Preliminary evaluation of the candidate international reference for Epstein–Barr virus capsid antigen immunoglobulin A in China

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Research Article

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Abstract

Background: Epstein–Barr capsid antigen (VCA) immunoglobulin A (IgA) detection is widely used for nasopharyngeal carcinoma (NPC), but reference standard for VCA-IgA has not yet been determined. For uniform and quantitative detection of VCA- IgA, reference standard is in urgent need.

Methods: A mixed reference serum from three patients with NPC diluted with healthy subjects serum was made as a possible first international standard for VCA-IgA. Four ELISA kits and two chemiluminescent immunoassays (CLIA) kits were used to detect VCA-IgA in twenty NPC patients using the reference as a calibration curve. The performance of six kits was evaluated, and the quantitative results were compared.

Results: Our results shown the reference has good linearity in different kits. Without reference, the difference of total CV (from 3.98% to 43.11%) and Within-run CV (from 2.47% to 19.66%) was large in 6 kits. The positive and negative coincidence rate between 6 kits and IFA for NPC diagnosis was 75% overall agreement, but there was a difference among the six kits ranged from 55%-90%. Concentration of 20 NPC samples divided into three sample categories was shown significant difference in a few subgroup of 6 methods. But a good correlation ($R^2=986$) was observed between Antu and Beier ELISA kits.

Conclusions: The reference serum may be used as a reference standard and better compared results from different methods/laboratories. However, due to the diversity of VCA antigens, the quantitative results of some kits are still inconsistent.

Introduction

South China is a highly endemic area of Nasopharyngeal carcinoma (NPC). The ASIRW (age-standardized incidence rates of world) in South China (9.69/100,000) was 3.4 times higher than that in Southwest China (2.85/100,000), in which the incidence is the second highest. [1, 2] Epstein–Barr virus (EBV) infection is one of the most important factor for NPC onset. [3] EBV associated antibodies, such as virus capsid antigen (VCA-IgA) and Epstein-Barr virus nuclear antigen 1 (EBNA1-IgA), exert an important role in the screening and diagnosis of NPC. [4] VCA-IgA is one of the most widely antibody markers used for assisting in monitoring efficacy of NPC and screening NPC. [5–8] It was usually measured by indirect immunofluorescence (IFA) [9] or enzyme-linked immunosorbent assay (ELISA) [10]. IFA was used as the "gold standard" for detection of VCA-IgA antibody. In virtue of enzyme-labeled antibodies instead of fluorescent antibodies, IEA did not need a fluorescence microscope to interpret the results which the semi-quantitative results were reported by titers was widely used in southern China. [2, 3].

The titer of VCA-IgA was closely related to the longterm curative effect and prognosis of NPC. [11, 12] Low titer has better efficacy and prognosis. Moreover, ascending EBV antibodies titers are strongly associated with an increased risk for NPC. [13, 14] Although, the titer detected by traditional IEA or IFA has considerable prognostic value, it is not suitable for mass NPC screening because of the need for manual interpretation. ELISA-based assays have similar detection sensitivity and specificity to IFA, [4, 15] and have other advantages. ELISA-based assays are easier to be standardized, with greater false tolerance to interference, and are also more labor-saving when analyzing a large number of specimens. Chemiluminescent immunoassay (CLIA) has advantages similar to ELISA have been developed in China, but no literature has been reported yet.

In summary, ELISA and CLIA are convenient and automated. But the performance and standardization of ELISAs and CLIAs used different peptide segments of VCA are still a matter of debate and concern. In addition, while new

methodologies and new platforms (automated vs. manual, ELISA vs. CLIA) for the detection of VCA-IgA have become available, proper interassay simultaneous comparisons have not been carried out. One important topic of discussion is whether to use nature reference preparations to calibrate these assays for quantitative testing. And the proper cross-validation and evaluation of the available for different methods and brands of kits are lacking.

But so far, reference standard for VCA-IgA has not yet been determined by the World Health Organization (WHO) International Laboratory and Collaborating Centers for Biological Standards. Most commercial ELISA kits only generate either negative or positive results.[16] Although our previous studies have confirmed that the rOD value of ELISA shows a good correlation with the titer from IEA. [2] At present, VCA-IgA detected by ELISA is unable to be used as a prognostic factor for NPC as IFA. As VCA-IgA ELISA is widely used in clinical laboratories, calibrator is in urgent need. Recently, at the request of the Guangdong Provincial Anticancer Association (<http://www.gdaca.org.cn/>), Southern China Tumor Markers Standardization Alliance (Guangzhou, Guangdong, P.R.China) was given a potential reference sample from Zhong He (Guangzhou, China) and Sun Yat-sen University Cancer Center (SYSUCC, Guangzhou, China), obtained from three patients with NPC and mixed with serum samples from healthy subjects for dilution purposes. This will be made available as the first international VCA-IgA reference reagent if it fulfils certain criteria for such use. We have performed a collaborative study in six clinical laboratories to evaluate different immunoassays in the quantitative detection of VCA-IgA and verify whether improve comparability of the results using this reference.

Material And Methods

Preparation of the reference serum

The VCA-IgA reference sample was prepared using serum from three strongly VCA-IgA positive NPC patients who were histologically confirmed by biopsy. Each patient took 20 ml of blood and added CaCl_2 to a final concentration of 18 mmol/L. Subsequently, the solution was incubated for 2 h at 37 °C and the insoluble material was removed by centrifugation (30 min at 10000 rpm). Finally, the supernatant was filtered with a 0.2 µm pore size nitrocellulose filter.

The VCA-IgA reference material was prepared by diluting the NPC serum by testing a series of dilutions in the ELISA assay from Euroimmun AG (Lubeck, Germany) and chemiluminescent immunoassay (CLIA) from New Industries (Shenzhen, China). The concentration closed to upper limit of detection method was identified as the best dilution concentration. And this optimum concentration was assigned to a target value of 16000 U/ml. Serum samples obtained from healthy blood donors were mixed to a pool as a dilutions. The donors' serum samples were individually tested in the Euroimmun IFA assay and were all found to be VCA-IgA negative. All serum samples were also found to be negative for anti-HIV 1 + 2, HbsAg and anti-HCV (Hepatitis C Virus).

The bulk material was produced by diluting the VCA-IgA-positive NPC serum in pooled serum from 50 blood donors, using the optimal dilution factor as determined above. Finally, 1 liter bulk reference serum of VCA-IgA was prepared. Subsequently, the bulk prepared reference serum was divided into 0.2 ml portions and preserved in -80°C refrigerator.

Patient And Public Involvement

Twenty patients with NPC were confirmed by histologically biopsy and further tests, including head and neck MRI and chest X-rays. Negativity and low and medium/high positivity was defined as result of VCA-IgA titer detected by VCA-IgA Euroimmun IFA. Seven were negative(titer < 1:20), seven were low positive($1:20 \leq \text{titer} \leq 1:80$), and six were medium/high positive(titer > 1:80)for VCA-IgA.

All patients were informed at the initial visit that the remaining samples of routine test items may be used for medical research, but these studies will not cause additional cost and damage to them and obtain their written consent. And the ethics committees approvedthis consent procedure.

VCA-IgA Measurements

For the evaluation of VCA-IgA levels, we used 6 commercial kits to analyze the reference and sera from 20 patients with NPC. Four immuno-enzymatic (ELISA) commercial kits produced by the following manufacturers: Euroimmun AG (Lubeck,Germany), Tarcine BioMed (Beijing, China), Antubio (Zhengzhou, China), Beier (Beijing, China). And 2 chemiluminescent immunoassay (CLIA) kits produced by the following manufacturers and tested by matching detection systems: New Industries Biomedical Engineering (Maglumi 4000 Plus System, Shenzhen, China) and YHLO Biotech (iFlash 3000 CLIA System ,Shenzhen, China). Antigen peptides, substrate, incubation time, volume and dilution of serum, type of conjugate, enzymatic substrate and cut-off level of six kits are not exactly the same. (Table 1)

Table 1
Characteristics of the 7 commercial kits for detecting VCA-IgA antibodies .

Manufacturer	Method	Antigen	Chromogenic / luminescence reaction system	Sample dilution	Testing laboratory
Euroimmun	IFA	P3HR1	FITC	1:10	SYSUCC
	ELISA	gp125	HRP/TMB	1:100	SAHGUCM
Beier	ELISA	p18	HRP/TMB	1:10	CHSUMC
Tarcine	ELISA	p18	HRP/TMB	1:10	PHJ
Antu	ELISA	gp125,p18	HRP/TMB	1:10	ATHZU
New Industries	CLIA	p18,p23	ABEI	1:10	SYSUCC
YHLO	CLIA	p18,p23	Acridinium ester/ H2O2	1:10	LMCHCH
TMB, tetramethyl benzidine; ABEI, N-(4-Aminobutyl)-N-ethylisoluminol; SYSUCC, Sun Yat-sen University Cancer Center; SAHGUCM, Second Affiliated Hospital of Guangzhou University of Chinese Medicine; CHSUMC, The cancer hospital of shantou University Medical college; PHJ, People's Hospital of Jieyang; ATHZU, Affiliated Tumor Hospital of Zhengzhou University; LMCHCH, Liuzhou Maternity and Child Health Care Hospital					

VCA-IgA titer of 20 patients were also tested by Immunofluorescence assay (IFA) as the gold standard method.

Linearity, Precision and Comparability

The reference serum was serially prediluted from 1:1 to 1:64 by dilutions in respective kits. The linearity of the measurements was calculated by means of linear regression statistical analysis (slope value with 95% CI, adjusted R^2) using the original reference and seven predilutions of the reference serum were further diluted according to the manufacturer's protocol, with each of the 6 commercial methods (Table 1).

The precision was evaluated according to the EP15-A2 protocol of the Clinical and Laboratory Standards Institute (CLSI).[17] The original reference and 1:16 predilution were analyzed three times per day during a period of five consecutive workdays ($n = 15$ per level). Precision was evaluated as the coefficient of variation (CV), which was calculated from the data series mean and standard deviation.

To evaluate the efficacy of harmonisation of antibody levels, sera from 20 NPC patients with different VCA-IgA concentrations (13 positive and seven negative, as measured in the IFA) were analyzed with 6 commercial kits. The VCA-IgA concentrations were calculated by calibration curve obtained by the original and seven dilutions of the reference serum. A concentration value of 16000 units (U/ml) was arbitrarily assigned to the original reference serum.

Statistical analysis

Scatter diagram and Linear regression analysis were performed by MedCalc version 11.4.2.0. The VCA-IgA concentration of each patient by 6 methods divided into three sample categories was compared via randomized block design ANOVA. Then, LSD test were used to make multiple comparisons between the groups. Quantitative results were correlated by the non-parametric Spearman test. The statistical analyses were performed using IBM SPSS Statistics version 19.0 (IBM Corp.). Statistical significance was considered present at $p < 0.05$.

Results

Linearity

Linear regression analysis was performed to evaluate the linearity of the quantitative data. Graphs and statistical values (slope with 95% CI, adjusted R^2) are shown in Fig. 1 and table S1. Coefficients of regression as a measure of linearity of the diluted reference serum tested with each one of the 6 commercial methods ranged from 0.00007181 to 11.276 (table S1). Except for Tarcine ($R^2 > 0.9367$), regression analysis for the other 5 commercial kits had achieved a good correlation coefficient ($R^2 > 0.96$) over the entire range tested. (Fig. 1, table S1)

To verify whether linearity was present even at low and high antibody concentrations, we diluted one low and one high VCA-IgA serum in the same way in which we had diluted the reference sample (predilutions from 1:0 to 1:64 followed by the dilution requested in each assay). The results showed that linearity was maintained even when low antibody concentrations, but did not maintained in upper high antibody concentrations (Figure S1).

Precision

The VCA-IgA concentrations of Euroimmun and Tarcine kits were calculated by one calibrator curve provided in each kit. The VCA-IgA concentrations of New Industries and YHLO kits were calculated by the calibration curve provided in each kit. Since the Antu and Beier ELISA kits do not have a self-made calibrator, precision was calculated by OD value of each detection when all the negative and positive controls met manufacturer requirements. In addition to

Beier ELISA kit, other 5 kits have excellent within-run CVs(CV < 8%) at the low and high VCA-IgA levels. Except for Antu and Beier ELISA kits, other 4 kits also have good total CVs(CV < 10%). (Table2)

Table 2

Levels of VCA-IgA antibodies detected in the VCA-IgA antibody reference serum by the 6 commercial methods.

Manufacturer		Cut-off value		Reference serum	Ratio†	Within-run CV		Total CV	
		(arbitrary units)*				High	Low	High	Low
Euroimmun	ELISA	1.1		13.0	11.8	3.98%	6.11%	6.24%	6.52%
Beier**	ELISA	1.1		3.2	2.9	5.03%	19.66%	13.77%	43.11%
Tarcine	ELISA	0.15		1.4	9.3	4.95%	7.58%	8.27%	7.22%
Antu**	ELISA	0.15		3.5	23.3	4.62%	6.45%	13.74%	16.85%
New Industries	CLIA	4		12.0	3.0	2.47%	4.34%	5.95%	9.86%
YHLO	CLIA	1.1		5.6	5.1	4.76%	5.22%	3.98%	4.87%
*VCA-IgA antibody levels detected in the reference serum at the highest concentration tested in each kit.									
†Ratio was calculated as reference serum arbitrary units divided by manufacturer cutoff value for each kit.									
**Precision was calculated by OD value of each test when all the negative and positive controls met manufacturer requirements									

Comparability

Table 2 shows the VCA-IgA values for the highest concentration of the reference serum obtained by the 6 commercial methods in each kit, the cut-off values proposed by each manufacturer, and their ratio. The VCA-IgA concentrations varied from 1.4 arbitrary units given by the Beier kit to 13 arbitrary units given by the Euroimmun kit, showing a very wide dispersion of the quantitative data.

The VCA-IgA concentration of each patient by different methods were shown in line chart. (Fig. 2A,B,C) All three sample categories (negative and low and medium/high positive) detected by 6 kits when results were calibrated by the reference material displayed significant difference between certain of six methods according to LSD test($P < 0.05$). (Fig. 2D,E,F) The value of New Industries and other kits were a little different. New Industries kit was different from other methods in medium/high positive categories by LSD test($P < 0.05$). (Fig. 2F) It was also different from Euroimmun, Beier and Antu kits in negative category. (Fig. 2D) And it was also different from Euroimmun kit in low positive category. (Fig. 2E)

Correlation Between Six Assays

Positive rates of each method were analyzed in 20 NPC patients.(Table 3) Euroimmun ELISA showed the highest positive rate in seven methods.The positive and negative coincidence rates between six method and gold standard method (IFA) are the same, but the differences between six method are great(ranged from 55%-90%). (Table 3) And the positive and negative coincidence rates of the four ELISA methods(Euroimmun and Beier and Tarcine and Antubio) were more than 70%.In addition, Beier and New Industries had the highest positive-negative coincidence rate, reaching 90%.

Table 3
Performance and coincidence rate of the different assays

Assay	Positive rate(%) ^a	Coincidence rate of positive and negative(%) ^b					
		Beier	Tarcine	Antubio	New Industries	YHLO	IFA
Euroimmun	90	70	75	70	85	75	75
Beier	60		75	80	65	55	75
Tarcine	65			85	90	60	75
Antubio	70				75	55	75
New Industries	70					70	75
YHLO	70						75
IFA	65						
a, Positive rate of assay in 20 patients.							
b, Coincidence rate of positive and negative in 20 patients.							

Table 4 show the Spearman correlation coefficientsbetween the different assays. Almost all assays gave a correlation coefficient lower than 0.80.Although quantitative results among different methods are not exactly the same, a good correlation of was observed between Antu and Beier ELISA kit(0.986), and between Tarcine ELISA kit and New Industries CLIA kit (0.897).(Fig. 3)

Table 4
Spearman correlations coefficients in the six assays.

Manufacturer	Euroimmun	Beier	Tarcine	Antubio	New Industries	YHLO
Euroimmun	1	0.4937	0.4646	0.4971	0.4396	0.5352
Beier		1	0.6951	0.9857	0.6232	0.6412
Tarcine			1	0.6347	0.9459	0.5415
Antu				1	0.5415	0.6171
New Industries					1	0.4467
YHLO						1
Bold represent results > 0.9						

Discussion

As the VCA-IgA was in widespread use in clinical laboratories as a major marker for NPC, three methods include IFA, ELISA and CLIA for VCA-IgA antibody detection are available. Numerous manufacturers used different peptides as VCA antigens with different assays has greatly contributed to the dissemination of the NPC screening in clinical laboratories, but the need for comparison of the results obtained with different kits and the urgent need for standardisation of the assay have become a priority. The main objective of this study was to evaluate and compare the performance of different commercial ELISAs and other new immunoassays for the quantitative detection of VCA-IgA. Another important objective was to evaluate the candidate reference serum by six different kits in six laboratories. To this end, a freeze reference sample was prepared by Zhong He (Guangzhou Zhong He Biotechnology Co., Ltd.) and proposed as a candidate reference serum for VCA-IgA detection to the Southern China Tumor Markers Standardization Alliance. This reference was provisionally tested and proved to be consistent with the requirements before and after freezing and thawing. (date not shown) The reference serum showed positive results in all the commercial VCA-IgA kits used. Linear analysis by reference standard curve obtained in all 6 kits showed that the reference sample can be used as a calibrator in assays using different antigenic substrates or different assays. Residual differences may be due to different assay reagents/procedure (dilution buffer, antigenic peptides, sample dilution, etc). Since the linearity of the method cannot be maintained at a higher concentration and the linearity(R^2) does not reach 0.99, it is recommended to use multi-point curves for each kit in the future.

Owing to the same reason of without reference, the precision of each kit is quite different. At present, the VCA-IgA kits can be classified into three categories according to the condition of the kit calibrator. First category, such as Beier and Antu VCA-IgA ELISA kits, they do not have any calibration material, but only negative and positive quality controls. The results were accepted when the negative and positive controls were acceptable within a certain range. Therefore, the results within the batch (Within-run CV) may not be very different, but the results of each batch (Total CV) are quite different. There is no comparison between the quantitative results of different test batches. The second category, such as Euroimmun and Tarcine VCA-IgA ELISA kits, the kit has a critical calibrator with its own. They have satisfactory within-run CV and total CV. But the disadvantage of single point calibrator is that $rODs(OD/cut-off\ OD)$ is difficult to accurately express all values because of imperfect linearity($R^2 = 0.9779$ and $R^2 = 0.9367$). The third category, such as New Industries and YHLO VCA-IgA CLIA kits, they own calibration curves and

provide quantitative results using their own units. Because of the advantages of methodology and matched testing system, they have better Within-run CV and Total CV.

Up to now, there is no systematic evaluation study on the quantitative and standardized research of all VCA-IgA methods. Previous studies on VCA-IgA detection generally used ELISA and IFA,[4] CLIA only related to VCA IgG or IgM antibody tested. In this study, we first included CLIA in VCA-IgA research.[18, 19]

The coincidence rate between the six methods and the gold standard method (IFA) was 75%. However, the coincidence rate of positive and negative between the six methods was quite different. (Table 3) Actually, the results obtained by different methods and expressed in arbitrary units differed so much that it was quite difficult to compare data from different studies and to understand whether VCA-IgA antibody levels could be used for monitoring therapy and prognostic purposes. Many studies have confirmed that VCA-IgA antibody level changes can be used for NPC prognosis and screening.[13, 20–22] Pretreatment serum EBV-VCA/IgA titer may be used as an independent prognostic marker of NPC.[11] Liuet al.'s study found that the geometric mean titer of anti-EBV/VCA IgA antibodies before and after radiotherapy was a significant difference.[23] People with ascending VCA-IgA antibody titers tended to have higher risks and shorter time to develop NPC, compared to those with a descending pattern.[13] For both the ELISA and CLIA, there was good agreement with IFA. Concordance between 6 kits and IFA for primary NPC diagnosis was 75% overall agreement.(Table 3) This result was similar to Karray's research which observed 81–91% overall agreement between ELISA(IgA anti-VCA-p18) and IFA for primary NPC diagnosis. They also found declining reactivity in patients in remission and increasing reactivity in patients with persistent disease or relapsed during follow-up monitoring both in ELISA and IFA detected antibody.[15] Our previous study also found an excellent correlation and a high degree of concordance between IgA titers(IEA) and rOD levels (ELISA),[2] However, Yao et al.'s study did not confirm the role of VCA-IgA as prognostic biomarkers among patients with NPC and undetectable EBV DNA. [24] A possible explanation for these contradictory results may be that the previous studies supporting the positive results use IFA, however this study uses ELISA(Beier kit). In the absence of calibrators, inter-assay differences in ELISA may affect outcome analysis, which is documented in the limitations of this article [24] and is confirmed in our study Total CV = 43.11%. This confirms the importance of the reference in VCA-IgA ELISA detection again.

Through reference, we may connect each different method and analyze the results which were not comparable before. We observed that the same patient has similar results with some different methods, or that the values are different but the trends are the same with different methods.(figure 2) The results of twenty patients tested by the kits calibrated on the reference serum divided into three sample categories was shown certain significant difference in 6 methods compared via randomized block design ANOVA and further analysis according to LSD test. (Fig. 2) New Industries kit is different from other methods in medium/high positive categories by LSD test($P < 0.05$). Moreover, New Industries and Euroimmun were different in the three groups.

Although there are still differences in quantitative results among different methods, the improvement of this study is obvious. The reference material can reduce the difference between different batches with the same kit. Furthermore, the quantitative results by reference standard curves are more accurate than the semi-quantitative results of IFA for monitoring efficacy and risk. IFA/IEA as the "gold standard" for detection of IgA to VCA, are laborious techniques, since they cannot be sufficiently automated to achieve a good level of output, and there is a certain degree of subjectivity in interpreting results. ELISA can achieve quantitative detection through calibration curve, and is easy to automate and detect a large scale of samples quickly. Finally, through reference materials, we may compare the results of different manufacturers' kits. A good correlation (correlations coefficient = 0.986) was observed between

Antu and Beier ELISA kits, which results calculated by reference standard curves. And the same correlation (correlations coefficient = 0.897) was observed between Tarcine and New Industries.

Although different kits calibrated by the reference serum displayed a certain consistency of VCA-IgA antibody results, there are still the low correlation between the results for some different commercial ELISA kit for IgA-VCA. For example, the quantification value of a particular patient was quick differences between some methods. This might be due to the different sources of antigens. This notion is also supported by Gu.[25] As regards measurements of IgA, all assays used the p18 peptide, except Euroimmun, which used gp125. In addition, the other three assays (Antu, New Industries, YHLO) which use the peptide p18 also contains other peptides. The results depend on the use of a range of different antigens, which can produce different serological responses. Although on the use of the same antigen, other factors, such as the different dilutions used for analysis maybe differences. [26] These differences may also lead to the different outcome of therapeutic and prognostic studies using different VCA-IgA kits.

Conclusion

In summary, the use of the reference serum may a certain reduce the differences between different kits and achieve a comparison between different kits. In subsequent studies, we should focus on the diversity between different antigens and try to unify them to promote the further development of VCA-related research.

List Of Abbreviations

nasopharyngeal carcinoma (NPC) virus capsid antigen (VCA) immunoglobulin A (IgA) chemiluminescent immunoassays (CLIA) Epstein-Barr virus nuclear antigen 1 (EBNA1-IgA) indirect immunofluorescence (IFA) enzyme-linked immunosorbent assay (ELISA) Epstein-Barr virus (EBV) early antigen (EA) immunoenzymatic assay (IEA) World Health Organization (WHO) age-standardized incidence rates of world ASIRW Sun Yat-sen University Cancer Center (SYSUCC)

Declarations

Ethics approval and consent to participate

The study follows the principles of the Declaration of Helsinki. This study was approved by the Institutional Review Board of Sun Yat-sen University Cancer Center. The authenticity of this article has been validated by uploading the key raw data onto the Research Data Deposit public platform (www.researchdata.org.cn), with the approval RDD number as RDDA2018000941

Consent for publication

All patients provided written-informed consent for the collection and publication of their medical information at the first visit to our center, which was filed in their medical records, and the ethics committees approved this consent procedure.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of Interests: NONE

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Authors' contributions

YWX and SLC conceived and designed the experiments.HC and QC and XBW and NX and YLD performed the experiments: HC and QHZ analyzed the data. HC and YWX wrote the paper.All authors read and approved the final manuscript.

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Additional Files

Table S1. A summary of linear regression studies

Figure S1. The regression curves obtained by diluted one more low and high VCA-IgA serum concentrations with each of the 6 commercial methods. Values of the ordinate are given in the units used by the assay kit, while the abscissa shows the arbitrary units of the reference sample.

Figures

Figure 1

The regression curves obtained with each of the 6 commercial VCA-IgA methods. Values of the ordinate are given in the units used by the assay kit, while the abscissa shows the arbitrary units of the reference sample.

Figure 2

VCA-IgA results of 20 patients calibrated by the reference according to three sample categories detected by 6 kits. Line chart of VCA-IgA results detected by each of 6 kits in three sample categories A, negative; B, low positive; C, medium/high positive. The mean VCA-IgA concentration of each patient by 6 methods divided into three sample categories were used LSD test to make multiple comparisons between the groups. D, negative; E, low positive; F, medium/high positive. It has been marked out, when the difference between the two groups is significant ($p < 0.05$). * New Industries group was different from other methods group in medium/high positive categories by LSD test ($P < 0.05$)

Figure 3

The correlations of VCA-IgA concentrations between different kits. A, Antu and Beier ELISA kits. B, Tarcine ELISA kit and New Industries CLIA kit. The low correlation ($R^2 < 0.8$) between other kits was not shown.

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