The Cut-off Point of Anti-dsDNA Test by EliA dsDNA

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ABTRACT

Objective: To determine the cut-off point of anti-dsDNA for screening by EliA dsDNA.
Methods: Serum specimens requested for anti-dsDNA between October and December 2007 were recruited and tested by the *Crithidia luciliae* immunofluorescence test (CLIFT) and automated fluorescence immunoassay (EliA dsDNA). The CLIFT was considered as the gold standard method. Different levels of sensitivity and specificity were determined and the cut-off point was selected from among them.
Results: Of the 133 specimens collected, 35 were positive whereas 98 were negative with the CLIFT. Of those 35 positive specimens, 2, 0, 2, 2 and 29 were, respectively, in ranges of < 5, 5 to 9.9, 10 to 14.9, 15 to 19.9 and > 20 IU/ml by EliA dsDNA. Also, of the 98 negative specimens, 73, 7, 4, 4 and 10 were, respectively, in ranges of < 5, 5 to 9.9, 10 to 14.9, 15 to 19.9 and > 20 IU/ml by EliA dsDNA. The sensitivity and specificity for each level were determined and the value of 11 IU/ml was selected as the cut-off point. Additionally, when clinical diagnosis was used in specimens with discrepant results, the sensitivity of EliA dsDNA was far better than the CLIFT, whereas the specificity of both methods was comparable.
Conclusion: The appropriate cut-off point of EliA dsDNA for screening was 11 IU/ml. Furtermore, the diagnostic value of EliA dsDNA was better than the CLIFT when clinical diagnosis was included in the gold standard criteria.

Keywords: EliA dsDNA, anti-dsDNA, *Crithidia luciliae*

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Anti-dsDNA autoantibodies were first found in 1957.[1,2] They are included in the classification criteria for systemic lupus erythematosus (SLE)[3,4] and their titers are correlated with the disease activity.[5,6] The methods for detecting anti-dsDNA include radioimmunoassay (Farr assay), the *Crithidia luciliae* immunofluorescence test (CLIFT) and the enzyme-linked immunosorbent assay (ELISA). The last two methods are the most widely used. The Farr assay is the standard reference method, but it is time-consuming and technically more difficult. The CLIFT is highly specific, but its sensitivity may be low and its use cumbersome when large numbers of samples are involved, and it is not quantitative. On the other hand, an automated system for anti-dsDNA is more convenient, straightforward, rapid, quantitative, and reproducible. In our laboratory unit, the CLIFT is the method used for anti-dsDNA detection and it is a tedious procedure for 2,000 tests per year. We are considering anti-dsDNA screening by an automated machine to reduce the workload. An automated fluorescence immunoassay (EliA dsDNA) is intended for the in vitro quantitative measurement of IgG antibodies directed to dsDNA with an automated machine. The purpose of this study was the determination of the cut-off point for anti-dsDNA screening, before confirmation with the CLIFT, which is the second best method when compared with other assays.

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MATERIALS AND METHODS

Serum specimens requested for anti-dsDNA test during between October and December 2007 were collected and kept at -20° or -70°. Specimens, which were hemolysed, lipemic, frozen and thawed or inadequate in quantity for the study, were excluded. The code for each specimen was used to protect sample identification.

The specimens recruited were tested for anti-dsDNA by both methods (CLIFT and EliA) with different technicians without their awareness of both

TABLE 1. The sensitivity and specificity of the EliA dsDNA test for each cut-off point.

EliA dsDNA (IU/ml)	CLIFT		Sensitivity % (95%CI)	Specificity % (95%CI)	PPV %	NPV %
	Positive	Negative				
< 5	2	73	100	0	26.32	0
5-9.9	0	7	94.29 (86.42-100)	74.49 (65.92-83.06)	56.90	97.33
10-14.9	2	4	94.29 (86.42-100)	81.63 (74.02-89.24)	64.71	97.56
15-19.9	2	4	88.57 (78.2-98.94)	85.71 (78.84-92.58)	68.89	95.46
≥ 20	29	10	82.86 (70.41-95.31)	89.80 (83.86-95.74)	74.36	93.62

Positive Predictive Value (PPV), Negative Predictive Value (NPV)

results. The EliA dsDNA assay (Phadia GmbH, Freiburg, Germany) was used according to the instructions of the manufacturer. The test uses a single polystylene well coated with double-stranded plasmid DNA and is completely automated and processed on the immuno CAP 100 instrument with software version 2.0 or higher. The results were expressed in IU/ml with a measuring range from 0.5 to 600 and printed automatically after the test was completed. The precision of intra-assay and inter-assay were < 5%. The CLIFT was used for measurement as described by Stanley P. Ballou with minute modification for appropriateness. It has been used for more than 15 years in our laboratory simultaneously with internal quality control for each run. In addition, external quality assurance (College of American Pathology survey) has also been applied during the last few years.

The sensitivity of EliA dsDNA test, which is higher than the CLIFT, ranges from 44% to 70% depending on the gold standard methods used, whereas the specificity is higher than 90% but less than the CLIFT.[7-9] Most of them compared the test results with clinical diagnosis of systemic lupus erythematosus or clinically active disease. No data comparing the sensitivity and specificity of EliA dsDNA with the CLIFT exists. Therefore, we assumed that the sensitivity and specificity of EliA dsDNA was about 90% because of the higher sensitivity, but lower specificity of EliA dsDNA test, as compared to the CLIFT.[8] Comparisons between methods (without clinical diagnosis) are estimated to fall within 10 percentage points of the true value, with a 95% confidence interval. The number of positive samples required was 35 specimens. The prevalence of positive anti-dsDNA specimens in routine practice was about 28%, as derived from our own data. Therefore, the total sample which needed to be collected was at least 125 specimens.

The cut-off point of the anti-dsDNA test by EliA dsDNA was determined and selected at the highest value of the accuracy (the summation of sensitivity and specificity) among the different levels of cut-off points. Then the agreement, kappa and the correlation between these two methods were also calculated and shown by using SPSS 11.1. Probabilities ≤ 0.05 were considered significant. The study was approved by the Siriraj Institutional Review Board, Faculty of Medicine, Siriraj Hospital.

RESULTS

The specimens collected for the study were 133 samples. Of those 133 specimens, 35 were positive whereas 98 were negative with the CLIFT. Of those 35 positive specimens, 2, 0, 2, 2 and 29 were, respectively, in ranges of < 5, 5 to 9.9, 10 to 14.9, 15 to 19.9 and > 20 IU/ml by EliA dsDNA. Also of those 98 negative specimens, 73, 7, 4, 4 and 10 were, respectively, in ranges of < 5, 5 to 9.9, 10 to 14.9, 15 to 19.9 and > 20 IU/ml by EliA dsDNA. The sensitivity and specificity of the EliA dsDNA for each cut-off point level are shown in Table 1.

The highest value of the summation of sensitivity and specificity was in the range of 10-14.9 IU/ml. There were two positive and four negative specimens in this range. Of those 6 specimens, the values were 11.1 and 14.5 IU/ml for positive specimens and were 10.3, 10.5, 12.8, 13.4 IU/ml for negative specimens. Additionally sublevels in the range of 10-14.9 were done to determine the sensitivity and specificity for each new sublevel and to select the appropriate cut-off point consistent with the purpose of the study as shown in Table 2. The highest value of the summation of sensitivity and specificity was 11 IU/ml and the second one was 14 IU/ml. Therefore, the cut-off point should be

TABLE 2. The sensitivity and specificity of EliA dsDNA for each level in range of 10-14.9%.

EliA dsDNA (IU/ml)	CLIFT		Sensitivity % (95%CI)	Specificity % (95%CI)	PPV %	NPV %
	Positive	Negative				
< 11	2	82	100	0	26.32	0
11-13.9	1	2	94.29 (86.42-100)	83.67 (76.41-90.93)	67.35	97.62
> 14	32	14	91.41 (81.93-100)	85.71 (78.84-92.58)	69.57	96.55

Positive Predictive Value (PPV), Negative Predictive Value (NPV)

TABLE 3. The discrepant results between two methods at the cut-off point of 11 IU/ml.

EliA dsDNA	The CLIFT	
	Positive	Negative
Positive	33	16
Negative	2	82

the value of 11 IU/ml. However, other factors such as the purpose of the study, the test application and cost should also be considered. The discrepant results at this cut-off point were shown in Table 3. The agreement and the kappa between these two methods at this cut-off point were 86.47% and 0.71, respectively. The correlation coefficient between these two methods was 0.545 and p-value was less than 0.001. Due to the fair results of both correlation and kappa, one test cannot be considered as a replacement for the other. The association between them is shown in Table 4 and in Fig 1.

DISCUSSION

In the case of 14 IU/ml selected for the cut-off point (Table 2), this value is similar to that of the manufacturer's recommendation. The ranges of negative, equivocal and positive by the manufacter's instructions were < 10, 10-15, and > 15 IU/ml respectively. Other studies[7,8] also applied the value of > 15 IU/ml for the cut-off point for the diagnosis and clinical management. However, in our case, the higher sensitivity and higher NPV with the acceptable specificity is more important because the purpose of the study is for a screening. Therefore, the value of 11 IU/ml is more appropriate for the cut-off point because this value gave the highest value of the summation of sensitivity and specificity and higher sensitivity and NPV compared with those of 14 IU/ml. This value will give higher false positive results; however, all those specimens will be confirmed with the CLIFT. The sensitivity of EliA dsDNA at this cut-off point is far higher whereas the specificity is lower than those from other studies.[9,10] These different results are due to different gold standard methods used and the objective of the study.

There were 18 specimens with discrepant results. Of those 18 specimens, 15 were diagnosed with SLE, 1 with vasculitis, 1 with cytomegalovirus infection and, for the other one, no information was found. Of those 15 specimens with SLE, 14 were positive with EliA dsDNA, but negative with the CLIFT and the other one was negative with EliA dsDNA, but positive with the CLIFT. If the gold standard criteria are changed from the CLIFT to include both similar results from both tests and the clinical diagnosis of SLE in specimens with discrepant results, the sensitivity and specificity will become 97.92%, and 97.62%, for EliA dsDNA, and 70.83% and 100%, for the CLIFT, respectively.

TABLE 4. The association of the results between both tests.

The CLIFT Titer	Number of samples	EliA dsDNA (IU/ml) Mean	Median	Minimum	Maximum
0	98	8.012	1.8	0.4	233
1:10	11	43.982	43.8	0.6	101
1:40	13	74.446	52.7	15.9	254
1:160	9	101.711	94.4	3.3	255
1:640	2	233.5	233.5	217	250

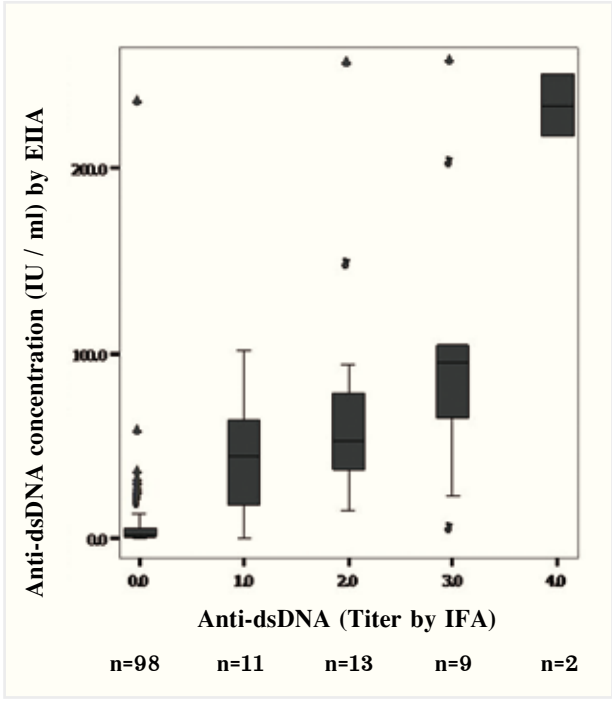

Fig 1. The correlation between two methods.
0 indicates negative; 1 indicates titer 1: 10; 2, titer 1: 40; 3, titer 1: 160; 4, titer 1: 640

The diagnostic values of EliA dsDNA are far better than those of the CLIFT. These results are similar to other studies[8,11] when clinical diagnosis is added to the gold standard criteria. Therefore, it is also valuable to replace the CLIFT with EliA dsDNA in routine practice because of its better diagnostic values. However, other perspectives such as cost-benefit or cost-effectiveness should be of concern and considered comprehensively before this change is made in routine practice.

The prevalence of false negative was 1.5% (2 out of 133 specimens) whereas the negative predictive value was 97.62%. Therefore; there were 2.38 % (2 out of 82 specimens) false negative. To decrease this problem, clinical information is required with the test request for an appropriate action. Of those two cases with false negative (2.44%), one was diagnosed with SLE, while for the other one no information was found. The positive predictive value (PPV) was 67.35% which is rather low, but it is not vital for clinical diagnosis because all these specimens with false positive will be retested with the CLIFT. Still, the cost for confirmation with the CLIFT should be of concern.

In conclusion, our results show that the appropriate cut-off point of the EliA dsDNA for screening was 11 IU/ml and its diagnostic values are better than those of the CLIFT when clinical diagnosis is included among the gold standard criteria.

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