Estimation of Reference Intervals for the Shizuoka Prefecture of Japan and Comparison with Those Obtained from Other Two Large-scale Studies of Reference Populations in Japan

Takashi KANNO¹; Kazutami TAUCHI²; Yoichi OHGUSHI³; Takeo SHIBATA³; Tetsuhei OGAWA⁴; Yoshinori TAKASHIMA⁵; Masahiro SUSUYAMA⁶; and Yoshihiro TAKAGI⁷

¹The Foundation of Hamamatsu Medical Center, ²Shizuoka Medical Service Center MEDIO, ³The Fundamental Section of Medicine, Tokai University, School of Medicine, ⁴Keiyu-kai, Johto Hospital, ⁵Department of Laboratory, Social Insurance Sakuragaoka General Hospitals, ⁶Shimizu Kosei General Hospitals, and ⁷Shizuoka Children's Hospital

ABSTRACT

Objective We calculated age- and sex-dependent reference intervals for 10 variables on the basis of health examination data of 156,565 individuals in Shizuoka prefecture of Japan. We compared these reference intervals with age- and sex-dependent reference intervals calculated from two other large scale studies across the country populations.

Design and Setting Data were obtained from three laboratories, and intervals were calculated by revised Hoffmanns fitting method. On the other hand, Shibata et al. calculated, by a non-linear optimization method (NLO), age- and sex-dependent reference intervals in 5-year age-based increments from data obtained approximately 750,000 reference individuals from 45 laboratories all around of Japan. Moreover, Suka et al. representing the Japan Association of Health Service (JAHS) established reference intervals and collected data from approximately 2.7 million individuals from metropolitan and branch offices in all prefectures of Japan with the basis of NCCLS criteria. We compared with these three large scale age- and sex-dependent reference intervals each others.

Results Reference intervals for all subjects were similar between three studies and especially the serum total cholesterol, uric acid and fasting blood sugar, albumin and lactate dehydrogenase were closely coincided between our study and the other two large scale studies. Levels of serum alkaline phosphatase and postmenopausal increases in this enzyme were similar between our study and Shibata's study.

Conclusion Our findings indicate that if compatibility between laboratories can be established by external quality control analysis, reliable reference intervals could be obtained in large-scale cooperative studies, and the use of these reference intervals by multiple laboratories would be possible.

Key Words Age, Nonlinear Optimization Method, Reference Intervals, Sex, Traceability

INTRODUCTION

The concept of reference interval was first proposed by Grasbeck and Saris in 1969^[1] and was adopted by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) in 1989.^[2] A guideline for the use of reference intervals (CA-28A) was later proposed by the National

Committee for Clinical Laboratory Standards (NCCLS).^[3] However, until the 1990s, inter-laboratory data differed widely, and joint utilization of laboratory data between laboratories was not possible. It became necessary for each laboratory to determine reference intervals based on its own reference population.

The concept of traceability in measurement systems was introduced in clinical laboratories in 2001.^[4] In Japan, assay procedures for six enzymes, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Lactate dehydrogenase (LD), Creatine kinase (CK), Alkaline phosphatase (ALP) and Gamma glutamyltranspeptidase (GGT), were recommended by the Japan Society of Clinical Chemistry (JSCC) during the period 1989 through 1990.^{[5]-[10]} Specifications for the practical use of standard materials in these enzyme assays were proposed by the same entity in 1997,^[11] and these standard materials were supplied by the Japan Association of Clinical Reagents Industries (JACRI) beginning in 1998.

The proposed reference methods and the supply of standard materials aided remarkably in the establishment of a traceability chain in Japan, and quality control analysis of the six enzyme assays by the Japan Medical Association (JMA) in 2002 showed a favorable within 2% coefficient of variation, indicating a decrease in inter-hospital variations in laboratory data. Thus, it is now possible to use reference intervals in clinical laboratories determined by cooperation of different laboratories. On the basis of these results, Shibata et al. calculated, by a non-linear optimization method (NLO), age- and sex-dependent reference intervals in 5-year age-based increments from data obtained in 2004 representing approximately 750,000 reference individuals from 45 laboratories all around of Japan.^[12] In addition, Suka et al. representing the Japan Association of Health Service (JAHS) established reference intervals and collected data from approximately 2.7 million individuals from metropolitan and branch offices in all prefectures of Japan.^[13] They calculated age- and sex-dependent reference intervals in 10-years age based increments from 730,000 non-smoking and non-drinking individuals based on the basis of the C-28A guideline.^{[3], [13]}

We determined age- and sex-dependent reference intervals for 10 clinical variables in 5 year increments from 156,565 individuals undergoing health care examinations in three lab-

Received: April 5, 2006

¹Address: The Foundation of Hamamatsu Medical Center, 328, Tomitsuka-cho, Hamamatsu City, Shizuoka, 432-8580 Japan. TEL: 81-53-451-2770, FAX: 81-53-451-2766, E-mail: kanno113@hmedc.or.jp

Table 1 Intra- and inter-laboratory coefficient of variation for three laboratories.

Variable	Values of samples	Intra-laboratory		Values of samples	Inter-Laboratory			Inter-laboratory	
		Lab. 1	n		Lab. 2	n	Lab. 3	n	CV
Total cholesterol (mmol/l)	4.24	0.71	105	6.49	0.63	105	0.66	104	0.81
Triglyceride (mmol/l)	0.89	0.9	105	1.16	0.72	105	0.72	104	1.68
Uric Acid (μmol/l)	343	0.91	105	459	0.93	105	0.71	104	1.38
Creatinine (μmol/l)	99	1.16	105	94.6	0.49	105	0.89	104	5.12
Aspartate Aminotransferase (U/l)	56.3	1.47	105	38.8	2.62	105	2.84	104	3.65
Alanine Aminotransferase (U/l)	50.4	0.98	105	28.3	1.65	105	3.97	104	0.79
Lactate Dehydrogenase (U/l)	155.8	1.23	105	147	1.24	105	2.18	104	4.45
Alkaline Phosphatase (U/l)	113.9	0.98	105	232	1.55	105	0.65	104	2.43
Gamma GT (U/l)	59.1	0.89	105	83.7	0.85	105	0.99	104	1.22
Total Protein (g/l)	68	0.65	105	73	0.88	105	0.99	104	1.77
Albumin (g/l)	4.2	0.88	105	45	0.63	105	0.93	104	1.09
Glucose (mmol/l)	5.58	0.82	105	5.08	0.9	105	1.35	104	1.08

All values were indicated as SI unit, except enzymes.
Enzymes were expressed as U/l.
CV: Coefficient of variation How to calculate are appeared in text.
Lab. 1: Sakuragaoka Helth care center
Lab. 2: Preventive Medicine Shizuoka Blanch
Lab. 3: Shimizu Kosei General Hospital
All laboratries were used the same control samples.

oratories using a revised Hoffmanns fitting method. Here, we report and compare our reference intervals with those of Shibata et al.^[12] who used a NLO method, and with those of the, JAHS which are based on the CA-28A guideline.^[13]

MATERIALS AND METHODS

Materials

Large-scale reference individuals were obtained from three laboratories for 156,565 individuals who underwent health examinations in Shizuoka prefecture during April of 2003 to March of 2004.

Pre-analytical condition: Well-established standard procedure for sample collection using Vacuteiner (Vacuum sampling system from Becton Dickinson Franklin Lakes NJ, USA) after a 12 hour fasting.

Inclusion criteria: The inclusion criteria for reference individuals were according to the guidelines for the Nordic Reference Interval Project 2000 (NORIP),^[14] except that of smoking. We excluded individuals for whom the values of objective variables fell more than three SDs outside the mean values. Approximately 1% of individuals were excluded from laboratory 1 (Social Insurance Sakuragaoka General Hospital) and fewer than 0.5% of individuals were excluded from laboratories 2 (Shizuoka Kosei General Hospitals) and laboratory 3 (Shizuoka branch of Japan Association of Health Service). Distribution of values of reference individuals for each laboratory was examined. We used Student’s *t*-test for uric acid (UA), total cholesterol (TC), fasting blood sugar (FBS), total protein (TP) and albumin levels, and we used the Mann-Whitney’s *U*-test for enzyme activities, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities. No differences in distribution were

Table 2 Methods used in this study.

	Analytes	Methods
1	Total cholesterol	COD/POD (enzymatic)
2	Triglyceride	GPO/GK (enzymatic)
3	Uric acid	Uricase/POD (enzymatic)
4	Creatinine	Creatininase/creatinase/SOD
5	Aspartate amionotransferase	JSCC consensus
6	Alanine aminotransferase	JSCC consensus
7	Lactate dehydrogenase	JSCC/IFCC consensus
8	Alkaline phosphatase	JSCC consensus
9	Gamma glutamyl transpeptidase	JSCC/IFCC consensus
10	Total protein	Biuret
11	Albumin	Bromcresol Green
12	Fasting blood sugar	Hexokinase/UV

COD: Cholesterol Oxidase
GPO: Glycerol-3-phosphate Oxidase
POD: Peroxidase
GK: Glycerol kinase
GIDH: Glycedrol dehydrogenase
SOD: Sarcosine oxudase
SI units were used in all subjects except Enzymes
Enzymes were expressed as U/l, not katal of SI system

observed between reference individuals from the three laboratories.

Analytical quality control: Intra- and inter-variation for the three laboratories during one year periods including experimental periods were tested with the use of refrigerated commercially available pooled serum (QAP-trol, 1QL-104 and 2QL 204, Sysmex Co. Ltd., Kobe, Japan.) The results are summarized in Table 1. Intra laboratory coefficients of variation of three laboratories were less than 2.0% except AST and ALT. Those of AST and ALT were less than 3.5% in QAT-trol 1QL 104 of low levels of control. The inter labora-

Table 3 Age- and sex-dependent reference intervals in Shizuoka prefecture.

Sex	Age	TC		TG		UA		Creatinine		AST		ALT	
		Lower RL	Upper RL	Lower RL	Upper RL	Lower RL	Upper RL	Upper RL	Upper RL	Lower RL	Upper RL	Lower RL	Upper RL
male	10-14	3.08	5.53	0.25	1.31					18	33	10	23
	15-19	2.97	5.43	0.26	1.32	253	475	54	94	12	29	9	28
	20-24	3.10	5.74	0.28	1.55	251	460	58	95	13	29	9	33
	25-29	3.28	6.18	0.29	1.84	253	462	59	95	13	29	9	34
	30-34	3.49	6.52	0.32	2.13	240	481	56	95	13	32	9	39
	35-39	3.59	6.78	0.35	2.18	240	501	54	95	13	32	9	47
	40-44	3.78	6.96	0.36	2.43	240	504	54	95	13	32	9	44
	45-49	3.80	7.03	0.38	2.45	235	501	54	98	13	34	10	43
	50-54	3.85	7.01	0.37	2.40	233	500	53	98	13	32	10	39
	55-59	3.83	7.01	0.38	2.40	232	499	52	98	14	35	10	38
	60-64	3.80	6.96	0.37	2.17	232	503	51	98	15	35	10	37
	65-69	3.83	6.80	0.37	2.13	234	510	52	100	15	35	10	34
	70-74	3.65	6.96	0.37	1.93	215	504	51	100	15	35	9	33
	75-79	3.49	6.75	0.36	1.64	214	503	50	105	14	35	7	29
	80-	3.03	6.31	0.36	1.58	178	465	46	114	13	32	6	24
female	10-14	3.23	5.74	0.27	1.32					15	29	7	20
	15-19	3.31	5.74	0.28	1.12	167	344	43	68	12	25	6	19
	20-24	3.26	5.77	0.26	1.05	164	335	44	67	12	24	6	20
	25-29	3.31	5.95	0.27	1.06	159	335	42	68	12	24	6	20
	30-34	3.34	6.05	0.26	1.08	156	338	42	67	12	24	6	22
	35-39	3.52	6.28	0.28	1.28	156	335	42	68	12	24	7	23
	40-44	3.62	6.52	0.28	1.32	153	334	42	68	12	25	7	23
	45-49	3.83	6.80	0.30	1.38	156	344	42	68	12	26	7	24
	50-54	4.11	7.29	0.33	1.61	162	369	41	68	13	29	9	28
	55-59	4.34	7.32	0.34	1.66	166	387	41	72	15	30	10	29
	60-64	4.29	7.29	0.36	1.86	165	388	41	72	15	32	10	29
	65-69	4.27	7.09	0.37	1.89	183	402	41	73	15	32	10	29
	70-74	4.16	7.24	0.41	1.84	179	396	40	73	15	30	10	28
	75-79	3.83	7.06	0.51	1.85	155	403	39	75	13	32	7	28
	80-	3.52	6.59	0.38	1.59	136	477	31	91	12	31	5	23
Sex	Age	LD		ALP		GGT		TP		Albumin		FBS	
		Lower RL	Upper RL	Lower RL	Upper RL	Lower RL	Upper RL	Lower RL	Upper RL	Lower RL	Upper RL	Lower RL	Upper RL
male	10-14												
	15-19	262	486	145	454	7	39	65	79			4.20	5.76
	20-24	139	496	139	349	7	41	67	81	41	50	4.20	5.68
	25-29	114	453	127	312	16	42	66	77	40	51	4.23	5.74
	30-34	137	472	130	313	13	56	66	78	41	50	4.28	5.80
	35-39	136	446	125	311	12	69	64	77	41	50	4.27	5.98
	40-44	159	443	125	310	12	71	64	77	40	50	4.32	6.09
	45-49	139	451	127	313	12	83	64	78	39	49	4.32	6.34
	50-54	129	452	126	315	12	82	65	78	39	49	4.32	6.44
	55-59	128	466	124	317	12	82	66	78	39	49	4.30	6.69
	60-64	116	478	129	318	12	71	66	80	39	50	4.34	6.68
	65-69	108	512	128	330	12	67	66	78	39	49	4.34	6.69
	70-74	104	390	127	330	14	55	63	77	38	50	4.46	6.42
	75-79	134	490	126	335	7	43	65	81	37	51	4.25	6.65
	80-	162	605	140	369	5	45		81	36	50	4.43	6.49
female	10-14							65	80				
	15-19	254	417	117	295	3	29	67	82			4.15	5.78
	20-24	252	388	105	257	3	30	66	79	40	54	4.17	5.45
	25-29	134	443	100	253	3	30	67	78	40	51	4.17	5.46
	30-34	138	448	100	249	3	31	66	78	39	50	4.17	5.49
	35-39	230	440	100	235	3	31	66	78	40	48	4.17	5.52
	40-44	230	444	99	235	4	32	66	77	39	47	4.20	5.72
	45-49	162	470	100	257	4	39	65	78	38	48	4.20	5.82
	50-54	161	514	121	331	5	41	65	79	39	49	4.22	6.02
	55-59	163	531	143	349	5	42	65	80	39	49	4.24	6.12
	60-64	139	581	144	354	6	42	66	81	39	50	4.28	6.13
	65-69	151	570	146	437	5	41	64	81	38	51	4.30	6.12
	70-74	147	530	147	355	5	40	61	82	39	49	4.33	6.14
	75-79	241	613	146	394	4	40	60	82	35	52	4.22	6.46
	80-	254	543	151	389	3	37	6	78	39	48	3.95	6.69

TC, TG and FBS were expressed as mmole// Creatinine, Uric Acid: $\mu\text{mol//}$ RL: Reference limit
Enzymes Units were expressed with U//, not katal of SI system. Total Protein, Albumin: g//
Units: All subjects were expressed as SI units except Enzymes

tory coefficient of variation between three laboratories was less than 5% in all variables for tested. The ability for providing the reference individuals for this study was confirmed.

Methods

Methods used to analyze the 10 variables are summarized in Table 2. FBS was measured by the enzymatic method using hexokinase recommended by the JSCC.^[15]

Statistics

Age- and sex-dependent reference intervals were calculated by the revised Hoffmann fitting method described by Usui and Kawai.^[16] We used software provided by Sysmex Co. Ltd. of Kobe, Japan. In brief, we first calculated mean $\pm$ SD values for the reference population and excluded data fell more than three SDs beyond the mean values. A histogram was then drawn. If the distribution was not normal, test data were transformed as log, square, third power, square root. The *chi*-square test was used to test for normal distribution. Data for which the *chi* square test yielded the smallest *p* values were used for the calculation. In our reference population, TC, TG, UA, creatinine, FBS fit normal distribution and six enzymes, AST, ALT, LD, CK, ALP and GGT fit log normal distribution. We used the mean $\pm$ 1.96 SD as the upper and lower reference limits. We then excluded data that fell outside upper and lower limits. We repeated this process until significant differences between the upper and lower limits were not observed between *x* times and *x*+1 times. We used the values obtained in this manner as the final upper and lower limits of the reference intervals. For other statistical examination, such as Student's *t*-test, we used Stat's View 5.0 (SAS Institute, Inc., San Diego, CA, USA).

RESULTS

Intra- and inter-laboratory variations

Intra- and inter-laboratory variations for three Shizuoka laboratories are summarized in Table 1. For all enzyme assays, the coefficients of variation were within 5% for intra-laboratory data and within 4.45% for inter-laboratory data. For other variables with the exception of creatinine, coefficients of variation were within 3.69% for intra-laboratory data and within 4.14% for inter-laboratory data.

Age- and sex-dependent reference limits

Age-dependent reference limits were calculated in 5-year increments from the age of 11 years to over 81 years. These values are summarized in Table 3. Distribution patterns for TC, FBS, UA, TP, and albumin were normal. Enzyme activity distribution patterns were log normal. Thus, we converted the values as logarithms, calculated the mean $\pm$ 1.96 SD, and used these values as reference intervals. The Units of variables were expressed as SI system except enzymes, and U// not katal were used for enzyme activities.

Comparison between our age- and sex-dependent reference intervals and those obtained from other two studies

The age- and sex-dependent reference intervals were calculated from the reference population of Shizuoka prefecture. Those obtained from total cholesterol, uric acid and

(A) Age- and sex-dependent reference intervals for TC.

(B) Age- and sex-dependent reference intervals for UA.

(C) Age- and sex-dependent reference intervals for FBS.

Fig. 1 Comparison of age- and sex-dependent reference intervals from Shibata et al.,^[12] Suka et al.^[13] and our study.

(A): Reference intervals for total cholesterol.

(B): Reference intervals for uric acid.

(C): Reference intervals for fasting blood sugar.

Units for total cholesterol and fasting blood sugar were expressed as SI Unit (mmole/L).

Units for uric acid was expressed as SI Unit (μmole/L)

fasting blood sugar were quite similar to those obtained by Shibata et al.^[12] and Suka et al.^[13] Postmenopausal (older than fifty years old women) age-dependent increases in the reference intervals for TC were observed in all three studies (Fig. 1(A)). Sex- dependent differences in UA were also

(A) Age- and sex-dependent reference intervals for AST.

(B) Age- and sex dependent reference intervals for ALT.

(C) Age- and sex-dependent reference intervals for γGT.

Fig. 2 Comparison of age- and sex-dependent reference intervals from Shibata *et al.* Machi *et al.*, and by us which showed similar tendencies but the limits of upper reference limits of reference intervals were not coincidence between three studies.

(A): Reference intervals for aspartate aminotransferase.
(B): Reference intervals for alanine aminotransferase.
(C): Reference intervals for γ-glutamyl transpeptidase.
The Units of these three enzymes were expressed as U/l, not katal of SI.

Fig. 3 Age- and sex-dependent reference intervals for alkaline phosphatase. Similar tendencies, relatively low upper limits of reference intervals for young adult women and higher upper limits for postmenopausal women were observed in both Shibata and our studies. Young adult women mean the women whose ages were over thirty years and less than forty nine years old. Postmenopausal women means the women whose ages were over fifty years old. The unit of alkaline phosphatase was expressed as U/l, not katal of SI.

observed in all three studies (Fig. 1(B)). The upper reference limits for FBS was less than 6.8 mmol/L in all three studies (Fig. 1(C)).

Reference intervals for LD were not analyzed by Suka *et al.*^[13] However, our age- and sex-dependent reference limits for LD and for albumin were quite similar to those obtained by Shibata *et al.*^[12] (data not shown).

As shown in Fig. 2, the upper reference limits for AST (Fig. 2(A)), ALT (Fig. 2(B)) and G-GT (Fig. 2(C)), were higher in all age groups in our study than in the Shibata's studies. Moreover, reference intervals of triglyceride showed same tendency for AST and ALT. The upper reference limits of Suka's data of AST, ALT, G-GT and triglyceride were highest in all three groups. (The data for triglyceride is not shown).

Reference intervals in serum ALP

As shown in Fig. 3, increases in upper reference limits for women more than 45 years of age, and lower tendencies of upper serum ALP were observed by us and by Shibata *et al.*^[12] Suka *et al.* did not calculate age- and sex-dependent reference limits for ALP.

DISCUSSION

Age- and sex-dependent reference intervals

We compared reference intervals of every 5 years of age with the data of Shibata *et al.*^[12] and of every 10 years of age with the data of Suka *et al.*^[13] Reference intervals, tendencies, and age- and sex-dependencies between studies were similar for TC, UA, FBS, LD, and albumin.

The reference individuals for whom data were analyzed by Shibata *et al.*^[12] and Suka *et al.*^[13] were from all areas of Japan. Our reference individuals, however, were restricted in Shizuoka prefecture only. Though values for TC, UA, and FBS were quite similar, these variables are closely related to diet. These results indicate similarities in dietary habits

throughout Japan.^[17] The results also indicate that similar values for age- and sex-dependent reference limits will be obtained for the Japanese population if they are obtained from large-scale studies of more than 100,000 reference individuals.

The differences of reference limits between studies

Differences in upper reference limits were observed between our study and the other two large-scale studies, particularly for AST, ALT, G-GT and TG. These variables are closely related to liver function and hyperlipidemia.^{[18], [19]} Thus, these variables should be carefully examined in large-scale studies of different groups in the Japanese population. A high upper reference limit for AST, ALT, G-GT and TG was observed in both males and females. Especially, highest upper reference intervals in Suka's study used the C-28A criteria of NCCLS. The collection of healthy reference individuals would include some problems. Further studies would be required to understand these phenomena. The accurate methods for correction of normal reference population and how to statistically calculate the reference intervals would be discussed in near future.

Post menopausal increases of serum ALP activities

We found an age-dependent increase in ALP activity in women whose age were over than fifty years old. These data were similar to that found by Shibata et al.^[12] In addition, the lower reference limits for young adult women for ALP in both our study and the Shibata et al. study.^[12]

Age-dependent changes in ALP in individuals less than 20 years of age have been reported by many investigators.^{[20], [21]} However, the increase in ALP in postmenopausal women was first observed by Shibata et al.^[12] and by us. The specific ALP isoenzyme expressed by postmenopausal women has not been clarified. Moreover, use of the same ALP reference limits for men and women less than 45 years of age will fail to account for hyperthyroidism which is frequently found in young women.

Comparison with other European countries

Comparison of reference intervals obtained in Japan with other European countries were performed with the data obtained from NORDIC countries.^[22] Except of AST, ALT and G-GT, the reference intervals of Japanese were not so large differences with those obtained from Nordic groups.

ACKNOWLEDGEMENT

We thank the Committee for External Quality Assessment in Shizuoka prefecture for providing laboratory data.

REFERENCES

- [1] Grasbeck R, Saris NE: Establishment and use of normal values. *Scand J Clin Invest Suppl* 110: 11-15, 1969
- [2] Expert Panel on Theory of Reference Values (IFCC): The concept of reference value (1978). *Clin Chem Acta* 87: 459F-465F, 1978
- [3] National Committee for Clinical Laboratory Standards (NCCLS): How to define, determine, and utilize reference intervals in the clinical laboratory. NCCLS Document C28-A March: 1992
- [4] ISO/TC212: In vitro diagnostic medical devices—measurement of quantities in samples of biological origins. ISO/DIS: July 2001
- [5] Committee on Enzyme, Japan Society of Clinical Chemistry: The reference procedure for the measurement of catalytic concentration of aspartate aminotransferase (AST), EC 2.6.1.1. in human serum (1989-08-30). The approved recommendation by the Japan Society of Clinical Chemistry (JSCC). *Jap J Clin Chem* 34: 14-29, 2005
- [6] Committee on Enzyme, Japan Society of Clinical Chemistry: The reference procedure for the measurement of catalytic concentration of alanine aminotransferase (ALT), EC 2.6.1.2. in human serum (1989-08-30). The approved recommendation by the Japan Society of Clinical Chemistry (JSCC). *Jap J Clin Chem* 35: 30-39, 2005
- [7] Committee on Enzyme, Japan Society of Clinical Chemistry: The reference procedure for the measurement of catalytic concentration of lactate dehydrogenase (LD), EC 1.1.1.27, in human serum (1989-08-30). The approved recommendation by the Japan Society of Clinical Chemistry (JSCC). *Jap J Clin Chem* 34: 40-57, 2005
- [8] Committee on Enzyme, Japan Society of Clinical Chemistry: The reference procedure for the measurement of catalytic concentration of creatine kinase (CK), EC 2.7.3.2, in human serum (1989-08-30). The approved recommendation by the Japan Society of Clinical Chemistry (JSCC). *Jap J Clin Chem* 34: 58-84, 2005
- [9] Committee on Enzyme, Japan Society of Clinical Chemistry: The reference procedure for the measurement of catalytic concentration of γ -glutamyltranspeptidase (γ -GT), in human serum (1989-08-30). The approved recommendation by the Japan Society of Clinical Chemistry (JSCC). *Jap J Clin Chem* 34: 85-101, 2005
- [10] Committee on Enzyme, Japan Society of Clinical Chemistry: The reference procedure for the measurement of catalytic concentration of alkaline phosphatase (ALP). In human serum (1989-08-30). The approved recommendation by the Japan Society of Clinical Chemistry (JSCC). *Jap J Clin Chem* 34: 102-119, 2005
- [11] Scientific Division of Japan Society of Clinical Chemistry: Proposed standard for certified enzyme reference materials (ERM) (1996-02-15). *Jap J Clin Chem* 34: 159-174, 2005
- [12] Shibata T, Ogushi Y, Ogawa T, Kanno T: Estimation of sex- and age-specific clinical reference ranges by nonlinear optimization method. *Studies in health technology and informatics (connecting medical informatics and bio-informatics IOS Press)* 116: 229-234, 2005
- [13] Suka M, Yoshida K, Kawai T, Aoki Y, Yamane N, Yamauchi K : Age- and sex-specific reference intervals for 10 health examination items: mega-data from a Japanese Health Service Association. *Rinsho Byori* 53: 599-607, 2005
- [14] Rustad P, Felding P, Lahti A: Proposal for guidelines to establish common biological reference intervals in large geographical areas for biochemical quantities measured frequently in serum and plasma. *Clin Chem Lab Med* 42: 783-791, 2004
- [15] Scientific committee for reagent in Japan Society of Clinical Chemistry: The recommended method for the serum glucose measurement (1991-4-30). *Jap J Clin Chem* 20: 247-254, 1991 (in Japanese)
- [16] Usui T, Kawai T: Estimation of normal range by the method of curve fitting to the histogram. *Japanese J Clin Chem* 8: 104-115, 1979 (in Japanese)
- [17] Minister of Health and Labor and Welfare, Office for life-style related diseases control, General affair division: Rec-

- ommended dietary allowance in Japanese. 2005 version
- [18] Takata Y, Nakanishi R, Isoda C, Niino M, Maeda Y: The effect of a health promotion program at the worksite—An approach to hyperlipoproteinemia in workers before middle age—. *San Ei Shi* 45: 43-44, 2003
- [19] Hata Y: Alcohol and serum lipid and lipoproteins. *Nihon Rinsho* 59(suppl): 207-213, 2001
- [20] Eastman JR, Bixler D: Serum alkaline phosphatase: normal values by sex and age. *Clin Chem* 23: 1769-1770, 1977
- [21] Feneka RI, Foli AK: Variation in total serum alkaline phosphatase activity with age and sex in adult and adolescent Ghaniana. *Clin Chim Acta* 60: 303-306, 1975
- [22] Rustad P, Felding P, Franzson L, Kairisto V, Lahti A, Martensson A, et al: The Nordic reference interval project 2000: recommended reference intervals for 25 common biochemical properties. *Scand J Clin Lab Invest* 64: 271-284, 2004