reference interval; SAA, serum amyloid A; SE, standard error; sTf-R, soluble transferrin receptor; TG, thyroglobulin; TP, protein, total; TRAP-5b, tartrate-resistant acid phosphatase 5b; TSH, thyroid-stimulating hormone; UIBC, unsaturated iron-binding capacity; UL, upper limit; VitB12, vitamin B12.

Introduction

There has been growing demand for the conduction of a multicenter study to derive reference intervals (RIs) of commonly ordered laboratory tests that have been globally standardized. The Asian multicenter study conducted in 2009 was unique in that it also attempted to derive common RIs for non-standardized analytes, such as protein hormones and tumor markers, most of which were measured by immunoassays [1]. The key strategy was to centralize measurement by transporting all serum specimens in a deep-frozen state to central laboratories located in Japan. A single reagent and platform for each of the analytes was used to detect regionality of test results without assay-related variations.

The policy was appropriate for the standardized analytes such as creatinine, uric acid, total cholesterol, and electrolytes. However, for the non-standardized analytes, each RI determined was only relevant to the laboratories that used the same reagents as the central laboratory. Therefore, a scheme was implemented to cross-compare test results between the central and collaborating laboratories. For this purpose, parts of the frozen aliquots of sera remained at each laboratory and were measured locally. We: 1) analyzed sex-, age-, and region-related changes in test results for 40 non-standardized analytes; 2) derived their RIs for the central laboratory; and 3) evaluated the transferability of the RIs to each collaborating laboratory on the basis of cross-check testing.

Materials and methods

Multicenter study conducted in East and Southeast Asian

The framework of the Asian multicenter study was described in the companion paper, Part 1 of this article [1]. In brief, 48 laboratories from 14 cities distributed widely from the north to the south of Japan, and 15 laboratories in nine cities outside Japan (Seoul, Beijing, Taipei, Tainan, Hong Kong, Macau, Ho Chi Minh City, Kuala Lumpur, and Jakarta) took part in the study. A total of 3541 healthy individuals (2082 from Japan and 1459 from outside Japan) between 20 and 64 years of age were recruited using common criteria, and samples of their blood were drawn at basal conditions. After a selection process for excluding those with apparent abnormal results in basic screening test items such as TG, HDL-C, glucose, etc., was performed, test results from a total of 3314 subjects (1438 males, 1876 females) were served for data analyses. Names, abbreviations, assay methods, and imprecision of the analytes for which test results were evaluated in this report are listed in Table 1.

Collection and measurement of specimens for cross-check testing

The standard amount of blood taken from each volunteer was 17 mL (8.5 mL/tube ×2). The tubes, Vacutainer SST (capacity: 8.5 mL) containing a clot-activator, were provided by Becton-Dickinson Corp. (Franklin Lakes, NJ, USA). Sera were separated into CryoTube cryovials

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

Abbr	Analytes	Method	Reagent	Analyzer	Within-day CV, %	Between-day CV, %
TP	Protein, total	Timed endpoint biuret method	BC	(1)	0.76	1.49
CysC	Cystatin C	Latex immunoturbidimetric method	NM	(1)	1.54	2.56
IP	Inorganic phosphate	Timed endpoint molybdate UV method	BC	(1)	1.55	0.00
Fe	Iron	Timed endpoint direct colorimetry	BC	(1)	1.97	1.46
UIBC	Unsaturated iron-binding capacity	Timed endpoint direct colorimetry	BC	(1)	1.74	2.18
Apo A1	Apolioprotein A-I	Turbidimetric method	BC	(1)	1.91	3.72
Apo B	Apolioprotein B	Turbidimetric method	BC	(1)	1.56	2.59
Apo E	Apolioprotein E	Turbidimetric immunoassay	NM	(1)	1.62	2.71
Lp(a)	Lipoprotein (a)	Turbidimetric immunoassay	NM	(1)	1.74	3.03
LIP	Lipase	Enzymatic rate method	BC	(1)	2.36	3.43
IgE	Immunoglobulin E	Chemiluminescent enzyme immunoassay	BC	(2)	2.61	2.17
SAA	Serum amyloid A	Latex immunoturbidimetric method	EM	(1)	1.64	2.63
RBP	Retinol-binding protein	Latex immunoturbidimetric method	NM	(1)	1.78	3.20
ASO	Anti-streptolysin O	Turbidimetric immunoassay	BC	(1)	1.83	3.34
sTf-R	Soluble transferin receptor	Chemiluminescent enzyme immunoassay	BC	(2)	1.30	1.50
Ferritin	Ferritin	Chemiluminescent enzyme immunoassay	BC	(2)	1.79	2.01
EPO	Erythropoiein	Chemiluminescent enzyme immunoassay	BC	(2)	1.74	1.76
VitB12	Vitamin B12	Chemiluminescent enzyme immunoassay	BC	(2)	3.33	2.36
Folate	Folic acid	Chemiluminescent enzyme immunoassay	BC	(2)	1.68	0.77
AFP	α-fetotein	Chemiluminescent enzyme immunoassay	BC	(2)	2.00	1.90
CEA	Carcinoembryonic antigen	Chemiluminescent enzyme immunoassay	BC	(2)	3.03	2.49
CA19-9	Carbohydrate antigen 19-9	Chemiluminescent enzyme immunoassay	BC	(2)	3.44	5.46
CA15-3	Carbohydrate antigen 15-3	Chemiluminescent enzyme immunoassay	BC	(2)	1.94	2.51
CA125	Carbohydrate antigen 125	Chemiluminescent enzyme immunoassay	BC	(2)	2.57	2.16
PSA	Prostate-specific antigen	Chemiluminescent enzyme immunoassay	BC	(2)	2.54	3.03
PG1	Pepsinogen I	Latex immunoturbidimetric method	EM	(3)	1.61	2.70
PG2	Pepsinogen II	Latex immunoturbidimetric method	EM	(3)	1.55	2.58
DHEA-S	Dehydroepiandrosterone sulfate	Chemiluminescent enzyme immunoassay	BC	(2)	1.52	1.46
PRL	Prolactin	Chemiluminescent enzyme immunoassay	BC	(2)	1.16	0.98
LH	Luteinizing hormone	Chemiluminescent enzyme immunoassay	BC	(2)	2.64	1.95
FSH	Follicle-stimulating hormone	Chemiluminescent enzyme immunoassay	BC	(2)	2.68	1.80
TSH	Thyroid-stimulating hormone	Chemiluminescent enzyme immunoassay	BC	(2)	2.23	1.53
FT4	Free thyroxine	Chemiluminescent enzyme immunoassay	BC	(2)	2.07	2.14
FT3	Free triiodothyronine	Chemiluminescent enzyme immunoassay	BC	(2)	1.95	1.05
Tg	Thyroglobulin	Chemiluminescent enzyme immunoassay	BC	(2)	1.95	1.88
Insulin	Insulin	Chemiluminescent enzyme immunoassay	BC	(2)	1.75	2.06
ADP	Adiponectin	Latex immunoturbidimetric method	OP	(3)	1.64	2.76
PTH	Intact parathyroid hormone	Chemiluminescent enzyme immunoassay	BC	(2)	1.92	1.76
BAP	Bone alkaline phosphatase	Chemiluminescent enzyme immunoassay	BC	(2)	1.85	1.98
TRAP-5b	Tartrate-resistant acid phosphatase 5b	Enzyme immunoassay	NM	(4)	1.62	2.71

BC, Beckman Coulter; EM, Eiken Medical; NM, Nitobo Medical; OP, Otsuka Pharma. (1) UniCell DxC, BC, Japan (2) UniCell DxI, BC, Japan (3) JCA-BM 6050, JEOL Ltd, Japan (4) Manual.

(capacity: 4.8 mL) (Nunc, Roskilde, Denmark), and put into deep freezer immediately as described in the first part of this paper.

Among the 63 collaborating laboratories, 44 voluntarily took part in the exercise of cross-check testing for secondary transference of RIs. Those volunteers who agreed to cooperate in the cross-check testing scheme gave one additional tube of blood (8.5 mL). These additional specimens were processed and stored identically with the other specimens to be transported to the central laboratory in Japan. The number of specimens used for the cross-check ranged from 9 to 73 (average 22.2). A total of 65 analytes, including standardized analytes for which RIs and related data analysis were reported [1], were available for the cross-comparison of test results.

The frozen specimens stored at −80°C were thawed and measured identically with specimens for routine assay under well-controlled condition during the period when the collective measurements were being conducted. The test results recorded in an Excel file together with assay methods, reagents, and assay platforms were sent to the Secretariat of this study located at the Ichihara Research Laboratory in the Yamaguchi University Graduate School of Medicine, Ube, Japan.

Quality control of the assays

Quality control (QC) monitoring in the central laboratory was carried out chiefly by use of a set of four fresh-frozen pooled sera from healthy individuals, stored in aliquots at −80°C. Additional seven sets of commercially available QC specimens with artificially adjusted matrix were also measured as a supplemental monitoring procedure (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). Each set of QC specimens consisted of two to three levels (below, within, or above the normal level). Two sets were for chemically measured tests and the other five were for the immunologically measured tests. The same sets of specimens were also distributed to the local laboratories that took part in the cross-check study. The test results of commercial QC sera were reported together with those of specimens for the cross check.

Statistical analysis

Analysis of sources of variation and derivation of RIs were performed in the exact same way as described in Part 1 of this paper [1]. The linear structural relation of test results between the central laboratory and each local laboratory was determined by use of the major axis regression method [2–4], expressed as follows with $\bar{x}$ and $\bar{y}$ and b denoting the averages of x (test result from the central laboratory) and y (corresponding result from a local laboratory), and the slope, respectively.

$$(y-\bar{y})=b\times(x-\bar{x}) \tag{1}$$

The slope b is determined by the following formula:

$$b=\sqrt{\frac{S_{yy}}{S_{xx}}} \tag{2}$$

$$S_{xx}=\sum_{i=1}^{n}(x_i-\bar{x})^2 \qquad S_{yy}=\sum_{i=1}^{n}(y_i-\bar{y})^2$$

where n, S_{xx}, and S_{yy} denote the sample size, and sums of squared-deviations of values from each of the central and local laboratories, respectively.

Using the regression Equation (1), the lower limit (LL) and upper limit (UL) of a RI centrally derived can be converted to those of a local laboratory (LL′ and UL′) by use of the following formulae.

$$\mathrm{LL}'=\bar{y}+b\times(LL-\bar{x})$$
$$\mathrm{UL}'=\bar{y}+b\times(UL-\bar{x})$$

The standard error of the slope b, SE(b), was determined by the bootstrap method by re-sampling the same number of data from the original dataset [5]. Mathematical derivation of SE(b) is known to be difficult but it is said to be approximated by the standard error of the slope b', SE(b′), computed by the ordinary least square method using the following formula (2, 4) (see Appendix 1).

$$y-\bar{y}=b'(x-\bar{x}) \tag{3}$$

$$b'=\frac{Sxy}{Sxx}, \quad S_{xy}=\sum_{i=1}^{n}(x_i-\bar{x})(y_i-\bar{y}) \tag{4}$$

$$SE(b)\cong SE(b')=\sqrt{\frac{(1-r^2)S_{yy}}{(n-2)S_{xx}}}=b\sqrt{\frac{1-r^2}{n-2}} \tag{5}$$

For standardized comparison of the SE(b), it was expressed as the coefficient of variation of slope b [CV(b)] by taking its ratio to 'b' as follows:

$$CV(b)=\frac{SE(b)}{b}\times 100\simeq 100\sqrt{\frac{1-r^2}{n-2}} \tag{6}$$

CV(b) was used as a guide to judge convertibility of the RIs derived from the central laboratory to those of the participating laboratories. Although many factors have to be considered in deciding when to allow the conversion, as explained in the Discussion, we empirically set CV(b)=10% as the cut-off value for the conversion.

Results

Sources of variations of test results

The magnitudes of sex-, age-, and region-specific differences in test results examined by three-level nested ANOVA are as shown in Table 2. We regarded a SD ratio (SDR) of ≥0.3 of any given factor as significant for consideration of partitioning RIs by the factor, as we did in Part 1 of this paper. With regard to between-sex difference, we compared the judgment by SDR for sex [SDR-sex] (third rightmost column in Table 2) with that by the Harris-Boyd method employing the adjusted z score [az] (fourth rightmost column in Table 2), whose critical limit is usually set as 3.0. The two approaches matched quite well. Significant sex-related change was identified by az score and SDR-sex in 20 and 17 of 40 analytes, respectively, including prominent examples of RBP, ferritin, and PSA. Similarly, significant between-region differences were found by SDR for between-region (SDR-reg) in nine analytes, including folate, CA15-3, adiponectin, and PTH as notable examples (Figure 1). Similar figures are provided for all the analytes in Supplemental Figure 1. Age-related changes were noted in 15 analytes including ApoB, DHEA-S, LH, and FSH. As the magnitude of between-region and -age differences often differ between the two sexes, we performed three-level nested ANOVA after separating the dataset by sex and introducing BMI as a third factor for the analysis (Supplementary Table 2). The two values shown within the parenthesis in the last two columns in Table 2 represent corresponding SDR-reg and -age values for males and females, respectively. We observed unmatched region- and age-related changes between the two sexes in three analytes (CysC, ferritin, and TRAP-5b) and seven

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)
TP	g/L		1437	71.4	3.9	1871	71.4	4.1	0.22	0.269	0.1	0.00	0.36 (0.31, 0.39)	0.23 (0.22, 0.23)
CysC	mg/L		1424	0.94	0.13	1846	0.81	0.12	27.55	0.271	7.5	0.72	0.28 (0.33, 0.20)	0.33 (0.28, 0.37)
IP	mmol/L		1437	1.18	0.16	1871	1.26	0.15	14.31	0.269	3.9	0.36	0.00 (0.00, 0.00)	0.34 (0.35, 0.32)
Fe	µmol/L		1437	19.1	6.9	1871	15.9	7.2	12.83	0.269	3.5	0.31	0.06 (0.00, 0.07)	0.03 (0.00, 0.06)
UIBC	µmol/L		1437	37.1	9.8	1871	45.1	12.7	20.41	0.269	5.5	0.49	0.14 (0.21, 0.07)	0.16 (0.02, 0.20)
ApoA1	mg/L		1436	1404	205	1866	1544	225	18.59	0.270	5.0	0.40	0.45 (0.55, 0.41)	0.20 (0.17, 0.13)
ApoB	mg/L		1436	931	215	1868	849	202	11.26	0.270	3.0	0.26	0.00 (0.00, 0.00)	0.58 (0.51, 0.63)
ApoE	mg/L	log	1436	41.1	10.5	1871	43.7	11.4	6.96	0.269	1.9	0.16	0.07 (0.08, 0.06)	0.20 (0.21, 0.19)
LP(a)	mg/L	log	1436	152	169	1871	166	170	2.36	0.269	0.6	0.05	0.10 (0.14, 0.11)	0.11 (0.11, 0.14)
LIP	U/L	log	1437	25.4	7.6	1871	26.2	7.6	3.30	0.269	0.9	0.00	0.11 (0.08, 0.15)	0.24 (0.31, 0.11)
IgE	$\times 10^3$ IU/L		1436	204	397	1868	158	339	3.53	0.270	1.0	0.13	0.21 (0.17, 0.14)	0.19 (0.15, 0.12)
SAA	mg/L	log	1424	7.2	16.2	1850	6.9	17.0	0.59	0.271	0.2	0.00	0.31 (0.27, 0.34)	0.04 (0.00, 0.04)
RBP	mg/L		1436	34.3	7.5	1871	26.0	6.2	33.86	0.269	9.1	0.94	0.16 (0.25, 0.00)	0.49 (0.47, 0.50)
ASO	IU/mL		1435	66.7	85.8	1870	61.6	75.5	1.78	0.269	0.5	0.00	0.30 (0.25, 0.33)	0.23 (0.28, 0.17)
sTf-R	nmol/L	log	1436	15.7	4.4	1869	17.2	6.9	7.40	0.269	2.0	0.08	0.20 (0.32, 0.11)	0.11 (0.06, 0.11)
Ferritin	µg/L	log	1436	135	97	1869	37	39	36.37	0.269	9.8	1.38	0.29 (0.44, 0.21)	0.38 (0.17, 0.44)
EPO	IU/L	log	1436	8.4	3.8	1869	11.1	8.6	12.07	0.269	3.3	0.27	0.13 (0.07, 0.15)	0.15 (0.17, 0.15)
VitB12	ng/L	log	1436	165	60	1868	191	69	11.29	0.270	3.0	0.26	0.18 (0.20, 0.17)	0.21 (0.18, 0.23)
Folate	µg/L	log	1434	15.0	7.1	1867	19.3	9.8	14.63	0.270	3.9	0.08	0.68 (0.71, 0.66)	0.26 (0.24, 0.27)
AFP	µg/L	log	1436	3.06	1.70	1869	2.88	1.69	3.09	0.269	0.8	0.00	0.00 (0.00, 0.09)	0.43 (0.41, 0.44)
CEA	µg/L	log	1436	1.85	1.24	1869	1.42	2.95	5.63	0.269	1.5	0.32	0.10 (0.18, 0.00)	0.23 (0.18, 0.26)
CA19-9	$\times 10^3$ IU/L	log	1436	7.2	7.6	1869	10.0	9.1	9.46	0.269	2.6	0.18	0.14 (0.21, 0.05)	0.05 (0.05, 0.00)
CA125	$\times 10^3$ IU/L	log	1436	8.3	3.8	1869	16.1	19.7	16.92	0.269	4.6	0.65	0.00 (0.08, 0.00)	0.37 (0.09, 0.42)
CA15-3	$\times 10^3$ IU/L	log	1436	8.1	3.6	1869	7.3	3.3	6.70	0.269	1.8	0.00	0.35 (0.38, 0.32)	0.09 (0.09, 0.07)
PSA	µg/L	log	1436	1.09	0.92	1869	0.01	0.08	44.40	0.269	12.0	17.29	0.24 (0.00, 0.08)	0.10 (0.08, 0.07)
PG1	µg/L	log	1424	50.2	19.6	1850	43.3	20.6	9.71	0.271	2.6	0.29	0.06 (0.12, 0.00)	0.36 (0.29, 0.36)
PG2	µg/L	log	1424	11.0	7.7	1850	10.5	9.2	1.67	0.271	0.5	0.00	0.00 (0.08, 0.00)	0.47 (0.42, 0.50)
DHEA-S	mmol/L		1436	2385	948	1869	1535	804	27.23	0.269	7.3	0.77	0.00 (0.00, 0.00)	0.66 (0.54, 0.80)
PRL	µg/L	log	1436	9.73	7.03	1869	14.00	10.23	14.21	0.269	3.8	0.43	0.04 (0.21, 0.00)	0.38 (0.21, 0.45)
LH	IU/L	log	1436	3.50	2.22	1869	13.30	15.03	27.81	0.269	7.5	0.76	0.00 (0.30, 0.00)	0.63 (0.16, 0.67)
FSH	IU/L	log	1436	6.35	5.30	1869	23.15	34.28	20.86	0.269	5.6	0.55	0.00 (0.00, 0.00)	1.28 (0.57, 1.48)
TSH	mU/L	log	1436	1.61	1.00	1868	1.72	1.05	3.17	0.270	0.9	0.00	0.16 (0.19, 0.15)	0.08 (0.05, 0.11)
FT4	pmol/L		1436	12.0	1.5	1869	11.5	1.4	9.99	0.269	2.7	0.23	0.14 (0.06, 0.19)	0.19 (0.21, 0.12)
FT3	pmol/L		1436	4.77	0.42	1869	4.47	0.41	20.79	0.269	5.6	0.52	0.00 (0.00, 0.08)	0.23 (0.29, 0.15)
Tg	µg/L	log	1436	11.0	10.7	1868	12.8	18.5	3.52	0.270	0.9	0.00	0.14 (0.14, 0.14)	0.06 (0.00, 0.04)
Insulin	mIU/L	log	1433	6.1	6.0	1867	5.4	4.3	3.83	0.270	1.0	0.00	0.24 (0.21, 0.28)	0.15 (0.00, 0.00)
Adiponectin	mg/L	log	1424	8.0	3.5	1850	12.1	5.2	27.08	0.271	7.3	0.65	0.36 (0.32, 0.38)	0.15 (0.00, 0.00)

(**Table 2** Continued)

Analytes	Units	Trans	Male (M)			Female (F)			Harris-Boyd method[a]			Nested ANOVA[b]		
			n	Mean	SD	n	Mean	SD	z	k	az=kxz	SDR-sex	SDR-reg (M, F)	SDR-age (M, F)
PTH	ng/L	log	1436	47.2	18.6	1869	49.3	20.7	2.97	0.269	0.8	0.00	0.41 (0.35, 0.45)	0.24 (0.22, 0.22)
BoneALP	µg/L	log	1436	13.4	4.3	1869	11.2	3.7	14.89	0.269	4.0	0.41	0.00 (0.00, 0.00)	0.38 (0.19, 0.48)
TRAP-5b	U/L	log	1411	2.09	0.79	1831	1.81	0.85	9.65	0.272	2.6	0.23	0.30 (0.43, 0.17)	0.46 (0.11, 0.59)

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 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, az≥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, -region or -age difference.

analytes (LIP, CA125, PRL, LH, FSH, BoneALP, and TRAP-5b), respectively.

Reference intervals

The RIs derived by the same procedures as described in Part 1 of this paper are listed in Table 3, and in Supplementary Table 3 for full results for region-specific RIs. The age-specific RIs derived for each sex and decade of age are listed in Supplementary Table 4.

Assay reagents or platforms involved in the cross-check testing

In this report, those laboratories using <15 specimens for the comparison were omitted due to lack of adequate statistical power in the evaluation. Thus, the actual datasets used for the evaluation were reduced to those from 37 of the original 44 laboratories. Furthermore, those analytes with participation of less than eight laboratories for cross-check testing were also omitted, thus reducing the number of analytes actually evaluated to 22 from the original 34.

The combinations of reagents and assay platforms actually used for the measurements by local laboratories were so divergent that we did not list them here. However, those analytes measured by immunoassays could be subgrouped by manufacturers of assay reagents/platforms into a small number of categories as listed in Table 4 with Roche, Abbott, and Tosoh accounting for the majority of the immunoassays.

Typical two-dimensional scattergrams for the cross-check testing

Typical cross-check results for four non-standardized analytes (AFP, CA19-9, PSA, and TSH) are illustrated in Figure 2. The x-axis represents test results by the central laboratory, and the y-axis represents results obtained at three local laboratories (Laboratories 1104, 1501, and 4103). These results were chosen to demonstrate representative results, and their reagents and assay platforms differed from those of the central laboratory. The comparisons of the values were made based on conventional units because the figures are just meant to demonstrate comparability of the values.

CV(b)s shown in the left top corner of the graph inversely correlated with the correlation coefficients shown in the right bottom. However, it should be noted

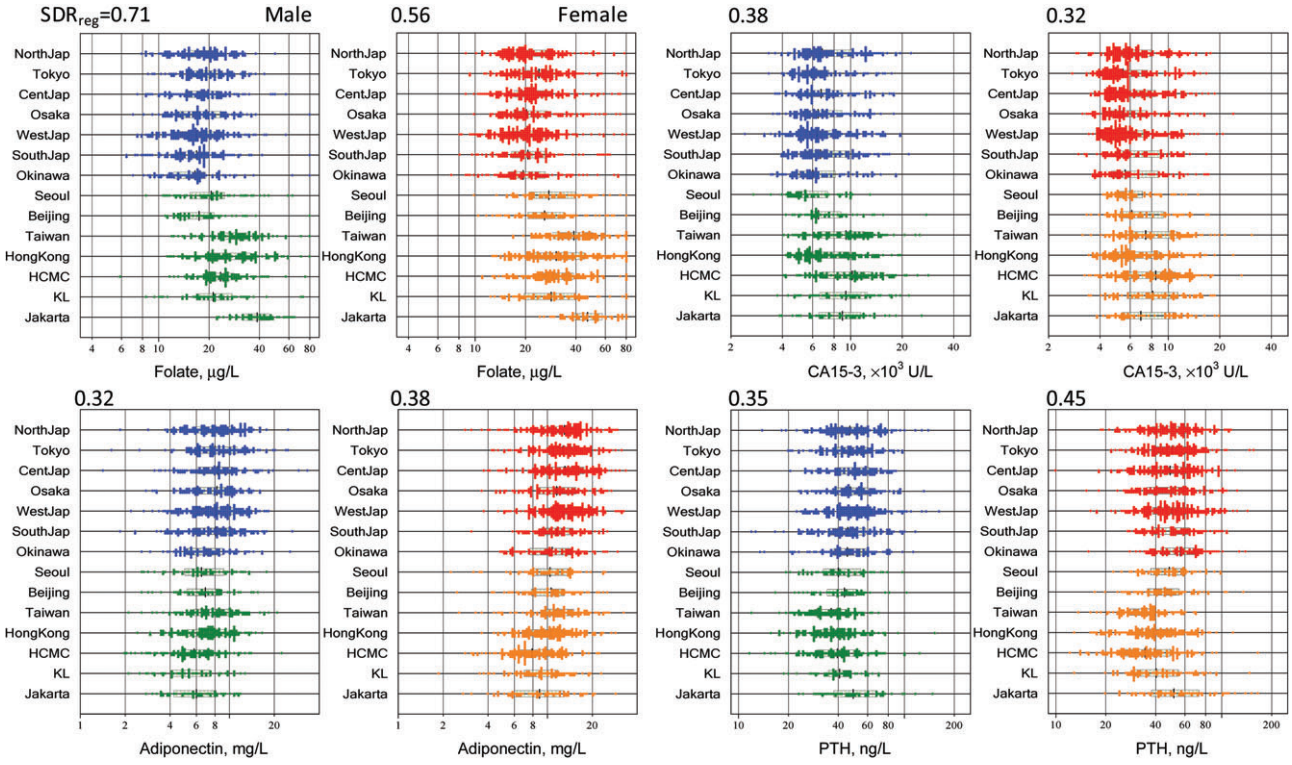

Figure 1 Regionality of test results observed in four analytes.
Each panel consists of two figures, one for males and one for females. Test results were sub-grouped into 14 areas: seven from Japan (blue, male; red, female) and another seven from East and Southeast Asia (green, male; orange, female). The regions were aligned from the north to the south. The box within the scattergrams represents the central 50% range, and the vertical line in the middle of the box indicates the median.

that CV(b) decreases proportionately with the square root of the sample size used for the linear regression as expected from Equation (6). As described below, the cross-comparison for AFP, PSA, and TSH generally resulted in close correlations, although regression lines mostly did not match with the line of concordance (y=x). However, the graphs for CA19-9 showed a large scatter around regression lines with larger CV(b)s.

The CV(b) list

The list of CV(b) computed from the regression line for the cross-check between the central laboratory and each local laboratory is shown in Table 5. The first column represents the sequence number of the local laboratories listed. The second column represents the number of samples actually measured by each local laboratory. Cells are colored according to the magnitude of CV(b). The average CV(b) and the percentage of laboratories that received the transference service of RIs with CV(b) <10% are shown in the two bottom rows. A high rate of transferability of RI (>80%) was attained among the participating laboratories in the vast majority of analytes. Notable exceptions were those for TP, CysC, CA19-9, FT4, and FT3, which showed <80% transferability. The list of CV(b) for the standardized analytes are shown in Supplementary Table 5. Their CV(b) were generally low except for Alb, Na, K, Cl, and Ca, with a CV(b) exceeding 10% in most of the cases.

Commutability of commercial QC specimens in the cross-comparison

We had expected that commercial QC specimens would give much the same results in the cross-comparison of test results between the central and local laboratories. However, prominent differences were observed with the use of the QC specimens in test results for chloride, inorganic phosphate, HDL-C, LDL-C, amylase, and, partly, AST, ALP, sodium, potassium, and total protein. Figure 3 shows typical results of the cross-comparison plotted for two datasets: one for individual sera (black dots), as in

Table 3 List of RIs derived.

International unit					Male + Female				Male				Female			
Analytes	**Units**	**SDR-reg**	**Area**	**n**	**LL**	**Me**	**UL**	**n**	**LL**	**Me**	**UL**	**n**	**LL**	**Me**	**UL**
TP	g/L	0.36	Asia All	2891	64	71	79	1279	64	71	79	1611	64	71	79
			Japanese	1732	64	71	78	784	64	71	78	948	63	71	78
			Chinese	601	64	71	79	266	65	71	78	334	64	71	80
			SE Asia	479	66	74	82	196	66	74	82	283	66	74	82
CysC	mg/L	0.28	Asia All	2884	0.63	0.85	1.16	1274	0.71	0.92	1.21	1605	0.61	0.80	1.05
			Japanese	1725	0.62	0.83	1.12	782	0.69	0.90	1.17	944	0.61	0.78	1.03
			Chinese	600	0.65	0.87	1.22	267	0.74	0.95	1.31	331	0.62	0.80	1.05
			SE Asia	477	0.65	0.90	1.21	196	0.79	0.98	1.29	282	0.63	0.84	1.10
IP	mmol/L	0.00	Asia All	2889	0.93	1.22	1.54	1278	0.88	1.18	1.50	1610	0.98	1.26	1.55
Fe	mmol/L	0.06	Asia All	2888	5	17	33	1271	8	18	34	1609	4	15	32
UIBC	mmol/L	0.14	Asia All	2889	21	40	69	1276	19	37	55	1612	24	44	75
ApoA1	mg/L	0.45	Asia All	2883	1112	1462	1967	1275	1072	1383	1839	1606	1173	1531	2000
			Japanese	1724	1175	1513	2017	778	1144	1442	1913	943	1240	1574	2052
			Chinese	598	1089	1399	1878	265	1036	1323	1628	333	1150	1484	1968
			SE Asia	471	1078	1361	1864	195	1012	1266	1585	283	1108	1435	1922
ApoB	mg/L	0.00	Asia All	2884	541	849	1329	1278	570	908	1371	1608	522	810	1256
ApoE	mg/L	0.07	Asia All	2872	25	41	64	1275	25	39	62	1602	26	42	66
LP(a)	mg/L	0.10	Asia All	2891	2	99	689	1278	1	90	692	1612	3	108	648
LIP	U/L	0.11	Asia All	2875	13	24	39	1270	13	23	41	1595	14	25	39
IgE	$\times 10^3$ IU/L	0.21	Asia All	1259	2	42	528	559	3	61	633	700	2	33	390
SAA	mg/L	0.31	Asia All	2846	1.0	3.8	20.4	1257	1.1	3.7	23.5	1589	1.0	3.9	18.3
			Japanese	1698	0.9	3.2	16.4	767	0.9	3.3	16.4	930	1.0	3.2	15.9
			Chinese	595	1.4	4.8	22.9	265	1.3	4.7	27.8	331	1.4	4.9	21.3
			SE Asia	477	1.4	5.7	24.7	193	1.4	5.5	22.3	281	1.6	5.7	25.2
RBP	mg/L	0.16	Asia All	2887	17.4	28.2	47.5	1273	21.9	33.4	49.7	1604	17.3	24.5	40.3
ASO	$\times 10^3$ IU/L	0.30	Asia All	2879	2	37	293	1272	3	37	315	1611	1	39	248
			Japanese	1730	1	38	292	778	2	37	343	946	2	36	295
			Chinese	592	1	27	159	264	2	29	193	334	1	26	163
			SE Asia	477	10	60	332	195	8	61	316	281	11	63	308
sTf-R	nmol/L	0.20	Asia All	2853	10.2	15.0	28.3	1262	10.4	14.9	23.9	1574	10.7	15.5	34.4
Ferritin	µg/L	0.29	Asia All					1266	22	115	348	1606	3	23	124
			Japanese					764	24	102	268	949	3	18	121
			Chinese					265	28	152	442	332	3	28	158
			SE Asia					191	39	162	499	282	5	38	159
EPO	IU/L	0.13	Asia All	2859	4.0	8.4	22.1	1269	3.8	7.7	15.8	1586	3.9	9.1	25.4
VitB12	ng/L	0.18	Asia All	2869	135	347	779	1270	123	312	705	1599	152	377	821
Folate	µg/L	0.68	Asia All	2840	10.1	21.8	56.4	1262	9.1	19.5	44.6	1591	11.5	24.0	67.2
			Japanese	1701	9.4	19.2	40.0	777	8.7	17.4	34.7	930	10.8	20.8	46.0
			Chinese	599	12.6	28.3	72.8	267	11.8	24.6	58.4	332	14.1	31.6	82.2
			SE Asia	477	13.3	29.7	72.5	195	12.0	25.5	61.1	282	14.5	33.5	74.2
AFP	µg/L	0.00	Asia All	2859	1.1	2.5	6.5	1271	1.2	2.7	6.8	1590	1.0	2.4	6.4
CEA	µg/L	0.10	Asia All	2880	0.4	1.3	4.1	1264	0.4	1.6	4.4	1606	0.4	1.1	3.4
CA19-9	$\times 10^3$ IU/L	0.14	Asia All	2887	0.8	6.4	30.0	1276	0.8	5.2	24.5	1608	0.9	7.6	33.3
CA125	$\times 10^3$ IU/L	0.00	Asia All					1267	3.2	7.5	16.2	1588	4.2	11.9	42.4
CA15-3	$\times 10^3$ IU/L	0.35	Asia All	2853	4.0	6.5	19.2	1276	4.0	7.0	18.8	1583	3.9	6.1	19.3
			Japanese	1726	3.8	6.1	17.4	775	4.1	6.7	17.8	942	3.8	5.7	16.4
			Chinese	592	4.2	6.9	20.4	266	4.5	7.3	19.6	331	3.9	6.5	20.0
			SE Asia	478	4.0	8.3	19.9	196	4.4	9.2	21.3	284	3.8	7.6	20.4
PSA	µg/L	0.04	Asia All					1271	0.32	0.88	2.77	1596	0.000	0.005	0.038
PG1	µg/L	0.06	Asia All	2834	22	42	85	1255	25	46	95	1584	22	39	81
PG2	µg/L	0.00	Asia All	2842	4	8	29	1248	4	9	26	1585	4	8	30
PG1/PG2		0.00	Asia All	2850	2.1	5.1	7.9	1260	2.0	5.3	8.1	1590	1.9	5.0	7.6
DHEA-S	mmol/L	0.00	Asia All					1273	2.4	6.1	12.1	1602	1.0	3.9	9.1
PRL	µg/L	0.04	Asia All	2862	4	10	29	1272	4	9	21	1591	5	12	33
LH	IU/L	0.00	Asia All					1272	1	3	7	1588	1	7	71

(**Table 3** Continued)

International unit				n	Male + Female			n	Male			n	Female		
Analytes	**Units**	**SDR-reg**	**Area**		**LL**	**Me**	**UL**		**LL**	**Me**	**UL**		**LL**	**Me**	**UL**
FSH	IU/L	0.00	Asia All					1263	2	5	14	1597	2	8	173
TSH	mU/L	0.16	Asia All	2872	0.4	1.4	4.0	1263	0.4	1.4	3.8	1599	0.4	1.5	3.9
FT4	pmol/L	0.04	Asia All	2879	9.2	11.7	14.6	1273	9.4	11.9	14.9	1607	9.1	11.5	14.2
FT3	pmol/L	0.00	Asia All	2872	38.6	4.56	5.50	1272	4.05	4.73	5.90	1596	3.80	4.43	5.31
Tg	µg/L	0.14	Asia All	2869	1	9	30	1248	2	9	30	1597	1	9	32
Insulin	mIU/L	0.24	Asia All	2858	1.8	4.6	11.8	1239	2.1	4.8	13.5	1599	1.9	4.4	10.8
Adiponectin	mg/L	0.36	Asia All	2861	3.7	9.5	22.8	1254	3.4	7.5	16.2	1590	4.7	11.5	24.0
			Japanese	1296	4.2	10.9	23.6	467	3.7	8.3	17.2	824	5.7	12.7	25.6
			Chinese	527	3.7	9.5	19.4	216	3.3	7.6	14.5	311	5.5	11.2	22.2
			SE Asia	414	3.0	7.5	16.3	159	2.6	5.9	12.4	258	3.8	8.6	18.8
PTH	ng/L	0.41	Asia All	2866	21	45	92	1271	21	44	89	1598	21	45	97
			Japanese	1720	23	48	96	779	23	48	93	943	24	49	101
			Chinese	594	18	38	70	265	19	37	68	329	18	38	73
			SE Asia	475	17	40	94	193	20	42	83	280	16	39	98
BoneALP	µg/L	0.00	Asia All	2869	6.4	11.4	20.6	1267	7.5	12.5	22.5	1601	6.2	10.5	19.1
TRAP-5b	U/L	0.30	Asia All	2814	0.8	1.8	3.7	1247	0.9	2.0	3.8	1563	0.7	1.6	3.5
			Japanese	1676	0.8	1.9	4.0	764	1.0	2.1	4.2	915	0.8	1.7	3.8
			Chinese	585	0.7	1.5	3.1	263	0.8	1.7	3.2	325	0.6	1.4	3.1
			SE Asia	471	0.7	1.7	3.7	193	1.1	2.0	3.8	277	0.7	1.5	3.6

When the standard deviation ratio due to region (SDR-reg) is 0.25, the RIs are computed in four ways: from the entire data (Asia All), Japanese, Chinese, and Southeast Asian (including Ho Chi Minh City, Kuala Lumpur, and Jakarta) (SE Asia).

Table 4 Reagent manufacturers involved in the cross-comparison of results measured by immunoassays.

	Roche	Abbott	Tosoh	Siemens	BC	Fuji	Misc
Ferritin	6	8	2	1	1	1	5
AFP	10	9	2	3	2	3	2
CEA	10	9	2	3	2	3	2
CA19-9	11	8	1	1	1	3	2
CA125	12	9	1	1	0	3	2
PSA	11	10	1	0	2	1	2
Estradiol	7	5	2	2	0	0	1
Progesterone	3	3	1	1	0	0	0
Testosterone	3	2	2	2	0	0	0
PRL	5	7	3	2	2	0	1
FSH	5	8	3	2	2	0	1
LH	5	8	3	2	2	0	1
TSH	11	12	2	4	0	0	1
FT4	11	12	2	4	0	0	1
FT3	9	11	2	3	0	0	1
Insulin	6	6	6	2	1	2	1
Cortisol	3	0	3	1	3	0	2
PTH	7	0	1	3	0	0	0
	135	127	39	37	18	16	25

Abbott, Abbott Diagnostics; BC, Beckman Coulter; Fuji, Fujirebio Diagnostics; Misc, miscellaneous; Roche, Roche Diagnostics; Siemens, Siemens Healthcare Diagnostics; Tosoh, Tosoh Bioscience.

those of Figure 2, and the other for the commercial QC specimens (red circle or cross). These results clearly indicate that commutable specimens such as freshly prepared sera from healthy volunteers are essential to make cross-comparisons of test results aimed at transferring RIs by the regression procedure.

Discussion

A multicenter study for derivation of RIs can be conducted by either of two schemes. One is by individual measurement by collaborating laboratories and integration of test results to derive common RIs. The other is by centralized measurement and derivation of RIs, followed by transference of RIs to the collaborating laboratories with or without cross-comparison of test results. The former scheme can be applied to a study that only targets standardized analytes. However, when non-standardized analytes are included, it is essential to apply the latter scheme. The present study is the first multicenter study of reference values to include non-standardized analytes measured by immunoassays. There was, however, some

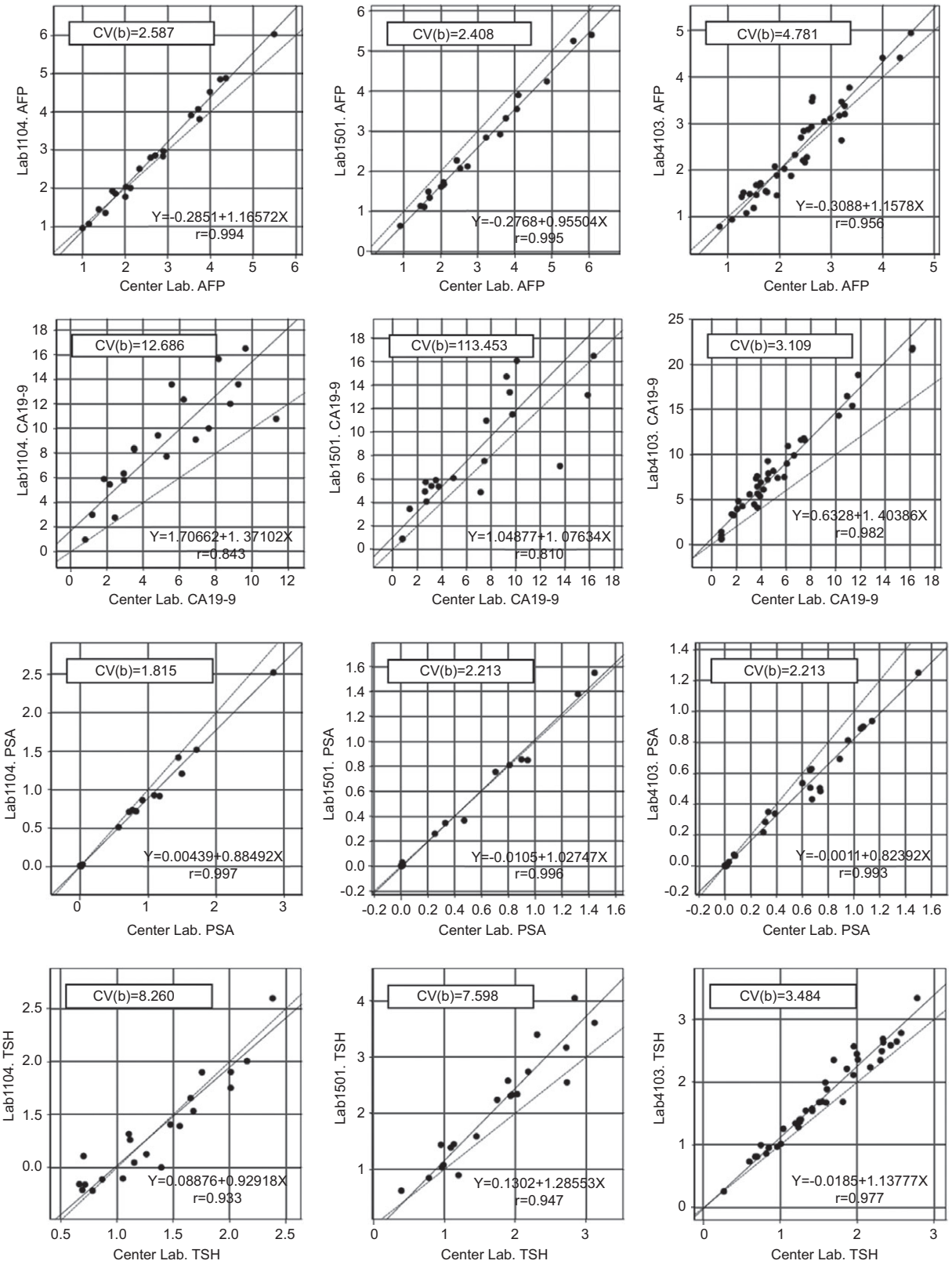

Figure 2 Cross-comparison of test results between the central and three local laboratories.
Typical results of cross-comparison of four non-standardized analytes (AFP, CA19-9, PSA, and TSH). The x-axis represents test results by the central laboratory, and the y-axis represents results obtained at the three local laboratories.The comparison was made in conventional unit.

Table 5 CV(b) tabulated by analyte name and laboratory ID.

Lab ID	n	TP	IP	Fe	UIBC	LIP	IgE	ASO	CysC	Ferritin	AFP	CEA	CA19-9	CA125	PSA	PRL	FSH	LH	TSH	FT4	FT3	Insulin	PTH
1	25	14.0	5.6	2.1	2.8				8.1	3.1	4.2	4.9	16.4	4.4					3.3	12.6	23.4	5.5	
2	72	10.8	3.2	1.1	2.3		1.3	3.6	10.2		4.7	6.6	10.1	6.2	1.0	2.4	1.1	2.4	4.2	29.5	14.8	3.4	
3	22	12.9		1.8																			
4	22	4.4	2.0	0.8		4.4		2.4															
5	17	12.4	4.2	2.4		7.9	3.5	2.5		1.7	2.4	6.7	16.6	4.1	2.2	3.7	2.5	2.6	8.0	23.9	18.0	10.6	
6	38	10.4	2.9	1.1	2.6	11.3	3.1	2.1		1.4	4.6	3.7			0.9	2.8	3.6	4.0	4.9	15.5	17.5	3.3	
7	44	10.8	2.2	1.2		4.7	0.8			2.3	4.7	6.7	7.1	8.9	0.7	1.9	3.1	4.5	4.3	13.4	16.8	5.4	3.0
8	32	6.5	2.6	0.7																			
9	15	9.5	3.6	0.9	2.3		2.6	3.1		1.8	2.2	11.1	8.0	7.0	0.6	6.4	3.1	2.7	2.4	16.2	26.0	7.0	7.7
10	31	17.9	3.8	2.5	4.6	8.0			12.2	2.2	7.3	23.6	16.2	4.5	1.1	1.7	1.7	2.2	3.3	13.5	17.5	6.9	4.1
11	21	7.4	4.8	1.3		3.6		2.6			4.5	5.2	7.6	12.8	1.4				8.3	27.0	13.9	3.2	
12	27	8.9	1.6	1.8	3.0					2.2	4.6	11.7	8.3						11.0	14.5	24.6	5.3	
13	29	8.3	3.4	1.3		11.2		6.0		2.1	3.7	8.4	42.4	10.0	1.9				3.7	21.5	41.3	10.4	
14	24	8.7	2.1	0.8	1.6	3.7	1.9		7.7	1.1	3.2	5.3	9.1	7.1	0.2	2.2	2.8	2.4	7.4	26.2	19.6	8.1	
15	21	11.1	3.0	1.6	1.6				11.5	1.1	4.1	9.2	18.6	4.5					8.4	28.6	22.0		
16	20	7.6	5.3	2.3	3.6					1.6	3.5	8.6	10.8	7.4	2.5		2.4	1.9	4.1	13.3	16.6	7.4	
17	20	7.3	3.3	1.2			2.5			2.3	4.9	14.5	12.0	13.3	1.6	1.9	2.5	6.8	4.2	30.5	65.7	7.1	
18	16	14.9	8.4	1.2	5.0		3.5	6.9	12.5	2.7	3.6	6.7	7.8	8.6	1.4	3.9	3.6	5.6	4.3	10.1	24.7	8.6	21.6
19	20	32.5	8.9	3.1	15.9																		
20	27	15.5	4.0	2.0	5.4	7.8	1.4			0.7	3.7	6.5	12.0	9.2	1.5	5.0	6.5	7.7	2.9	19.8	26.7	4.7	10.1
21	25	6.7	3.6	1.2		3.8	1.6	2.2	7.5	1.8	4.8	6.4	14.7	6.7	0.9	3.5	4.3	2.4	5.0	13.2	13.6	4.0	
22	23	18.4	2.7	1.2	4.7	5.6		3.3	13.1	1.7	3.2	5.1	2.0	8.6	0.6	2.4	2.7	1.1	5.4	15.7		22.6	6.0
23	15	23.3	3.4	1.6		5.8		5.8	14.3	1.4	3.8	3.2	5.3	11.0	1.2				4.8	12.6	16.7	5.3	
24	15	8.9	4.2	2.0		22.3	3.4	3.7	18.3	1.4	5.0	5.7	3.2	5.6	2.0	3.6	2.1	2.7	3.6	14.6		3.3	7.2
25	15	10.6	2.5	1.8		9.0	3.4	2.7															
26	15	19.7	13.4	1.4	6.9		2.3	3.9			3.4	5.3	4.9	7.6	1.8								
27	27	20.4	3.4	2.3		7.6				2.1	4.3	11.0		5.7	1.6	6.0	2.2	4.4	5.1	29.1			
28	21	9.0	5.0	1.5							2.6	5.0	12.2	6.8	0.6	3.0	4.3	3.3	3.0	11.7			8.3
29	20										10.6	10.0							9.3	28.7	72.0		
30	45	14.2	5.4								4.2	5.9		21.8	3.2				5.9	13.7	15.7		
31	20	10.6								1.9	5.5	7.0	7.4	8.7	7.2	3.5	5.0	1.7	19.1	15.0	19.8		
32	20	15.1	11.8	1.4	6.6		2.3	3.9			3.3	5.2	4.8	7.2	1.8								
33	20	15.4	3.4	2.3		7.2				2.1	4.2	10.1	23.6	5.6	1.6	5.8	2.2	4.3	5.0	18.6			
34	20	8.4	4.9	1.5							2.6	4.9	10.9	6.5	0.6	3.0	4.2	3.2	2.9	10.5			7.8
35	20										9.7	9.2							8.6	18.2	22.4		
36	23	12.2	5.3								4.1	5.8		19.6	3.2				5.7	11.7	12.8		
37	20	9.7	22.4							1.9	5.4	6.7	7.0	8.2	7.0	3.5	4.9	1.7	14.8	12.6	15.3		
average CV(b)		12.4	5.0	1.6	4.6	7.7	2.4	3.7	11.5	1.9	4.5	7.7	11.5	8.5	1.9	3.5	3.2	3.4	6.1	18.1	24.1	7.0	8.4
% of CV(b)<10%		40	91	100	93	81	100	100	30	100	97	81	50	79	100	100	100	100	90	0	0	84	78

The index of reliability of transference, or coefficient of variation (CV) of the regression line of slope b [CV(b)], is tabulated according to analyte name and the sequence number of each collaborating laboratory. Cells with CV(b) ≥ 5.0 are drawn with a gradation color according to their magnitude.

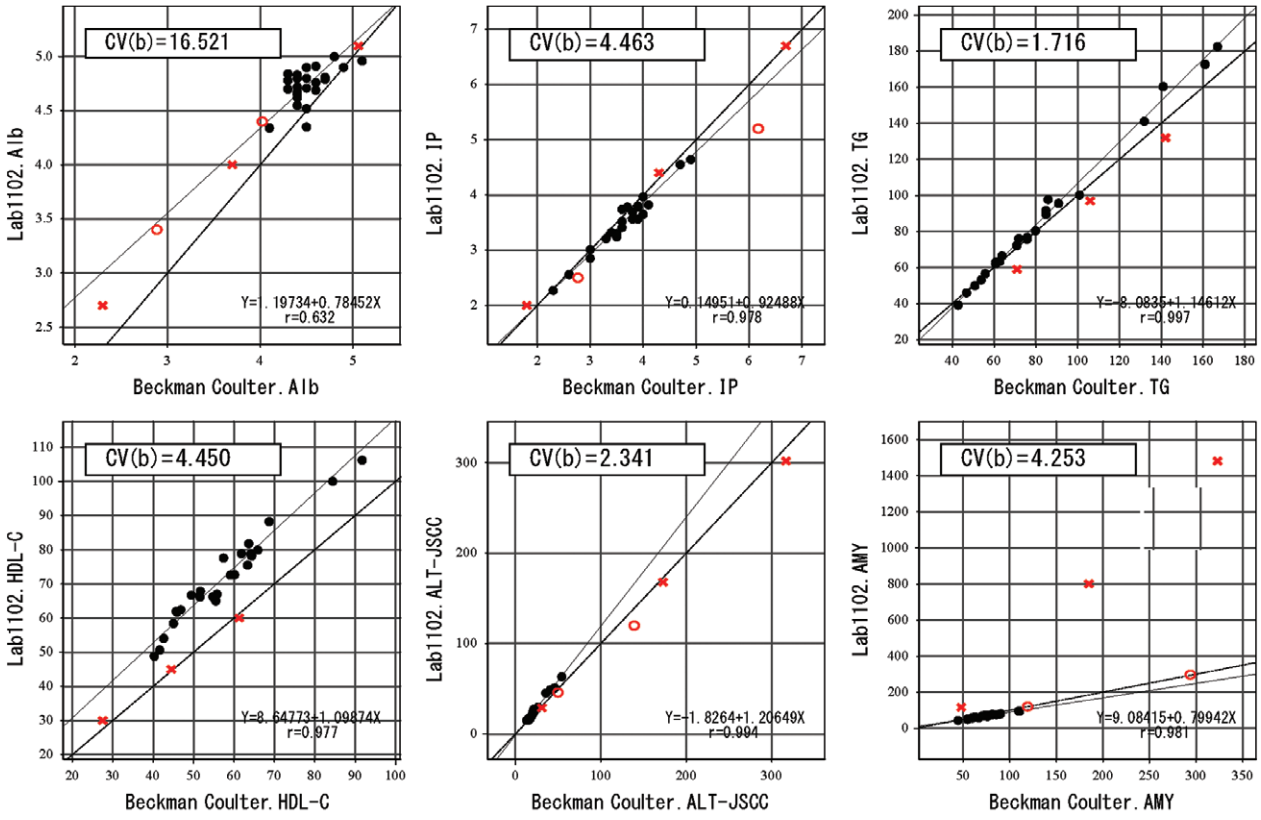

Figure 3 Commutability of commercial QC specimens in the cross-comparison.
Typical results of the cross-comparison plotted for two datasets: one for individual sera (black dots), as in those of Figure 1, and the other for the commercial QC specimens (red circle or cross, distinguishing the sources). The comparison was made in conventional unit.

hesitancy among would-be collaborating laboratories to take part in this study with no expected benefit unless the same reagents/platforms as those in the central laboratory were used. Therefore, it was needed to cross-compare test results between the central and local laboratories, and a service for transferring RIs to the participating laboratories based on the cross-comparison results by use of the reduced major axis regression was planned.

The precision of converting RIs depends primarily on the SE of the slope, SE(b), which in turn depends on both the correlation coefficient and the sample size used for the comparison, as expected from Equation (5) (also refer to Appendix 1). It is obvious that with increases in the data size n and/or the correlation coefficient r, the precision of the conversion improves. For the standardized comparison, we chose to use *CV(b)* by dividing it by the slope b.

Regarding the critical limit for providing the conversion service, it is difficult to set a fixed value that is suitable to all situations. This is because the precision depends not only on n and r described above but also on analytical variations and the range of the RI (LL–UL) relative to its median (see below). Therefore, we inspected all the cross-check test results and checked for the behavior of CV(b) in relation to the data size and the degree of scatter around regression line. Then, we set CV(b)=10% empirically, as the critical value by consensus among the coordinators of the study. To demonstrate the performance of CV(b) and the implication its cut-off value, all pair-wise comparison made for four analytes are displayed in Supplementary Figure 2 as a reference. On the basis of the approximated formula, Equation (6), for the typical data size of n=20, 30, and 40, CV(b)=10% corresponds to r=0.906, 0.849, and 0.787, respectively, indicating that uncertainty of the conversion is equivalent for the three cases despite relatively large change in the r value.

The number of specimens used for the comparison was in most cases <40, less than that described in the CLSI guideline (EP9-A2) entitled 'Method comparison and bias estimation using patient samples' [6]. Duplicate measurement as recommended in the guideline was also not done for the comparison. However, we obtained very low CV(b) of below 5% in most of the standardized analytes, a situation where we apparently do not need that many

specimens for the comparison, with the notable exception of sodium (Na), chloride (Cl), potassium (K), total calcium (Ca), and albumin (Alb), which showed CV(b) of >10%, as discussed below.

At any rate, we probably have to stratify the level of strictness in setting the acceptability criteria for the cross-comparison in the multicenter study. It varies from a situation where one just needs to confirm transferability of the RIs centrally determined to a situation where a regulatory group requests strict criteria for the transference. In fact, what we aimed at by the cross-comparison was to meet the need of the former situation, and thus, most laboratories were pleased with provision of the transference service.

However, we realized that it was very important not to measure all specimens for the cross-comparison in a single day. They should have been measured in two or more parts on separate days so that between-day variations of test results be considered in deriving the regression line.

As expected, the test results for the non-standardized analytes depended on reagent types and showed systematic bias between the central and local laboratories in the majority of cases. However, CV(b)s observed for the analytes mostly measured by immunoassays were as low as those for the standardized analytes, with 43% of them attaining a CV(b) of <5%. This observation should have great relevance in considering harmonization of test results of those analytes. In fact, an international forum called 'Improving Clinical Laboratory Testing through Harmonization' was held in October, 2010, in Gaithersburg, MD, USA, and the topic of harmonization of immunoassays has become an urgent global issue in the clinical chemistry societies [7]. It appears obvious that an indispensable step for harmonization is comparative measurement of a panel of commutable specimens or a set of multiple individual sera across reagents/platforms in the market. Then, the clinical chemical societies can find a way to build consensus among reagent manufacturers on how to align test results based on the comparison.

The large scatter around the regression line observed for TP, Alb, and the electrolytes (Supplementary Table 5) is mostly attributable to the narrow range (UL–LL) of each RI relative to the mid-point ((LL+UL)/2) of the RI, as was predicted by Linnet based on his simulation study to evaluate required number for samples for the method comparison [8]. This implies that a small change in the test result causes a large relative fluctuation in the cross-comparison using specimens limited to healthy individuals. Actually, the unit for reporting values of Na and Cl is 1 mmol/L, and their RIs cover a range of only 6 units (140–146 mmol/L) and 8 units (100–108 mmol/L), respectively. Thus, there exists a large round-up error for the last digit in reporting test results: i.e., a difference of one unit (1 mmol/L) corresponds to 16.7% and 12.5% of the range of RIs for Na and Cl, respectively. Similar situations exist for TP, Alb, K, and total Ca: a 1-unit change in the reported value makes a difference of 6.7%, 11.1%, 9.1%, and 7.7% of the range of RIs, respectively.

From these theoretical considerations, the success of the transference of RIs on the basis of cross-comparison using specimens from healthy individuals depends on: 1) a wider range of the RI (UL–LL) relative to the median of the RI; 2) a larger sample size *n* used for the comparison; and 3) a higher correlation coefficient *r*. It is also important to make a visual check for outlying data points which affect the regression line.

As for the regionality prominently observed for folate, we assume this will be attributable to the genetic differences in enzymes that are involved in processing folate in the body [9]. This is because supplementation of folate is not a common practice in East and Southeast Asia, even for women in the reproductive age. Actually, the pattern of regionality (higher values in Taiwan, Hong Kong, Ho Chi Minh City, and Jakarta) appears similar for both males and females. The possibility of higher consumption of green vegetables in those countries appears unlikely because regionality is still clearly observed even after adjusting for the level of vegetable consumption recorded in the questionnaire by multiple regression analysis (this finding will be reported in the third companion paper to be submitted in this series). In regard to the regionality observed for CA15-3, PTH, and adiponectin, we can only attribute them to genetic differences in concentrations of those proteins in blood.

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Appendix 1

Estimation of standard error of slope *b* by the reduced major axis regression

Assuming a dataset consisting of n points of data pair (x and y), the reduced major axis regression is expressed as follows:

$$y-\bar{y}=b(x-\bar{x})$$

where $\bar{x}, \bar{y}$ represents the means of x and y. The slope b is derived as follows:

$$b=\sqrt{\frac{S_{yy}}{S_{xx}}} \qquad \left[S_{xx}=\sum_{i=1}^{n}(x_i-\bar{x})^2 \;\; S_{yy}=\sum_{i=1}^{n}(y_i-\bar{y})^2\right]$$

The mathematical method to derive standard error (SE) of b, $SE(b)$, is not known and commonly approximated by SE of slope b', $SE(b')$, by the ordinary least-square method by the following formula (references 2, 4).

$$y-\bar{y}=b'(x-\bar{x})$$

$$b'=\frac{S_{xy}}{S_{xx}} \qquad \left[S_{xy}=\sum_{i=1}^{n}(x_i-\bar{x})(y_i-\bar{y})\right]$$

$$SE(b)\simeq SE(b')=\frac{s}{\sqrt{S_{xx}}}$$

where s represents the standard deviation of data points around the ordinary least-square regression line. s is derived as follows with Y representing a predicted y for a given x, or $Y=\bar{y}+b'(x-\bar{x})$, and r representing the correlation coefficient.

$$s=\sqrt{\frac{\sum(y_i-Y_i)^2}{n-2}}=\sqrt{\frac{\sum\left((y_i-\bar{y})-b(x_i-\bar{x})\right)^2}{n-2}}$$

$$=\sqrt{\frac{\sum(y_i-\bar{y})^2-2b'\sum(x_i-\bar{x})(y_i-\bar{y})+b'^2\sum(x_i-\bar{x})^2}{n-2}}$$

$$=\sqrt{\frac{S_{yy}-2b'S_{xy}+b'^2S_{xx}}{n-2}} \quad \Leftarrow b'=\frac{S_{xy}}{S_{xx}}$$

$$=\sqrt{\frac{S_{yy}-S_{xy}b'}{n-2}}=\sqrt{\frac{S_{yy}-S_{xy}^2/S_{xx}}{n-2}} \quad \Leftarrow r=\frac{S_{xy}}{\sqrt{S_{xx}S_{yy}}}$$

$$=\sqrt{\frac{(1-r^2)S_{yy}}{n-2}}$$

Using the last formula, approximated $SE(b)$ can be expressed as follows.

$$SE(b) \simeq SE(b') = \frac{s}{\sqrt{S_{xx}}} = \sqrt{\frac{(1-r^2)S_{yy}}{(n-2)S_{xx}}} \quad \Leftarrow b = \sqrt{\frac{S_{yy}}{S_{xx}}}$$
$$= b\sqrt{\frac{1-r^2}{n-2}}$$

Therefore, CV of slope b, or $CV(b)$, can be expressed as follows:

$$CV(b) = \frac{SE(b)}{b} \times 100 \simeq 100\sqrt{\frac{1-r^2}{n-2}}$$

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