COMPARATIVE STUDY OF ANTI-DOUBLE STRANDED DNA DETECTION BY ELISA AND *CRITHIDIA LUCILIAE* IMMUNOFLUORESCENCE

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Abstract. Two commercial enzyme-linked immunosorbent assays (ELISA) and two commercial *Crithidia luciliae* immunofluorescence tests (CLIF) were reevaluated as to the efficiency and degree of correlation of anti-double stranded DNA (anti-dsDNA) detection in systemic lupus erythematosus (SLE). The two ELISAs exhibited an overall agreement of 95% and significantly correlated with each other ($r= 0.91$, $p<0.001$). They were comparable in sensitivity (64%, 61%) and had the same specificity (95%, 95%). The sensitivity of the two CLIFs was 39% and 29% with corresponding specificities of 100% and 97%, and an overall agreement with each other of 94%. The two ELISAs had comparable specificity to the CLIFs with good agreement (84%, 79%) while they had a much greater sensitivity than the CLIFs. These findings suggest that ELISA is a useful laboratory test for anti-dsDNA detection of SLE due to its simplicity, quantitative results, sensitivity, specificity and cost, as compared to CLIFs.

INTRODUCTION

Anti-double stranded DNA (anti-dsDNA) antibodies are highly specific for systemic lupus erythematosus (SLE) and correlate with disease activity. The detection of anti-dsDNA is of value for the diagnosis and prognosis of SLE (Ter Borg *et al*, 1990; Bootsma *et al*, 1996; Arbuckle *et al*, 2001). The presence of antibodies to dsDNA has been included as one of the criteria for the disease classification of SLE by the American Rheumatism Association (Tan *et al*, 1982).

A variety of commercially available tests incorporate different techniques for the detection of anti-dsDNA. The Farr assay (Wold *et al*, 1968) is quite specific and has been advocated as the most reliable assay, however, it is time-consuming, technically difficult and involves the use of radioactive material. *Crithidia luciliae* immunofluorescence (CLIF) (Aarden *et al*, 1975) is highly specific, but lacks sensitivity and requires a fluorescence microscope to interpret results, often unavailable in many laboratories. Enzyme-linked immunosorbent assay (ELISA) is technically simple and quantitative. Previous reports have demonstrated varying efficiencies of ELISA with different sensitivity and specificity levels (Tipping *et al*, 1991; Hylkema *et al*, 1994; Avina-Zubieta *et al*, 1995; Takeuchi *et al*, 1997; Wong *et al*, 1998; Tan *et al*, 1999). Most results showed higher sensitivity but much lower specificity than CLIF and seemed to show varying standardization (Tipping *et al*, 1991; Avina-Zubieta *et al*, 1995; Wong *et al*, 1998). More recently, new ELISA commercial kits for anti-dsDNA detection have been developed by many manufacturers to improve sensitivity, specificity and standardization of results. The objective of this study is to reevaluate the efficiency of two commercial ELISAs compared to CLIFs for the sensitivity, specificity, predictive value, accuracy and cost effectiveness of anti-dsDNA detection for the diagnosis of SLE.

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MATERIALS AND METHODS

Sera

We studied 28 patients with a diagnosis of SLE, 28 with other rheumatic diseases (ORD):

20 with rheumatoid arthritis (RA), 4 with mixed connective tissue diseases (MCTD), 2 scleroderma, 1 ankylosing spondylitis and 1 CREST syndrome patient, enlisted from the rheumatology out-patient clinic of the Bhumipol Adulyadej Hospital. Thirty control sera were identified from normal human subjects (NHS) individuals whose antinuclear antibodies (ANA) were negative without known autoimmune diseases. All 86 sera were stored at -70°C until used.

Anti-dsDNA detection by commercial ELISA kits

Two commercial ELISA kits with different antigens were selected for use. One was the "ETI-dsDNA" anti-dsDNA kit from DiaSorin, Italy (ELISA I) and the other was the "BINDAZYME" anti-dsDNA kit from The Binding Site, UK (ELISA II). The procedures specified in both kits were followed according to manufacturers' recommendations. For ELISA I, recombinant dsDNA (*Escherichia coli*) was used as the antigen coated plate. Serum samples diluted at 1:100 with serum diluent, serum controls and calibrators were added to wells and incubated for 30 minutes at room temperature (18º-25°C). The plates were washed 3 times with wash buffer. One hundred microliters of horseradish peroxidase-labeled anti-human IgG was applied to each well and then incubated for 30 minutes at room temperature. The plates were washed and then 100 µl of substrate containing hydrogen peroxide and TMB substrate was added. After 10 minutes of incubation, 1 N sulfuric acid was added to stop the reaction. The optical density (OD) was read at 450 nm with the reference at 620 nm. The results were calculated exactly as described by the manufacturer. Anti-dsDNA antibody levels of more than or equal to 20 IU/ml were considered positive results.

ELISA II was a purified calf thymus dsDNA antigen kit. The assay was performed by adding 100 µl of control, 1:100 diluted serum samples and each calibrator to the antigen coated plate. After incubation at room temperature for 30 minutes, the plate was washed 3 times with wash diluent. Peroxidase labeled antibody to human IgG was added in each well and incubated at room temperature for 30 minutes. The plate was washed again and TMB substrate solution was added for 30 minutes. The reaction was stopped by adding 3 M phosphoric acid. The optical density of each well was read at 450 nm. Anti-dsDNA antibody levels of more than or equal to 30 IU/ml were considered positive results.

Anti-dsDNA detection by *Crithidia luciliae* immunofluorescence

Two commercial kits for anti-dsDNA detection by *Crithidia luciliae* immunofluorescence were selected for testing. One was from DiaSorin, Italy, the "nDNA Fluoro-kit" (CLIF I). The other was from The Binding Site, UK, the "Crithidia lucilliae dsDNA" (CLIF II). In brief, *Crithidia luciliae* was used as the substrate antigen and a 1: 10 diluted serum sample was added to each well of substrate coated slide. After incubation, the slide was washed and the antigen-antibody reaction was determined by FITC-labeled anti-human gamma globulin conjugate. The slide was observed using a fluorescence microscope. A test was considered positive with a titer of 1:10 or above.

Statistical analysis

Sensitivity, specificity, positive and negative predictive values and accuracy were calculated in accordance with standard methods. The correlation between the two ELISAs was performed by simple linear correlation.

RESULTS

The results of ELISAs for anti-dsDNA detection in a total of 86 sera are shown in Table 1. Seventeen (64%) and sixteen (61%) of twenty-eight SLE were positive by ELISA I and ELISA II, respectively. No false positive results were found in normal human subjects (NHS) by the two ELISAs. Three of twenty-eight (11%) other rheumatic diseases (ORD) gave positive results by both ELISA commercial kits. ELISA I had a slightly higher sensitivity than ELISA II (64%, 61%) while both had the same specificity (95%, 95%). The two ELISAs showed high positive predictive value (PPV) (86%, 85%), negative predictive value (NPV) (85%, 83%) and accuracy (84%, 84%).

No false positive results were found in NHS

Table 1
Results of the frequency of anti-dsDNA detection in systemic lupus erythematosus (SLE), other rheumatic disease (ORD) patients and normal human subjects (NHS) with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy for SLE diagnosis among tests.

	Number positive (%)			
	ELISA I	ELISA II	CLIF I	CLIF II
SLE (n = 28)	18 (64)	17 (61)	11 (39)	8 (29)
NHS (n = 30)	0 (0)	0 (0)	0 (0)	0 (0)
ORD (n = 28)	3 (11)	3 (11)	0 (0)	2 (7)
Sensitivity (%)	64	61	39	29
Specificity (%)	95	95	100	97
PPV (%)	86	85	100	80
NPV (%)	85	83	77	74
Accuracy (%)	84	84	80	74

by the two CLIFs. Two of twenty-eight ORD (7%) gave false positive results by CLIF II, but no false positive results were found in ORD detected by CLIF I. There were positive results in eleven and eight of twenty-eight SLE detected by CLIF I and CLIF II respectively. CLIF I had greater sensitivity (39%, 29%) and also had a greater specificity (100%,97%) than CLIF II as shown in Table 1. The PPV of CLIFs was very high (100%, 80%) but rather low for the NPV (77%, 74%).

Fig 1 gives the correlation between the two ELISAs in the overall samples tested. The two commercial kits had very good correlation with each other ($r = 0.91$, $p<0.001$). The concordance of the results of anti-dsDNA detection by each ELISA is shown in Table 2. The two ELISA and two CLIF commercial kits had very good agreement at 95% and 94% respectively. CLIF I and ELISA I and CLIF II and ELISA II, which are two different techniques from the same manufacturer, had good agreement at 84% and 79% respectively.

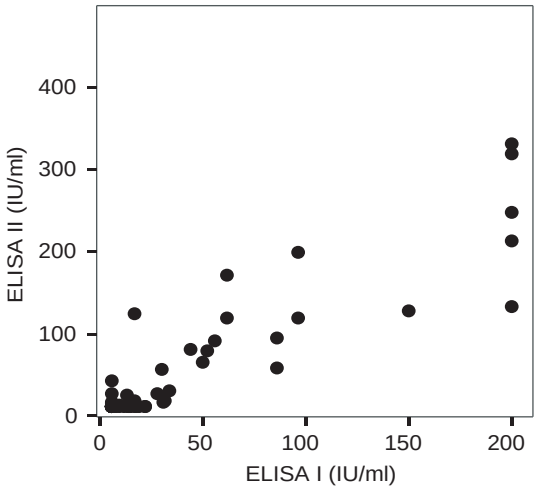

Fig 1–The correlation between two commercial ELISA kits for anti-dsDNA detection in 86 serum samples ($r=0.91$, $p<0.001$).

DISCUSSION

Measurement of anti-dsDNA is widely used to assess disease diagnosis and management in SLE patients. ELISA and CLIF are the most commonly used methods in the clinical laboratory. However, most ELISA testing demonstrates high sensitivity but rather low specificity compared to CLIF (Tipping *et al*1991; Wong *et al* 1998). Since ELISA is technically easier to perform, the ELISA has been developed for greater effectiveness in diagnosing SLE. Two commercial ELISA kits, using recombinant dsDNA (ELISA I) and purified calf thymus dsDNA (ELISA II) as antigens were selected for investigation of efficiency in the diagnosis of SLE. Although previous reports demonstrated great variation in sensitivity and specificity in ELISA kits for the detection of anti-

Table 2
Concordance and discordance of anti-dsDNA detection by various tests.

Test performed	Pos/Pos	Neg/Neg	Pos/Neg	Neg/Pos	Overall agreement (%)
ELISA I/ELISA II	18	63	3	2	95
CLIF I /CLIF II	8	73	3	2	94
CLIF I / ELISA I	9	63	12	2	84
CLIF II / ELISA II	4	62	6	14	79

Pos: Positive, Neg: Negative.

dsDNA (Tipping *et al*, 1991; Avina-Zubieta *et al*, 1995; Takeuchi *et al*, 1997; Wong *et al*, 1998; Tan *et al*, 1999), there was a strong correlation between both commercial ELISA kits tested ($r = 0.91$, $p<0.001$) with a high level of agreement (95%). The two commercial ELISA kits had comparable sensitivity (64%, 61%) and the same specificity of 95%. This indicated that both commercial ELISA kits provided a standard measure, which may be due to the detection of the same group of anti-dsDNA autoantibodies, even though they used different sources of antigens. These two ELISAs had a higher sensitivity and specificity when compared to most previous reports (Tipping *et al*, 1991; Avina-Zubieta, *et al* 1995; Wong *et al*,1998; Tan *et al*, 1999) Tipping *et al* (1991) reported a commercial ELISA for anti-dsDNA detection with a sensitivity and specificity of 42% and 94% respectively. Wong *et al* (1998) reported a commercial ELISA for anti-dsDNA detection with a sensitivity and specificity of 68% and 80% respectively and an in-house ELISA with a sensitivity and specificity of 32% and 89% respectively. The sensitivity of these two ELISAs was lower than expected. This may be due to the differences in the SLE patients' sera tested. Most of the SLE patients in our study had undergone treatment before testing, which may have had an effect on the level of anti-dsDNA. Some reports show decreasing levels of anti-dsDNA after treatment or in inactive stages (Gladmann *et al*, 1979; Ter Borg *et al*, 1990; Bootsma *et al*, 1996).

The efficiency of CLIF was also evaluated. We found that the two CLIFs had very high specificity (100%, 97%), and had good concordance (94%). Their sensitivities were much lower than the two ELISAs tested. These differences may be due to the differences in technical procedures and sources of antigen. In our study, the CLIFs showed rather low sensitivity, this may be due to the differences in SLE patients' sera tested. As previously mentioned, because most SLE patients had undergone treatment before testing, the level of anti-dsDNA may have been affected and could not be detected with CLIF. Some reports show decreasing levels of anti-dsDNA after treatment or in inactive stages and this may have resulted in lower an anti-dsDNA detection by CLIF.

The method of choice used in the detection of anti-dsDNA often depends on the availability of technical support, cost and quality of the test. We compared ELISA and CLIF for efficiency in diagnosis of SLE and cost effectiveness. CLIF had the higher specificity (100%) but lower sensitivity (27%), whereas ELISA had slightly lower specificity (95%) but much higher sensitivity (64%). The cost of CLIF for a single dilution and ELISA for one quantitative assay are about 120 Baht (US$3) and 180 Baht (US$4.5) per test, respectively.

In conclusion, the results showed that different antigens used in two commercial ELISA kits were not different in anti-dsDNA detection for SLE diagnosis. The detection of anti-dsDNA by CLIF had very low sensitivity and needed a fluorescence microscope, a major limitation in many laboratories. This study suggests that ELISA produces standard results, is easy to perform and no more expensive than CLIF. It is effective in detecting anti-dsDNA for the diagnosis of SLE in the routine clinical laboratory.

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REFERENCES

Aarden LA, de Groot ER, Feltkamp TEW. Immunology of DNA III *Crithidia luciliae*, a simple substrate for the detection of anti-dsDNA with immunofluorescence technique. *Ann NY Acad Sci* 1975; 254: 505-15.

Arbuckle MR, James JA, Kohlhase KF, Robertone MV, Dennis GJ, Harley JB. Development of anti-dsDNA autoantibodies prior to clinical diagnosis of systemic lupus erythematosus. *Scand J Immunol* 2001; 54: 211-9.

Avina-Zubieta JA, Galindo-Rodriguez G, Kwan-Yeung L, Davis P, Russell AS. Clinical evaluation of various selected ELISA kits for the detection of anti-DNA antibodies. *Lupus* 1995; 4: 370-4.

Bootsma H, Spronk PE, Hummel EJ, *et al.* Anti-double stranded DNA antibodies in systemic lupus erythematosus: detection and clinical relevance of IgM-class antibodies. *Scand J Rheumatol* 1996; 25; 352-9.

Gladman DD, Urowitz MB, Keystone EC. Serologically active clinically quiescent systemic lupus erythematosus; a discordance between clinical and serological features. *Am J Med* 1979; 48: 937-44.

Hylkema MN, Huygen H, Kramers C, *et al*. Clinical evaluation of a modified ELISA, using photobiotinylated DNA, for the detection of anti-DNA antibodies. *J Immunol Methods* 1994; 170: 93-102.

Tan EM, Cohen AS, Fried JF, *et al*. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982; 25: 1271-7.

Tan EM, Smolen JS, McDougal JS, *et al*. A critical evaluation of enzyme immunoassays for detection of antinuclear autoantibodies of defined specificities. *Arthritis Rheum* 1999; 42: 455-64.

Takeuchi Y, Ishikawa O, Miyachi Y. The comparative study of anti-double stranded DNA antibody levels measured by radioimmunoassay and enzyme-linked immunosorbent assay in systemic lupus erythematosus. *J Dermatol* 1997; 24: 297-300.

Ter Borg EJ, Horst G, Hummel FJ, Limburg PC, Kallemberg CG, *et al*. Measurement of increases in anti-double-stranded DNA antibody levels as a predictor of disease exacerbation in systemic lupus erythematosus. A long term prospective study. *Arthritis Rheum* 1990; 5: 634-43.

Tipping PG, Buchanan RC, Riglar AG, Dimech WJ, Littlejohn GO, Holdsworth SR. Measurement of anti-DNA antibodies by ELISA: a comparative study with *Crithidia luciliae* and a Farr assay. *Pathology* 1991; 23: 21-4.

Wold RT, Young FE, Tan EM, Farr RS. Deoxyribonucleic acid antibody: A method to detect its primary interaction with deoxyribonucleic acid. *Science* 1968; 167: 806 –7.

Wong KH, Lawton JW, Cheng SK, Lee SS, Lau CS. Measurement of anti-dsDNA: a comparative study of two ELISA and the Crithidia assay. *Pathology* 1998; 30: 57-61.