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Evaluation of a novel serum IgG4 assay and determination of reference interval for the Japanese population

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ABSTRACT

Background: IgG4-related disease (IgG4-RD) is a new syndrome characterized by elevated serum IgG4 concentration and tissue infiltration of IgG4-positive plasma cells. Here, we evaluated the analytical performance of a new IgG4 assay reagent featuring a wide dynamic range, highly specific monoclonal antibody, and the reversed passive latex agglutination assay and determined the IgG4 reference interval (RI) for the Japanese population.
Methods: Performance evaluations were conducted on precision, linearity, sensitivity, interference, and method comparison with The Binding Site (TBS) and Siemens reagents. The RI was derived by the parametric method from 619 apparently healthy Japanese 18 to 65 years of age.
Results: Between-day precisions ranged from 1.99 to 5.52 CV%. Linearity was confirmed up to 5.0 g/l. The limit of quantitation was 0.085 g/l. Interfering substances did not significantly influence values. Method comparison among the 3 reagents yielded correlation coefficients between 0.973 and 0.988. Values for the new reagent matched those of TBS reagent except at a higher concentration range, where reactivity dissociated. The RI was 0.11–1.21 g/l without distinction by sex and age.
Conclusion: The novel IgG4 assay reagent demonstrated satisfactory analytical performance for clinical use. Because of matched value with TBS reagent at low concentrations, it is possible to use the IgG4-RD cut-off value determined by TBS reagent.

1. Introduction

IgG4-related disease (IgG4-RD) is a new disease entity discovered in 2001 that is characterized by IgG4-positive plasma cell infiltration and elevated serum IgG4 concentration. Clinical features of IgG4-RD are fibrous degeneration and dysfunction of affected organs. The lesion occurs in almost any organ, including lachrymal/salivary glands, thyroid gland, lung, pancreas, kidney, prostate gland, retroperitoneum, lymph nodes, and artery [1–3]. As IgG4-RD has become more widely recognized, the number of requests for serum IgG4 measurements from clinicians has recently dramatically increased.

Currently, reagents from 2 companies [The Binding Site (TBS) and Siemens Healthcare Diagnostics (Siemens)] are mainly used to measure serum IgG4 worldwide. However, there is a large discrepancy in test results between the 2 reagents. The upper limit of the reference interval is 1.05 g/l for TBS reagent and 2.01 g/l for Siemens reagent. The cut-off value of IgG4-RD (1.35 g/l) was calculated from TBS reagent values for Japanese patients [4]. The cut-off value for Siemens reagent was reported as 2.48 g/l [5]. Although worldwide standardization of laboratory results for major plasma proteins have been achieved by distribution of reference materials developed by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) [6], reference material with certified values for IgG subclasses including IgG4 is not available. Therefore, each company independently set the value of the IgG4 calibrator, which is the cause for the discrepancy.

Existing IgG4 measurement reagents were designed to measure

Abbreviations: C-RIDL, the Committee on Reference Intervals and Decision Limits; Drk, drinking habbit; Exer, exercise; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; IgG4-RD, IgG4-related disease; IRB, institutional review board; LAVE, latent abnormal values exclusion; LL, lower limit; Obj Var, object variable; rp, partial regression coefficient; rs, Spearman's correlation coefficient; SDR, standard deviation ratio; Smk, smoking; UL, upper limit

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values at a low concentration range accurately. IgG4 is the least abundant of IgG subclasses, and the principal aim of IgG4 measurement has been to diagnose IgG4 deficiency, which is especially observed in children with severe recurrent respiratory tract infections [7]. However, IgG4 concentrations can be 100 times higher in IgG4-RD patients than those of healthy individuals. Thus, in such cases, existing reagents have yielded falsely low values due to the prozone phenomenon [8]. Because current analyzers have a system for detecting the prozone phenomenon, the frequency of falsely low values has been reduced, although not eliminated.

To overcome these limitations, a novel IgG4 assay was developed based on the principle of reverse passive latex agglutination that can eliminate the occurrence of the prozone phenomenon and enables a wider dynamic range of the assay. It also features the use of monoclonal antibody that allows highly specific detection of IgG4. The assay principle is described in Materials and Methods. In addition, to enhance the accuracy of the quantitation, the IgG4 calibrator was value assigned by using mass spectrometry that can quantify proteins without high-level purification in reference to internal standard.

2. Materials and methods

2.1. Assay principle

N-assay LA IgG4 Nittobo (Nittobo Medical Co., Ltd.) is a kit for reversed passive latex agglutination assay composed of a mouse anti-human IgG4 monoclonal antibody and IgG4-coated latex particles. Namely, serum IgG4 reacts with the monoclonal antibody in the first reagent, and excessive antibodies react with human IgG4-coated latex particles in the second reagent, finally causing latex agglutination. Serum IgG4 concentration was calculated from the absorbance of the resulting immune complexes using a calibration curve generated by measuring standard IgG4 samples prepared from normal human sera of known concentrations. The primary calibtrator was prepared by purification of IgG4 in human pooled serum through consecutive use of ion exchange chromatography, 3 types of affinity chromatography containing protein A and 2 different mouse anti-human IgG4 monoclonal antibodies, and gel filtration. The IgG4 concentration of the primary calibrator was determined by mass spectrometry in reference to synthesized peptides that have IgG4-specific amino acid sequences.

2.2. Evaluation of analytical performance

Serum IgG4 was measured using the JCA-BM6070 chemistry analyzer (Japan Electron Optics Laboratory) using the Nittobo reagent. For method comparison, serum IgG4 was also measured by the BN-II nephelometer (Siemens Healthcare Diagnostics Products GmbH) using BS-NIA IgG4 (TBS reagent; The Binding Site) and N Latex IgG4 (Siemens reagent; Siemens Healthcare Diagnostics Products GmbH). All assays were performed in our laboratory according to the respective manufacturer's instructions. Dynamic ranges were 0.12–3.86 g/l for TBS; 0.057–3.65 g/l for Siemens. High concentration serum above the upper limit of the dynamic ranges were automatically diluted using sample diluent included in BN-II systems, which was used for TBS and Siemens assays.

2.2.1. Precision

For evaluation of within-run precision, 2 manufactured quality controls and 2 pooled sera were analyzed 20 times. For between-day precision, duplicate measurements of each sample were carried out for 20 days.

2.2.2. Linearity

The dynamic range of N-assay LA IgG4 Nittobo was 5.0 g/l. For high concentration serum above 5.0 g/l of IgG4, Nittobo system dilutes the specimen using diluent with bovine serum albumin (details not disclosed) for routine assay. Therefore, for conducting linearity experiment in a stricter condition, we deliberately prepared pooled serum of low IgG4 (0.17 g/l) for serial dilution. Two pooled sera with approximately 5.0 g/l and 10.0 g/l of IgG4 were serially diluted using the low IgG4 serum to prepare specimens with ten graded concentrations. The concentration of each diluted specimen was measured in quadruplicate and the mean value of each specimen was plotted against its expected value.

2.2.3. Sensitivity

The limit of blank (LoB) was determined by measurements of the zero concentration standard in 12 replicates over 5 days. To determine the limit of detection (LoD), approximately 0.2 g/l serum specimen was serially diluted to make 8 concentrations and measured for 8 days. To determine the limit of quantitation (LoQ), 12 low-level sera (approximately 0.02–0.12 g/l) were measured for 6 days. CV of each sample was calculated and the LoQ was determined as the lowest IgG4 concentration within a CV of 20%.

2.2.4. Specificity

The specificity was confirmed using human IgG1 (Calbiochem), IgG2 (Calbiochem), IgG3 (Sigma-Aldrich). The specimen of each IgG subclass protein arbitrarily prepared to a concentration 5 to 20 times above the upper limit of each IgG subclasses were tested for IgG4 in triplicate and the rate of cross reaction was calculated.

2.2.5. Interference

The influence of interfering substances was verified using Interference Check A Plus and Interference Check RF Plus (Sysmex Co., Ltd.). Pooled serum was mixed with free bilirubin, conjugated bilirubin, hemoglobin, chyle material, or Rheumatoid factor (RF) according to the manufacturer's protocol. IgG4 was measured in each adulterated specimen in duplicate.

2.2.6. Method comparison

IgG4 concentration was measured in 100 consecutive serum samples from patients with suspected IgG4-RD at Shinshu University Hospital. Method comparison was carried out using the major axis-linear regression analysis. Altman's difference plot analysis was also performed to evaluate the concentration-dependent bias in test results.

2.3. Reference individuals

From January to March 2012, 651 healthy volunteers were recruited as a part of the global multicenter study coordinated by the Committee on Reference Intervals and Decision Limits of International Federation of Clinical Chemistry and Laboratory Medicine (IFCC/C-RIDL) in four cities in western Japan. The target age range was 18–65 y with sex and age distribution set nearly even where possible. The actual sex and age composition (stratified as 18–29, 30–39, 40–49 50–59, and 60–75 years) were 357 males (n = 61, 62, 70, 85, and 79, respectively) and 294 females (n = 54, 54, 52, 73 and 61, respectively). Inclusion/exclusion criteria specified in the IFCC/C-RIDL protocol [10] were applied. In brief, volunteers had to consider themselves healthy for participation. Subjects who had diabetes mellitus requiring drug treatment, chronic renal or hepatic diseases, or other major diseases and those who were in pregnancy, within one year after childbirth, or within 2 weeks after recovery from conditions requiring hospitalization were excluded. Those who were under medical treatment with ≤ 2 minor drugs, such as statins, antihypertensives, analgesics, and antihistamines, were allowed to participate. Sample processing and storage were carried out according to the IFCC protocol. Six 2-milliliter serum aliquots were prepared from each volunteer, frozen immediately, and then stored at $-80\,^{\circ}C$ until measurement.

In this study, serum aliquots from 619 volunteers that remained after completing the primary study were used. The primary study

protocol was approved by the Ethics Committee of Yamaguchi University School of Medicine, Faculty of Health Sciences. Possible use of serum aliquots that remained for the purpose of deriving reference intervals (RIs) for newer tests was explicitly written both in the explanatory leaflet and the informed consent form presented to would-be volunteers. The clinical protocol and design of this study were approved by the IRB of Shinshu University School of Medicine (Matsumoto, Japan).

2.4. Sources of IgG4 variations and correlation with related analytes

For assessment of sources of IgG4 variation and other immunoglobulins, multiple regression analysis was performed. Each analyte was set as the objective variable, while explanatory variables were constantly set to sex, age, Body Mass index (BMI), smoking habit (in four levels), alcohol consumption (in five levels), and regular exercise (in 7 levels) [11].

To evaluate the need for partitioning reference values by sex or age, standard deviation (SD) or SD ratio (SDR) was computed [9,11,12]. In its computation, SD representing between-sex variations (SDsex) and between-age variations (SDage) were calculated based on nested ANOVA, and their ratio to between-individual variations was taken and expressed as SDRsex and SDRage, respectively. We considered a need for partitioning reference values by the condition of SDR ≥ 0.4 [9].

2.5. Derivation of reference intervals

RIs for IgG4 and other immunoglobulins were derived by the parametric method after normalizing data using the modified Box-Cox power transformation [9]. For optimal selection of reference individuals, the latent abnormal values exclusion (LAVE) method was applied, allowing one abnormal result in the reference tests [9]. The following markers related to IgG4 were chosen for reference tests: IgG, IgA, IgM, IgE, Albumin, globulin (calculated as total protein minus albumin), transthyretin, C-reactive protein, alanine transaminase, and gamma-glutamyltransferase, which belong to inflammatory markers and/or nutritional/hepatic makers. These analytes had been tested in the primary study and the analytical methods used were shown in the interim report of the global study [11]. The 90% confidence interval (CI) of the lower limit (LL) and upper limit (UL) of RIs were calculated based on the bootstrap method through random resampling of the same dataset 100 times. The final LL and UL were smoothed by adopting the means of repetitively derived LLs and ULs, respectively.

3. Results

3.1. Analytical performance

The results of the precision study are summarized in Table 1. Within-run and between-run precisions ranged from 1.54 to 3.72 and 1.99 to 5.52 CV%, respectively. In the dilution experiment using low IgG4 diluent serum, the linearity of the IgG4 measurement was confirmed (Supplemental Fig. 1). The LoB, LoD, and LoQ at a CV of 20% were determined to be 0.042 g/l, 0.066 g/l, and 0.085 g/l, respectively. None of the interfering substances tested had a significant influence on values (Supplemental Fig. 2). Changes in IgG4 test results caused by the addition of 0.19 g/l of free bilirubin, 0.20 g/l of conjugated bilirubin, 4.90 g/l of hemoglobin, 1,790 formazine unit of chyle, and 5.5 g/l of RF were all < 10%. The rate of cross reaction by solutions of 3 IgG subclasses at high concentrations were all < 0.2% (Supplemental Table 1).

Table 1
Precision of N-assay LA IgG4 Nittobo. Between-run and within-run SD and CV were calculated for 2 levels of quality controls (QC) and pooled sera based on duplicate measurements over 20 days.

Sample	Within-run			Between-run		
	Mean	SD	CV	Mean	SD	CV
	(g/L)	(g/L)	(%)	(g/L)	(g/L)	(%)
QC Low	0.54	0.02	3.72	0.51	0.03	5.52
QC High	1.52	0.03	2.10	1.49	0.05	3.04
Pooled serum 1	1.30	0.03	2.54	1.27	0.03	2.42
Pooled serum 2	2.91	0.05	1.54	2.72	0.05	1.99

3.2. Correlations among the 3 assays for IgG4

Correlations among the 3 assays (Nittobo, TBS, and Siemens) for 100 clinical specimens were examined as shown in Fig. 1. Deviations of major-axis regression lines from the diagonal line of equality (shown in broken line) are obvious for any combination of comparison, pointing to sheer lack of agreement among the measured values by the 3 reagents. For the comparison between Nittobo and TBS (panel A), dissociation of the two values gets more prominent at higher IgG4 level with a profile of slight curvilinear relationship. However, when limiting the specimens to those below 2.0 g/l by Nittobo, it is notable that nearly perfect matching of values was observed as shown magnified in the panel D. For the comparison between Nittobo and Siemens (panel B), the relationship of two values was again slightly curvilinear. At lower IgG4 level, the slope was computed as 0.55, implying values of Nittobo is 0.55 times lower than those of Siemens. For the comparison between Siemens and TBS (panel C), values were almost linear for the observed range up to 12 g/l by Siemens. The slope of 1.944 indicates, Siemens values were nearly twice those of TBS. Possible causes of this lack of agreement in values among the 3 reagents is discussed in the Discussion.

3.3. Sources of IgG4 variation in comparison with other immunoglobulins

The results of multiple regression analysis shown in Table 2 revealed that IgG4 showed a weak but statistically significant sex-related change with a partial regression coefficient (rp) of − 0.135, implying IgG4 is slightly low in females. However, IgG4 did not show any association with age, BMI, smoking habit, alcohol consumption, and exercise. Regarding results for other immunoglobulins, only notable associations were found for sex and age. In brief, IgG and IgM values were higher in females (rp = 0.173 and 0.335, respectively), whereas IgA values (rp = −0.138) were higher in males. An age-related increase was noted for IgA (rp = 0.200), while age-related decreases were noted for IgM (rp = −0.314) and IgE (rp = −0.206).

Correlations among immunoglobulins were calculated pairwise by use of Spearman's correlation coefficient (rs) as shown in Table 3. IgG4 showed a moderate, positive correlation with IgG (rs = 0.242) and a weak, positive correlation with IgE (rs = 0.172).

To assess the need for partitioning reference values by sex and age, magnitudes of between-sex and between-age variations were calculated as SDRsex and SDRage, respectively (Table 4). Both SDRs were below the critical level of 0.4, and thus we chose to derive the RI for IgG4 without partitioning by sex and age. For other immunoglobulins, IgM was judged as requiring sex-specific RI and age-specific RIs only for females.

4. Reference intervals

Fig. 2 illustrates the distribution of reference values (males + females) of IgG4 drawn before and after logarithmic transformation. Notably, the distribution was very close to a logarithmic Gaussian shape judged from the matched shape of the histogram to the theoretical Gaussian curve and from the linearity on the probability paper plot. RIs were derived by the parametric method with or without the LAVE method for all immunoglobulins except IgE, for which the RI was

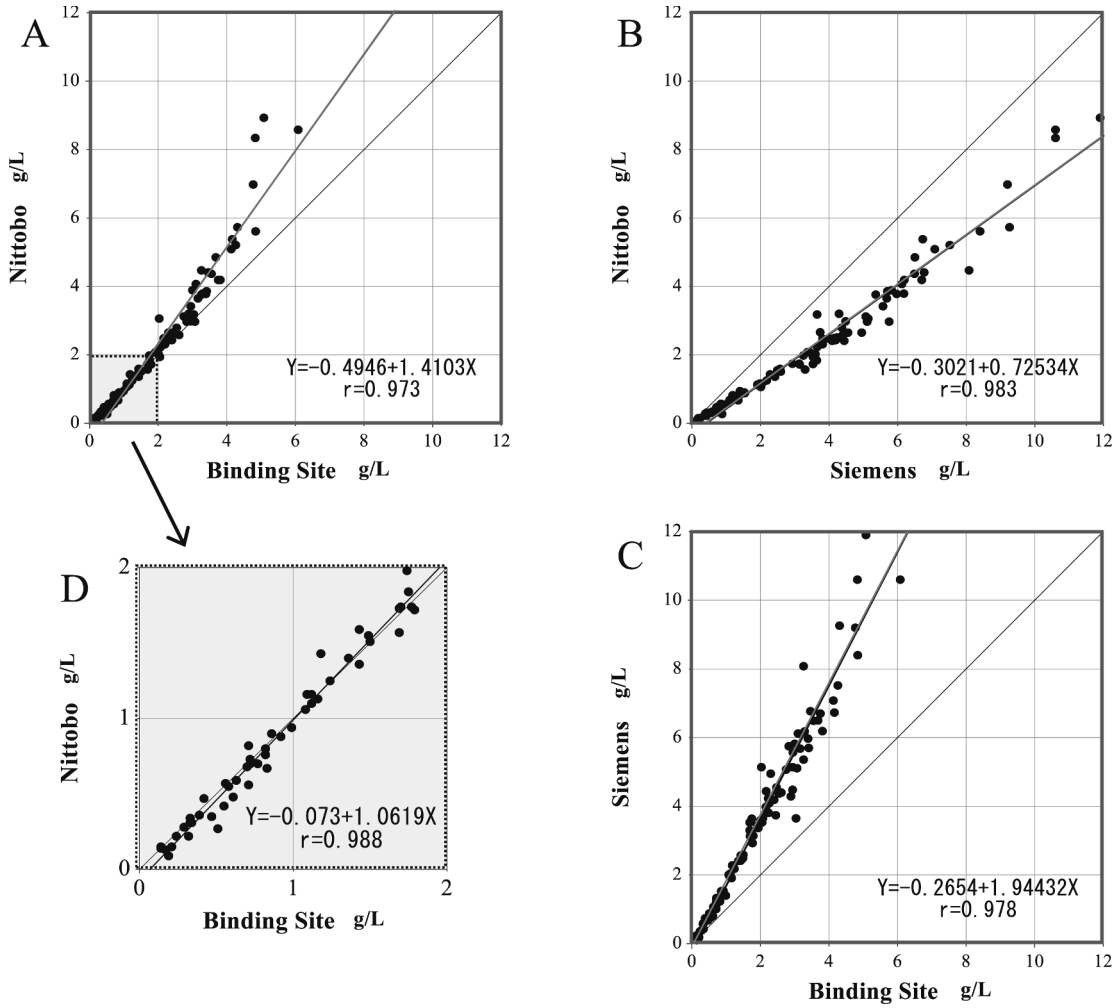

Fig. 1. Correlations among the 3 reagents (Nittobo, TBS, and Siemens). Using test results of 100 clinical specimens measured by 3 reagents, 2-dimensional scattergrams were drawn in 3 combinations (panel A, B, and C). Regression lines were calculated by major-axis linear regression and drawn as the solid line together with the small dotted line of equality (Y = X). In panel D, comparison between Nittobo and TBS values are shown by limiting specimens with IgG4 < 2.0 g/l by Nittobo (n = 50) to demonstrate matched values of 2 reagents at the low concentration range.

calculated with/without exclusion of individuals with self-reported status of allergic diathesis (i.e., pollinosis, atopic dermatitis, or asthma).

The effect of this secondary exclusion was judged from the bias ratio (BR) of difference in UL with/without exclusion (bias UL) based on the following formula:

$$\text{bias ratio (BR) of UL} = \frac{UL_{+} - UL_{-}}{(UL_{+} - LL_{+})/3.92}$$

where UL_{+} and UL_{-} represent UL with/without secondary exclusion, respectively, and LL_{+} denotes LL with the exclusion.

Based on the theory of allowable analytical bias [13], we set 0.25 as the critical value, we found it necessary to use LAVE in deriving the RI for IgG4 (with bias UL = 0.27) and to exclude individuals with allergy in deriving the RI for IgE (with BR of UL = 1.16).

Regarding the need for partitioning reference values by sex or age, we judged it based on SDR ≥ 0.4 (12). Partitioning was found necessary

Table 2
Sources of variation evaluated by multiple regression analysis. Obj Var = object variable, R = SmkLvl = smoking level, DrkLvl = alcohol consumption level, ExerLvl = physical exercise level. The values listed under each source of variation are partial regression coefficient (rp) with its P-value shown in parethesis where appropriate.

Obj Var	n	R	Sex	Age	BMI	SmkLvl	DrkLvl	ExerLvl
IgG4	614	0.171	−0.135	0.050	0.019	0.074	−0.042	0.021
			(0.0020)	–	–	–	–	–
IgG	614	0.193	0.173	0.084	0.044	0.041	0.011	−0.034
			(0.0001)	(0.0385)	–	–	–	–
IgA	614	0.243	−0.138	0.200	−0.006	−0.001	0.032	−0.035
			(0.0013)	(0.0000)	–	–	–	–
IgM	614	0.456	0.335	−0.314	0.022	−0.014	−0.063	−0.013
			(0.0000)	(0.0000)	–	–	–	–
IgE	614	0.243	−0.089	−0.206	0.063	0.016	−0.051	−0.049
			(0.0380)	(0.0000)	–	–	–	–

Table 3
Pairwise Spearman's correlation coefficients among immunoglobulins.

	IgG4	IgG	IgA	IgM	IgE
IgG4	–	0.2419	0.1055	−0.0634	0.1718
IgG	0.2419	–	0.2449	0.1265	−0.0138
IgA	0.1055	0.2449	–	−0.1045	0.1370
IgM	−0.0634	0.1265	−0.1045	–	0.0765
IgE	0.1718	−0.0138	0.1370	0.0765	–

Table 4
Magnitude of between-sex and between-age variations. SDR = standard deviation ratio. Magnitudes of between-sex and between-age variations were calculated as SDRsex and SDRage, respectively. The value greater than the critical of 0.4 were highlighted by bold letter.

	SDRsex	SDRage M	SDRage F
IgG4	0.197	0.000	0.084
IgG	0.214	0.000	0.000
IgA	0.207	0.241	0.000
IgM	**0.489**	0.231	**0.508**
IgE	0.117	0.084	0.317

only for IgM both by sex and age with SDRsex of 0.45 and SDRage for females of 0.55.

Accordingly, final RIs were determined as highlighted by values in bold letter in Table 5. The RI for IgG4 was 0.11–1.21 g/l.

5. Discussion

The goal of this study was to evaluate the analytical performance of the new serum IgG4 assay (Nittobo reagent) and to derive its RI for the Japanese population. The results demonstrated that the new reagent has satisfactory analytical performance for clinical use with high precision and is unaffected by common interfering substances.

The assay principle of the novel reagent is reversed passive latex agglutination, in which the prozone phenomenon does not occur. Therefore, the new reagent is free from yielding falsely low values at a range of extremely high IgG4 concentrations. Thus, the precision of the assay was improved for the higher concentration range with no need for retesting by dilution up to the concenration covered by the standards.

As previously reported [14], there were large differences in test results between the two existing reagents (TBS and Siemens). The values of the new reagent (Nittobo) matched those of TBS reagent at concentrations < 2.0 g/l. Therefore, it is possible to use the IgG4-RD cut-off value calculated from TBS reagent values for the Nittobo reagent [4]. Whereas, at the higher range of values, interpretation of measured values dissociates between reagents, which causes some confusion among clinicians. Harmonization of values among the reagents should be done as soon as possible.

The slope of the regression line between the Nittobo and TBS reagents varied depending on the concentration range. Because there was no problem in linearity of the two reagents in each dynamic range, the cause of the slope change at the higher range seems to be the difference in reactivity of anti-human IgG4 antibodies used in the two reagents: i.e., monoclonal antibody in Nittobo reagent vs. polyclonal antibody in TBS and Siemens reagents. It was reported that IgG4 binds to other IgG subclasses (IgG1, IgG2, and IgG3) through each Fc portion, and the frequency of this between-subclass binding increases in proportion to the IgG4 concentration [15]. Therefore, this binding can result in a concentration dependent differences in the reactivity of anti-IgG4 antibodies, which should differ between polyclonal and monoclonal antibodies.

There are a few reports [16] on RI and biological sources of IgG4 variations in healthy individuals. In this study, we found that there is a slight sex difference in serum IgG4; females have lower serum IgG4 levels than males, although the magnitude was not large enough to require a sex-specific RI for IgG4. Conversely, the RI for IgM was much higher in younger females. Moreover, although serum IgG has been reported to be lower in smokers [17], we did not observe any influence of smoking for IgG or IgG4.

Concerning the relation of serum IgG4 with other immunoglobulins

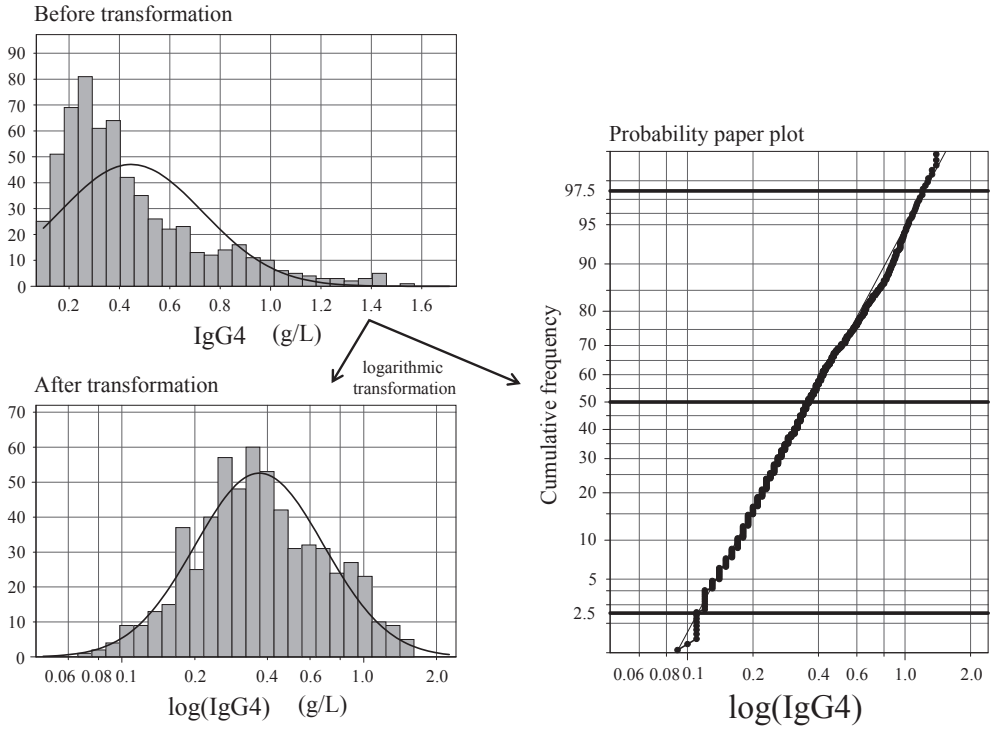

Fig. 2. Characteristics of the distribution of IgG4 reference values. The distribution of IgG4 reference values without sex distinction was drawn before and after logarithmic transformation.

Table 5
All RIs derived by parametric and nonparametric methods with/without the LAVE method. LL = lower limit, Me = median, UL = upper limit, LAVE = latent abnormal values exclusion.

Analyte	unit	LAVE[*1]	Sex	Age	n	LL	Me	UL	BR of UL[*3]
IgG4	g/L	LAVE(−)	MF	18–65	544	0.12	0.37	1.29	**0.27**
		LAVE(+)	MF	18–65	471	**0.11**	**0.36**	**1.21**	
IgG	g/L	LAVE(−)	MF	18–65	552	**8.01**	**12.24**	**17.61**	0.09
		LAVE(+)	MF	18–65	489	8.3	12.27	17.41	
IgA	g/L	LAVE(−)	MF	18–65	552	**1.04**	**2.14**	**4.19**	0.06
		LAVE(+)	MF	18–65	484	1.06	2.16	4.14	
IgM	g/L	LAVE(−)	M	18–65	248	**0.31**	**0.86**	**1.88**	−0.14
		LAVE(+)	M	18–65	217	0.32	0.86	1.94	
		LAVE(−)	F	18–44	147	0.65	1.34	2.83	**0.32**
		LAVE(+)	F	18–44	132	**0.64**	**1.34**	**2.67**	
		LAVE(−)	F	45–65	156	**0.39**	**1.03**	**2.28**	−0.15
		LAVE(+)	F	45–65	145	0.41	1.03	2.35	
Analyte	**unit**	**Allergy**[*2]	**Sex**	**Age**	**n**	**LL**	**Me**	**UL**	**BR of UL**[*3]
IgE	IU/mL	Included	MF	18–65	471	10	71	1057	**1.16**
		Excluded	MF	18–65	355	**8**	**60**	**818**	

RIs were derived by the parametric method after normalizing data using the modified Box-Cox power transformation with/without LAVE method. The RI for IgM was partitioned by age and sex, and that of IgE was partitioned by the status of allergy. The RI by the LAVE method was adopted when the Bias UL exceeded 0.25. The final RIs adopted were highlighted by numbers in bold letters.

*1 The LAVE method was applied by setting reference tests as albmin, IgG, IgA, IgM, IgE, globulin (calculated as total protein minus albmin), transthyretin, C-reactive protein, alanine transaminase, and gamma-glutamyltransferase.

*2 Allergy represents whether individuals with allergic diathesis were excluded or not.

*3 BR of UL represents a difference in ULs with/without exclusion divided by (UL − LL)/3.92.

in healthy individuals, we noted a weak but positive correlation with IgE. Production of both IgG4 and IgE is primarily induced by type 2 helper T cells. However, another regulatory mechanism is also involved, and thus which cytokines are more predominantly expressed determines the production balance of IgE and IgG4 [1]. This partially shared induction mechanism may explain the weak and positive association of IgG4 and IgE in healthy individuals. Furthermore, these associations account for the effect of the LAVE method in lowering the UL of the IgG4 RI.

In conclusion, the analytical performance of the novel IgG4 reagent was satisfactory for clinical use with wide dynamic range, straight linearity of values by serial dilution, and high specificity unaffected by the presence of other IgG subclasses. Because the measured Nittobo reagent values matched very well with those of TBS reagent at a lower concentration range, the IgG4-RD cut-off value can be used in the same manner. Using the LAVE method, we determined a more reliable RI for IgG4 unaffected by latent inflammatory conditions.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.10.032.

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