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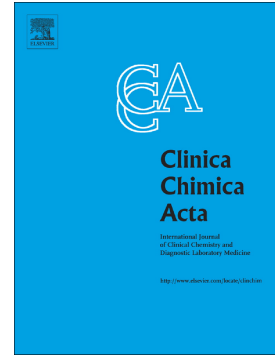
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**The standardization of 5 immunoassays for anti-toxoplasma immunoglobulin G(IgG).**

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**Abstract**

*Background:* Quantitative immunoassays to detect IgG antibodies are the most commonly used tests for diagnosing toxoplasmosis. We investigated the current state of standardization of quantitative immunoassays used to measure anti-Toxoplasma IgG levels.

*Methods:* Four fully automated immunoassays (Architect i4000ISR, Immulite 2000 Xpi, Siemens; Liaison, DiaSorin; Cobas e601, Roche) and one manual immunoassay (ELISA classic Toxo IgG, Virion Serion) were performed on the following: individual patient serum samples, the WHO international standards, control samples, and calibrators provided by 5 immunoassay manufacturers. Statistical analysis was used to illustrate the results.

*Results:* No perfect correlation (slope = 1.0) was found between any 2 assays. Large differences in anti-Toxoplasma IgG titers were observed among the 5 immunoassays using serum samples from individual patients. Using IS 01/600 as a calibrator minimized the inter-assay variability of anti-Toxoplasma IgG values

*Conclusions:* There is still significant effort needed towards standardization of anti-Toxoplasma IgG quantitative immunoassays.

**Keywords:** Toxoplasma gondii; IgG; immunoassays; standardization

## Introduction

*Toxoplasma gondii* (*T. gondii*) is an intracellular parasite that is capable of infecting virtually all warm-blooded animals via vertical or horizontal transmission, resulting in a disease known as toxoplasmosis [1-4]. The seroprevalence of *T. gondii* in the general population located in different regions of the world is variable, with warm and humid climates having higher seroprevalence [5,6]. For most healthy adults, the infection is typically asymptomatic; however, severe symptoms may occur in immunocompromised patients and in a minority of immunocompetent patients [7]. If a woman becomes infected while pregnant, *T. gondii* may be transmitted to the fetus transplacentally, which could lead to severe congenital disease or abortion [1].

Once an individual is infected with *T. gondii*, successive immunoglobulin (Ig) production is the basis for identifying stage of infection. Therefore, immunoassays to detect anti-Toxoplasma IgM and IgG levels are commonly used in the clinic for diagnosing toxoplasmosis [2]. Accurate interpretation of immunoassay results is essential for confirming a recent or past infection [8]. Inaccurate or poorly interpreted results may lead to poor patient outcomes, which is particularly problematic in cases of pregnancy [9]. Since serologic interpretation of IgM results, the results are in combination with other diagnostic means, such as IgG avidity detection or IgG titer analysis, is a better strategy. For IgG titer analysis, interval serum samples from the same patient are tested; increasing IgG titers suggests a recent infection [2].

Several commercial test kits and automated platforms are available for quantitative detection of anti-Toxoplasma IgG antibodies. To improve the harmonization and standardization of anti-Toxoplasma IgG antibody detection, the World Health Organization (WHO) released the second International Standard (IS) for human anti-Toxoplasma Ig in the 1980s [10]. The third human anti-Toxoplasma Ig IS (IS TOXM; 1000 IU of Ig/ampoule) was established in 1994. In 2004, the first IS for human anti-Toxoplasma IgG (IS 01/600; 20 IU of Ig/ampoule)

was established, followed by the fourth human anti-Toxoplasma Ig IS (IS 13/132; 160 IU of Ig/ampoule) in 2015 [11]. For most commercial quantitative IgG tests kits and automated platforms, calibrators are based on the WHO IS and the result unit for the IgG assay is reported in IU/ml.

## **Materials and methods**

### **Individual samples and ethics statement**

A total of **68** leftover serum samples from pregnant patients, each with anti-Toxoplasma IgG levels ranging from 3.0 to 105.0 IU/ml (DiaSorin Liaison Toxo IgG assay), were obtained from a clinical laboratory in a Beijing hospital. Each sample was divided into 5 aliquots and stored at  $-70^{\circ}\text{C}$  with a single freeze–thaw cycle before testing. Pooled normal sera were also collected from leftover human serum samples obtained from a Beijing hospital. The normal samples did not have any detectable anti-Toxoplasma IgG (DiaSorin Liaison Toxo IgG assay). The total volume obtained was approximate 50 ml and stored at  $-70^{\circ}\text{C}$ . Since we used leftover serum samples and did not require detailed patient information for our analyses, the Ethics Committee of the National Center for Clinical Laboratories agreed that it was not necessary for our study to provide written informed consent.

### **International Reference Preparation**

The 3<sup>rd</sup> WHO International Standard (IS) for anti-toxoplasma Serum Ig (code: TOXM, 1000 IU of anti-toxoplasma Ig per ampoule.), the 1st IS for anti-toxoplasma IgG (code: 01/600, 20 IU per ampoule) and the 4th IS for anti-toxoplasma (code: 13/132, 160 IU per ampoule) were provided by the National Institute for Biological Standards and Control (NIBSC). The control serum sample was a Chinese primary reference material for anti-Toxoplasma IgG (GBW09192) provided by the National Center for Clinical Laboratories.

IS 01/600 for Toxoplasma-specific IgG was traced to IS TOXM. The source for 01/600 was a late-stage convalescent phase sera, which contained specific IgG but lacked specific IgA or IgM, thereby making it possible to compare directly direct comparisons of IgG values from different assays.

IS TOXM was reconstituted with 1.0 ml of deionized water. It was then dissolved in pooled normal human plasma to 7.8, 15.6, 31.3, 62.5, and 125.0 IU/ml, followed by serial dilution in phosphate-buffered saline (PBS; sodium phosphate 0.01 mol/L, sodium chloride 0.15 mol/L, pH 7.5). IS 01/600 was reconstituted with 1.0 ml of deionized water, and subsequently diluted in pooled normal human plasma to 1.25, 2.5, 5.0, 10.0, and 20.0 IU/ml. IS 13/132 was reconstituted with 1.0 ml of deionized water, and subsequently diluted in pooled normal human plasma to 2.5, 5.0, 10.0, 20.0, 40.0, 80.0, and 160.0 IU/ml. All dilution procedures were checked by weight. The complete sets of samples were divided into 5 aliquots and stored at -70°C before testing.

### **Measurement of anti-Toxoplasma IgG by immunoassay**

The anti-Toxoplasma IgG titers obtained from individual patients, the pooled control sample from patients, the WHO IS controls, and manufacturer-supplied standards (calibrators) were investigated in triplicate by a multi-center study using 5 different immunoassays. The tests were performed in accordance with manufacturer protocols. Four immunoassays were fully automated (Architect i4000ISR and Immulite 2000 Xpi, Siemens; Liaison, DiaSorin; Cobas e601, Roche) and one immunoassay was performed manually (ELISA classic Toxo IgG, virion-serion). Technical information about immunoassays dependent calibrator and analytical specifications are provided in Table 1. The WHO IS controls and the calibrators from each manufacturer were interspersed between individual patient samples.

### **Data analysis**

Statistical analyses were performed using MedCalc software, SPSS 16.0, Graphpad 6.0, and Microsoft Excel. Immunoassay results of the individual serum samples, the WHO IS controls, and the calibrators were analyzed to examine inter-assay variability. Passing-Bablok regression analysis was used to evaluate the performance of each assay in comparison to the other assays using the mean values of triplicate tests. Pearson correlation coefficients were also calculated.

## Results

### Results for individual patient samples

Fig. 1 and Table 2 show the slopes, intercepts, and Pearson correlation coefficients with respective 95% confidence intervals (CIs). Passing-Bablok regression showed no perfect correlation with slope equaling to 1.0 was observed between the results of any two assays. Correlation coefficients for anti-Toxoplasma IgG results from each assay pair ranged from 0.7709 to 0.9607. The Elecsys assay (Roche) produced considerably higher values than the other assays. The Architect assay (Abbott) produced the lowest mean values compared to the other assays. These results demonstrate inter-assay variability using individual patient serum samples.

### Results for the WHO IS controls and the manufacturer-supplied calibrators

Three WHO ISs (TOXM, 01/600, and 13/132) were analyzed as unknown samples by 5 immunoassays. The results illustrate large inter-assay variability, even when the same IS was used (Fig. 2). The anti-Toxoplasma IgG values were higher using the Elecsys assay (Roche) when compared to the other assays. For IS 01/600 and IS 13/132, the Architect assay (Abbott) produced the lowest values. However, the Liaison assay (DiaSorin) produced the lowest values for IS TOXM.

Passing-Bablok regression showed differences in slope between IS TOXM in negative plasma and IS TOXM in PBS buffer. The Pearson correlation coefficient was  $> 0.97$  for each assay result, regardless of buffer solution used and y-intercepts were not significantly different from 0 (Table 3). The potency of IS TOXM in negative plasma was lower than IS TOXM in PBS buffer using the same immunoassay with the exception of Viron Serion, which illustrated that anti-Toxoplasma IgG values may be affected by the sample matrix.

We also examined the high-value calibrators of the immunoassays. The results for the high-value calibrators provided by each manufacturer for each assay are shown in Table 4. The estimated anti-Toxoplasma IgG values of the calibrators showed inter-assay variability. For each calibrator tested, the anti-Toxoplasma IgG values were underestimated by the other four immunoassays.

### **Results of control plasma before or after standardization with the WHO IS**

The anti-Toxoplasma IgG level means and their respective CVs of the control samples are shown in Table 5. The means varied from 11.83 to 228.33 (IU/ml). After standardization with IS 01/600 and then IS 13/132, the anti-Toxoplasma IgG level means varied from 7.57 to 10.95 (IU/ml) and from 20.65 to 137.72 (IU/ml), respectively.

### **Discussion**

Enzyme-linked immunosorbent assays (ELISA) and chemiluminescence assays (CIA) are routine screening methods for toxoplasmosis in clinical laboratories [12]. Several commercial ELISA test kits and automated platforms are available for quantitative detection of anti-Toxoplasma IgG antibodies. Results of quantitative anti-Toxoplasma IgG testing are typically reported in IU/ml because the manufacturer-supplied calibrators are traced to the WHO ISs. A previous study [13] compared the results of patient serum samples detected by six au-



tomated immunoassays for anti-Toxoplasma IgG. Study findings showed that inter-assay standardization was suboptimal. Of note, the second and third generation WHO ISs were used for calibration of the six automated immunoassays. Then, the WHO released the first IS for anti-Toxoplasma IgG (Code number: 01/600) with an assigned potency of 20 IU, followed by a fourth IS for anti-Toxoplasma Ig (Code number: 13/132). However, few studies have illustrated the harmonization or standardization of anti-Toxoplasma IgG detection using immunoassays currently implemented. The goal of our study was to characterize the standardization problem inherent to immunoassays used to detect anti-toxoplasma IgG. According to our study results, there is large inter-assay variability regarding estimated anti-Toxoplasma IgG values.

Table 2 summarizes the Passing-Bablok regression analysis of **68** individual patient serum samples for anti-Toxoplasma IgG measurements using 5 immunoassays. Results showed inter-assay variability regarding estimated anti-Toxoplasma IgG levels, and no perfect correlation (slope = 1.0) existed between any two assays. In general, the highest anti-Toxoplasma IgG values were obtained by the Elecsys (Roche) immunoassay, followed by values obtained by the Serion ELISA classic Toxo IgG (virion-serion), Immulite (Siemens), Liaison (DiaSorin), and Architect (Abbott) immunoassays.

We propose several reasons for lack of standardization among the immunoassays. First, the WHO ISs are “biological standards” (class B certified reference material; CRM) [14,15]. The standards deriving from pooled human sera were not pure or derived from purified analytes. The values were assigned and not calibrated by using a measurement system or reference method. Uncertainty estimations were not provided and no commutability studies were conducted. Deficiencies found in the WHO standardization process may influence further standardization of Toxoplasma-specific IgG assays. Second, the manufacturer-supplied calibrators of the 5 commercial immunoassays were all derived from WHO ISs (Table 1).

However, the indicated IS used for each calibrator varied. Two calibrators (Liaison Toxo IgG and Virion Serion) were derived from second IS, two calibrators (Elecsys Toxo IgG and Immulite) were derived from IS TOXM, and one calibrator (Architect Toxo IgG) was derived from IS 01/600. No details for the principles of traceability have been well described through a traceability chain, and no uncertainty estimations regarding traceability have been provided. Since a perfect correlation did not exist between any two assays, it is likely that the manufacturers' process of traceability is variable. Third, the differences between immunoassays may be also related to different antigens used (native antigen versus recombinant antigen). The advantages of using native antigen is real antigenic value, however, the main disadvantage with using native antigen is that it is difficult to standardize and is often contaminated with extra-parasitic material, which could result in inter-assay variability [16, 17]. Using recombinant antigen of the different infection stages improves standardization and increases the probability of accurately discriminating between a recent and past infection by *T. gondii* [16-18]. However, incorrect folding of the antigen during production could result in incorrect test outcomes. The immunoassays performed in our study used different antigens with various specificities and affinities for anti-Toxoplasma antibodies. Among the 5 immunoassays used, two fully automated immunoassays (Elecsys and Architect) used recombinant antigen, while the other three immunoassays used native antigen. It is also possible that lack of standardization in preparation and purification methods for each antigen could result in variable values in different lots for the same immunoassay. Fourth, the testing principles differ from one immunoassay to another. Four of the immunoassays adopt indirect formats, the exception being Elecsys Toxo IgG, which uses a sandwich format. The sample dilution factor varied among immunoassays using an indirect format, which may influence the quantitative testing results. Table 1 shows the differences between the immunoassays regarding interpretation of results. However, our study shows that inter-assay variability exists despite having a similar interpre-

tation range. For example, Architect Toxo IgG and Elecsys Toxo IgG share a common interpretation range for reactive results ( $\geq 3.0$  IU/ml), yet each assay produced different results for the same specimen.

Anti-Toxoplasma IgG values in a single patient serum sample may also vary between different immunoassays if the sample has a low level of specific IgG, as this increases the difficulty of interpretation of IgG results. Unfortunately, this is a frequent occurrence in clinical settings. Inaccurate or poorly interpreted results may lead to suboptimal clinical decision-making and poor patient outcomes. IgG titer analysis of serum samples from a single patient collected at intervals is best practice for confirming a recent or past *T. gondii* infection. An increasing anti-Toxoplasma IgG titer over time suggests a recently acquired infection. In such situations, two successive titrations from the patient should be analyzed using the same commercial immunoassay. In light of the reasons explained above, it is necessary to further standardize diagnostic immunoassays with standardized purified antigen and standardized traceability methods for calibrators.

For the 5 immunoassays used in the study, anti-Toxoplasma IgG values were calculated using calibrators. As mentioned previously, the calibrators have been standardized against WHO ISs, yet there is no uniformity in the IS used. Interestingly, there was inconsistency in results when examining the high-value calibrators of all 5 immunoassays (Table 4). For each immunoassay's calibrator, the IgG values were underestimated by the other four assays. In order to illustrate that usage of a common IS could minimize inter-assay variability in anti-Toxoplasma IgG results, we used pooled control sera and compared IgG values by Passing-Bablok regression analysis using IS 01/600 or 13/162 as a common standard (Table 5). Our results showed that variability in anti-Toxoplasma IgG values among the 5 immunoassays was minimized when IS 01/600 was used as a standard. However, inter-assay variability in anti-Toxoplasma IgG values were not minimized when calibrated by IS 13/162. It is im-

portant to point out that IS 13/162 contains high levels of IgG, IgA, and IgM with a potency higher than IS 01/600 (160 IU Ig/ampoule versus 20 IU Ig/ampoule) [11]. Consequently, standardization with IS 13/162 may lead to inter-assay variability in IgG results depending on the immunoassay's specificity in antibody testing (e.g., detects IgG only, detects multiple Ig classes). Specific IgM and IgG antibodies interact and may produce false-positive or false-negative results in immunoassays measuring specific IgG. As mentioned previously, the source for 01/600 was a late-stage convalescent phase sera, which contained specific IgG but lacked specific IgA or IgM, allowing for direct comparison of specific IgG from different assays [12]. Additionally, the avidity of IgG in IS 01/600 is higher than the avidity of IgG in IS 13/132 [11].

Given this information, it appears that using IS 01/600 for calibration would produce more accurate results and less inter-assay variability in results compared to using IS 13/132 as a reference standard.

We also investigated whether or not the diluent used had an effect on anti-Toxoplasma IgG values. Using Passing-Bablok regression analysis, we compared the differences in slope between series dilution of IS TOXM using negative sera versus PBS buffer solution. We found that the potency of IS TOXM differed between the two solutions despite using the same immunoassay. The most common method used for detection of anti-Toxoplasma IgG is the indirect detection method in which serum samples are diluted before testing. However, the buffer solution provided by manufacturers is variable, which, based on our results, may result in IgG value variability. Additional studies are necessary to further investigate the effects of manufacturer-supplied buffer solutions on immunoassay results, ideally using a single IS.

Three limitations in our study were identified. First, we used only 5 immunoassays, thus limiting our ability to compare a wide range of immunoassays. Second, we had a limited

number of individual patient serum samples and we had no follow-up seroconversion serum samples, which limited our data pool.

In conclusion, our study illustrated that there is still significant progress to be made towards standardization of anti-Toxoplasma IgG quantitative immunoassays. For further standardization, we recommend that standardization committees and organizations present a practical protocol for the direct value transfer of commercial calibrators from CRMs [19]. We also suggest that assay manufacturers establish calibrators using the same CRM (e.g., IS 01/600). Assay manufacturers should also ensure that the calibrators are traced to the CRM by metrological principles, as well as provide information for commutability and an uncertainty budget. An alternative approach would be to transition from quantitative assays to qualitative assays, which have greater clinical sensitivity and specificity [20].

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**Fig 1. Linear regression analysis of individual samples for anti-toxoplasma IgG in immunoassay pairs.**

Each sample was measured in triplicate for each assay.

**Fig 2. Linear regression analysis of WHO IS using various assays.**

(A) Dilution series of IS TOXM;(B) Dilution series of IS 01/600; (C) Dilution series of IS 13/132.

Each sample was detected in triplicate for each assay.

**Table1 Technical information for the 5 assays used for the measurement of anti-*Toxoplasma* IgG**

Immunoassay(Manufacturer)	Instrument used	Sample Volume	Antigen used	WHO IS used for calibration	Calibration curves	Reference range (IU/ml)	Interpretation of Results
Liaison Toxo IgG (DiaSorin )	LIAISON	20 $\mu$ L	Native antigen	Second	Two point	0.6–400	Nonreactive: < 7.2 IU/ml; Reactive: $\geq$ 8.8 IU/ml; Indeterminate: $\geq$ 7.2 to < 8.8 IU/ml



							Nonreactive: < 1.6 IU/ml;
Architect Toxo IgG (Abbott)	ARCHITECT i4000ISR	75 $\mu$ L	Recombinant antigen (P30 and P35)	01/600	Multipoint	0– 200	Reactive: $\geq$ 3.0 IU/ml; grayzone : $\geq$ 1.6 to < 3.0 IU/ml.
Elecsys Toxo IgG (Roche)	cobas e 601	10 $\mu$ L	Recombinant antigen	TOXM(T hird)	Two point	0.13– 650	Non-reactive: < 1 IU/ml Indeterminate: $\geq$ 1 to < 3 IU/ml Reactive: $\geq$ 3 IU/ml
Immulinite(Siemens)	Immulinite 2000 Xpi	10 $\mu$ L	Native antigen	TOXM(T hird)	Two point	5– 250	Non-reactive: < 6.0IU/ml Indeterminate: $\geq$ 6.0 to < 8.5 IU/ml Reactive: $\geq$ 8.5 IU/ml

							Non- reactive: < 10.0IU/ml Indeter- minate: ≥
Serion ELISA classic Toxo IgG (virion- serion)	Microplate spectropho- tometer	1 μL	Native antigen	Second	One point	5– 500	10.0 to < 20.0 IU/ml Reactive: ≥ 20.0 IU/ml

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**Table 2. Linear regression analysis of individual leftover serum samples for anti-Toxoplasma IgG measurements between 5 immunoassays**

Assay pair (x-y)	Slope (95% CI) <sup>a</sup>	Intercept (95% CI) <sup>a</sup>	$r^b$ (95% CI)
Diasorin- Abbott	0.3823 (0.3254–0.4632)	-0.7004(-1.3870–0.0242)	0.9137 (0.8634–0.9460)
Diasorin- Roche	13.0355 (10.0302–17.0212)	-42.0831 (-102.2237– -17.4550)	0.8013 (0.6958–0.8729)
Diasorin- Siemens	1.5122 (1.3991–1.7125)	-1.2721(-3.8507–0.1757)	0.9607 (0.9369–0.9757)
Diasorin-Viron Serion	2.5010 (1.9971–2.8766)	-0.5853 (-5.3676–3.4604)	0.9117 (0.8602–0.9447)
Abbott- Roche	30.1528(26.2373–35.5144)	0.2479 (-4.3626–10.6705)	0.8693 (0.7958–0.9176)
Abbott- Siemens	3.3551(2.9753–4.1889)	2.6310 (-0.2511–3.4826)	0.9017 (0.8449–0.9384)
Abbott- Viron Serion	5.9391(4.8630–7.5045)	4.2727 (-2.2403–8.8422)	0.8109 (0.7097–0.8793)
Roche- Siemens	0.1114(0.0942–0.1364)	3.7462 (1.2058–4.8775)	0.8630 (0.7864–0.9135)
Roche- Viron Serion	0.2040(0.1368–0.2845)	7.3689 (3.7033–10.7314)	0.7709 (0.6524–0.8526)
Siemens- Viron Serion	1.6225(1.4210–1.9161)	3.4802 (-0.5799–6.8887)	0.9402 (0.9046–0.9628)

<sup>a</sup> Passing-Bablok slopes and intercepts are expressed as means and 95% confidence intervals (CIs)

<sup>b</sup> Correlation coefficients,  $P < 0.0001$

**Table 3. Linear regression analysis of WHO TOXM using different dilution buffer**

Assay	WHO TOXM, IU /ml	
	In negative plasma	In PBS buffer
Diasorin	0.4387 (0.04646)	0.4943(0.03274)
Abbott	0.9268(0.02053)	0.9527 (0.04869)
Roche	1.7057 (0.02281)	1.7307 (0.00711)
Siemens	1.2320 (0.02420)	1.6119 (0.03933)
Viron Serion	1.45792 (0.01530)	1.3532(0.11808)

Values provided are slopes (and std. error of slopes).

Pearson correlation coefficients > 0.97 for all buffers.

Y-intercepts were not significantly different from 0.

**Table 4. Analysis of anti-toxoplasma IgG calibrators for 5 immunoassays**

Assay	Calibrators measured, IU/ml				
	Liaison (DiaSorin )	ARCHI- TECT (Ab- bott )	Elecsys (Roche)	Immulite(Siem ens)	Viron Serion
Target value	265.0	200.0	100.0	60.799	63.8
Liaison	—	20.37	6.98	<3.00	<3.00
Architect	5.37	—	1.27	0.83	0.10
Elecsys	60.36	29.47	—	38.07	5.42
Immulite	12.07	101.03	6.88	—	<5.00
Viron Serion	25.6	129.2	26.7	6.7	—

**Table 5. The results of control before and after standardization to the WHO IS**

Assay	Mean anti- Toxoplasma IgG before standardization, IU/ml (CV,%)	Regression equation for IS 01/600 <sup>a</sup>	Mean anti- Toxoplasma IgG after standardization, IU/ml (CV,%)	Regression equa- tion for IS 13/132 <sup>b</sup>	Mean anti- Toxoplasma IgG after standardization, IU/ml (CV,%)
Diasorin	28.10 (1.85)	$y = 3.0700x + 0.8192$	8.89 (1.90)	$y = 0.3232x + 9.5325$	57.45 (2.80)
Abbott	11.83 (0.49)	$y = 1.08709x - 0.0729$	10.95 (0.48)	$y = -0.4156x + 3.2525$	20.65 (0.67)
Roche	228.33 (2.74)	$y = 26.387x + 28.4762$	7.57 (3.13)	$y = 1.6126x + 6.2500$	137.72 (2.82)

Siemens		$y = 4.2723x -$		$y = 0.8741x -$	
	36.83 (3.81)	6.3583	10.11 (3.25)	2.7317	45.26 (3.55)
Viron		$y = 6.4577x +$		$y = 1.0359x +$	
	71.06 (1.96)	7.0125	9.92 (2.18)	18.6083	50.63 (2.66)
Serion					

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<sup>a</sup> IS 01/600 was serially diluted to 1.25, 2.5, 5, 10, and 20 IU/ml in the normal human plasma pool.  $x =$

IU/ml,  $y =$  IU/ml;

<sup>b</sup> IS 13/132 was serially diluted to 10, 20, 40, 80, and 160 IU/ml in the normal human plasma pool.  $x =$

IU/ml,  $y =$  IU/ml;

ACCEPTED MANUSCRIPT

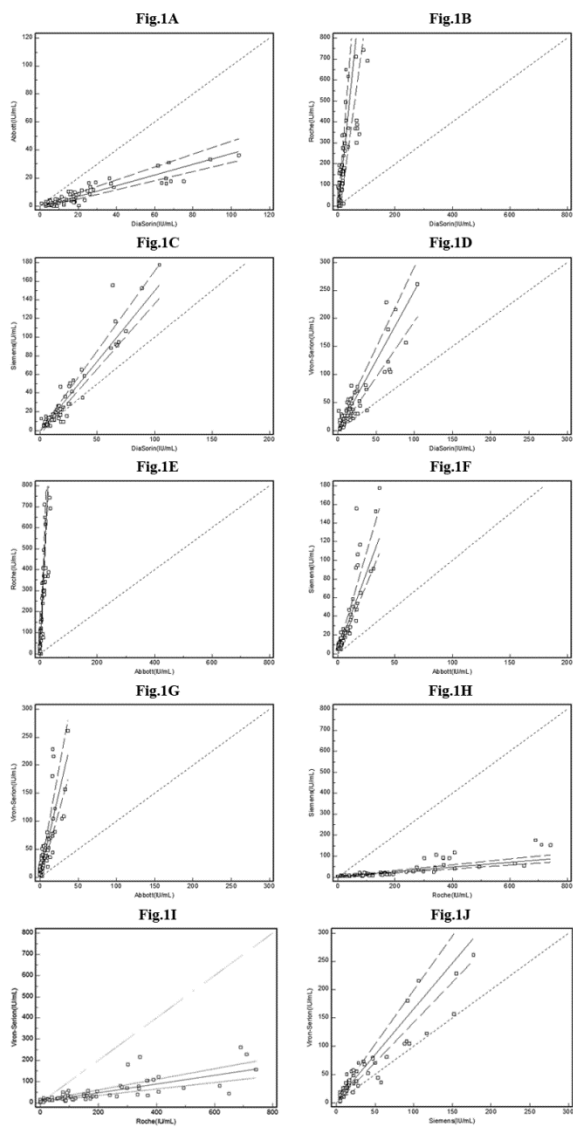


Fig. 1

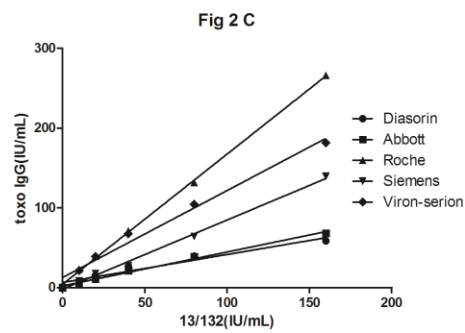
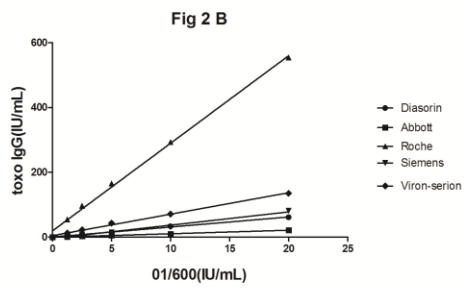
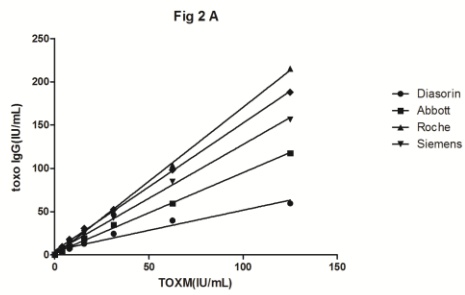


Fig. 2



### Highlights

1. No perfect correlation was found between any two-assay pair for sera samples.
2. Sample matrix may affect quantitative anti-toxoplasma IgG.
3. Standardization using IS 01/600 as a common standard, a good coincidence of anti-toxoplasma IgG by 5 immunoassay could be obtained.
4. There is still significant progress to be made towards the standardization for quantitative anti-toxoplasma IgG detection.