Evaluation of Five Commercial Assays for the Detection of Anti-dsDNA Antibodies: Three *Crithidia luciliae* Indirect Immunofluorescence Test Kits and Two Enzyme Immunoassay Kits

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Objective: *There are various methods for anti-dsDNA detection. Crithidia luciliae indirect immunofluorescence test (CLIFT) and enzyme immunoassay (EIA) are the most commonly used at present. A number of CLIFT and EIA kits are commercially available. The objective of the present study was to evaluate the diagnostic performance of three commercial CLIFT kits, two commercial EIA kits, and their combinations for anti-dsDNA detection.*

Material and Method: *One hundred thirty nine sera sent for anti-dsDNA testing were investigated. Three commercial CLIFT kits (kit C1, C2, and C3) and two commercial EIA kits (kit E1 and E2) were evaluated. Sensitivities and specificities were calculated. The gold standard methods were the consensus results of all five kits, together with the clinical diagnosis when the results of five kits were discrepant.*

Results: *Of 139 sera investigated, 94 (67.6%) sera showed concordant results for all five kits and 45 (32.4%) sera showed discordant results. Thirty-five of those 45 patients (77.7%) were diagnosed as SLE. Sensitivities and specificities of the kits were as follows, C1 82.1% and 94%, C2 46.4% and 100%, C3 78.6% and 98.8%, E1 71.4% and 94%, and E2 75% and 93.8%, respectively. Kit C3 yielded the maximum sum of sensitivity and specificity (177.4%). Sensitivities and specificities of the combinations of CLIFT and EIA kits were as follows, C1 + E1 89.3% and 90.4%, C1 + E2 98.2% and 87.9%, C2 + E1 73.2% and 94%, C2 + E2 82.1% and 92.8%, C3 + E1 85.7% and 94%, and C3 + E2 94.6% and 91.6%, respectively. The combination of kit C3 and E2 yielded the maximum sum of sensitivity and specificity (186.2%).*

Conclusion: *Kit C3 was the assay of choice for anti-dsDNA detection. EIA kits yielded lower sensitivities and specificities than two of three CLIFT kits. Therefore, they should not be used as the first assay for anti-dsDNA screening. When CLIFT and EIA assays were combined, sensitivities were increased. Kit E2 helped CLIFT kits to detect more SLE cases than E1.*

Keywords: *Anti-dsDNA antibodies, Crithidia luciliae indirect immunofluorescence test, CLIFT*

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The presence of anti-double-stranded DNA (anti-dsDNA) antibodies is one of the diagnostic criteria for systemic lupus erythematosus (SLE) according to the 1982 American College of Rheumatology (ACR) criteria[1]. Currently, there are various methods to detect anti-dsDNA antibodies in clinical laboratories, including Farr radioimmunoassay, *Crithidia luciliae* indirect immunofluorescence test (CLIFT), and enzyme immunoassay (EIA), etc.[2,3]. The Farr assay is the first method developed for anti-dsDNA detection. However, it is rarely used in clinical laboratories nowadays due to radioactive substance hazard. At present, the most commonly used techniques for anti-dsDNA detection are CLIFT and EIA.

CLIFT is an indirect immunofluorescence assay using the hemoflagellate *Crithidia luciliae* as a substrate. EIA uses purified or synthetic dsDNA as an antigen. CLIFT employs more cumbersome techniques because it requires a fluorescence microscope and slide reading skill. EIA is simpler and more automated method. CLIFT detects medium to high avidity antibodies that are more associated with SLE, whereas EIA detects both low and high avidity antibodies[3,4].

Many commercial kits have been provided by their manufacturers. The aim of the present study

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was to evaluate the diagnostic performance of commercial CLIFT and EIA kits for anti-dsDNA detection and determine the diagnostic performance when CLIFT and EIA kits were combined.

Material and Method

Patient samples

Sera sent to Clinical Pathology Laboratory at Siriraj Hospital for anti-dsDNA testing between June and November 2008 were randomly selected and were stored at -20°C in aliquots until analysis. Sera containing high level of hemolysis, lipemia, or icterus were excluded.

Only medical records of patients with sera showing discrepant results for all commercial kits were reviewed.

The present study was conducted following the protocol approved by the Siriraj Institutional Review Board (protocol number Si306/2010).

Laboratory measurements

Five commercial anti-dsDNA detection kits were evaluated. Three of them were CLIFT (IMTEC Immundiagnostika, Hemagen Diagnostics, and Euroimmun AG), and two were EIA (IMTEC Immundiagnostika and Euroimmun AG). Characteristics of the kits are shown in Table 1. The assays were performed according to the manufacturers' instructions and were considered positive or negative using the cut off values provided by the manufacturers.

Statistical analysis

The sensitivities and specificities with 95% confidence intervals (CI) for each kit and each combination of CLIFT and EIA kit were calculated. The gold standard methods were the consensus results of all five kits. If the five kits showed discrepant results, clinical diagnosis was used as the gold standard. The sums of sensitivities and specificities were also determined to demonstrate which kit and which combination gave the best performance.

Statistical analyses were performed using MedCalc for Windows, version 13.0.2 (MedCalc Software, Ostend, Belgium).

Results

One hundred thirty nine sera were included in the present study. Analyzing with five commercial kits, 94 sera (67.6%) gave concordant results: 21 (15.1%) were positive and 73 (52.5%) were negative, and 45 sera (32.4%) gave discrepant results for all kits. Thirty-five of those 45 discrepant sera (77.7%) were from patients diagnosed as SLE. Of those 10 non-SLE patients, two had immune thrombocytopenic purpura, one each had rheumatoid arthritis, IgA nephropathy, chronic urticaria, psoriasis, cerebral malaria, osteoarthritis, and thalassemia, and one was healthy.

Sensitivities and specificities of each kit are displayed in Table 2. Kit C1 gave the best sensitivities (82.1%; 95% CI, 69.6-91.1) and kit C2 gave the best specificity (100%; 95% CI, 95.6-100). However, kit C3 gave the maximum sum of sensitivity and specificity (177.4%). Two of the three CLIFT kits (C1 and C3) gave better sensitivities and specificities than both EIA kits.

When EIA kits were combined with CLIFT kits for anti-dsDNA testing, sensitivities were higher,

Table 1. Characteristics of anti-dsDNA detection kits in the study

Kit	Manufacturer	Technique	dsDNA antigen	Isotype detection	Sample dilution	Cut-off
C1	IMTEC Immundiagnostika, Inc. (Zepernick, Germany)	CLIFT	*Crithidia luciliae* kinetoplast	IgG, IgM, IgA	1:41	1:41
C2	Hemagen Diagnostics, Inc. (Columbia, Maryland)	CLIFT	*Crithidia luciliae* kinetoplast	IgG	1:10	1:10
C3	Euroimmun AG, Inc. (Luebeck Germany)	CLIFT	*Crithidia luciliae* kinetoplast	IgG	1:10	1:10
E1	IMTEC Immundiagnostika, Inc. (Zepernick, Germany)	EIA	Purified dsDNA	IgG	1:101	40 WHO-IU/ml
E2	Euroimmun AG, Inc. (Luebeck Germany)	EIA	Purified dsDNA with nucleosome	IgG	1:201	100 IU/ml

CLIFT = *Crithidia luciliae* indirect immunofluorescence test; EIA = enzyme immunoassay; IgG = immunoglobulin G; IgM = immunoglobulin M; IgA = immunoglobulin A; dsDNA = double stranded DNA

Table 2. Diagnostic performance of each commercial kit

Kit	Positive (n)	Negative (n)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
C1	51	88	82.1 (69.6-91.1)	94.0 (86.5-98.0)	90.2 (78.6-96.7)	88.6 (80.1-94.4)
C2	26	113	46.4 (33.0-60.3)	100 (95.6-100)	100 (86.7-100)	73.5 (64.3-81.3)
C3	45	94	78.6 (65.6-88.4)	98.8 (93.4-99.8)	97.8 (88.2-99.6)	87.2 (78.8-93.2)
E1	45	94	71.4 (57.8-82.7)	94.0 (86.5-98.0)	88.9 (75.9-96.3)	83.0 (73.8-89.9)
E2	48	91	75.0 (59.7-84.2)	93.8 (84.9-97.2)	87.2 (74.3-95.1)	83.7 (74.5-90.6)

PPV = positive predictive values; NPV = negative predictive values

Table 3. Diagnostic performance of each combination of CLIFT and EIA kits

Combination of kits	Positive (n)	Negative (n)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
C1 + E1	58	81	89.3 (78.1-95.9)	90.4 (81.8-95.7)	86.2 (74.6-93.8)	92.6 (84.6-97.2)
C1 + E2	65	74	98.2 (90.4-99.7)	87.9 (78.9-94.1)	84.6 (73.5-92.4)	98.7 (92.7-99.8)
C2 + E1	46	93	73.2 (59.7-84.2)	94.0 (86.5-98.0)	76.4 (76.4-96.3)	74.8 (74.8-90.7)
C2 + E2	52	87	82.1 (69.6-91.1)	92.8 (84.9-97.3)	88.5 (76.6-95.6)	88.5 (79.9-94.3)
C3 + E1	53	86	85.7 (73.8-93.6)	94.0 (86.5-98.0)	90.6 (79.3-96.8)	90.7 (82.5-95.9)
C3 + E2	60	79	94.6 (85.1-98.8)	91.6 (83.4-96.5)	88.3 (77.4-95.2)	96.2 (89.3-99.2)

PPV = positive predictive values; NPV = negative predictive values

while specificities were lower (Table 3). The combination of kit C3 and E2 yielded the maximum sum of sensitivity and specificity (186.2%). Kit E2 helped to detect more SLE cases than kit E1 when combined with CLIFT kits (9, 20 and 9 versus 4, 15 and 4, combining with C1, C2 and C3, respectively) (Table 4).

Discussion

Immunoassays for the detection of autoantibodies have been continuously developed. Clinical laboratories have many choices of commercial assays for their services. Different techniques have different diagnostic performances, advantages, and drawbacks. Each laboratory should select the assay giving the best performance and suiting the work of individual laboratory.

In the present study, five commercial anti-dsDNA detection kits, three CLIFT kits and two EIA kits, were evaluated for their diagnostic performances. Different sensitivities and specificities among different kits were demonstrated. It was found that 32.4% of sera showed discrepant results for all kits. These marked discrepancies might be because the studied sera were randomly selected from routine anti-dsDNA testing in the laboratory. The high concordance among different kits has been observed when applied to populations of SLE patients(5-7).

A variation in the results among kits, even based on the same techniques, could be explained by the source of antigen and the isotype detection. The preparation of *C. luciliae* substrate could affect CLIFT results. For example, the presence of histone in the kinetoplast gave false positive CLIFT(8-10). The source of DNA, including the purification of DNA, the method of antigen presentation and reaction conditions could be the causes of the discrepancies among EIA kits(7,11,12). Previous study reported that the false positive results of the EIA kit containing immobilized high-molecular-weight calf thymus DNA were attributable to anti-single-stranded DNA (ssDNA) antibodies(13). Moreover, polyvalent anti-human immunoglobulin used in kit C1 might provide the highest sensitivity.

EIA kits yielded lower sensitivities and specificities than CLIFT kits. This data suggested that they should not be used as a first assay for the screening of anti-dsDNA. However, they increased sensitivities when combined with CLIFT due to positive EIA but negative CLIFT results of SLE samples. Kit E2 helped to detect more SLE cases than kit E1. This could be explained by the nucleosome antigen used in kit E2. Anti-nucleosome antibodies have been reported to be good markers for the diagnosis of SLE(14). Previous study demonstrated the discrepancies between ELISA

Table 4. Distribution of clinical diagnosis of discrepant sera for each combination of CLIFT and EIA kits

CLIFT	EIA	SLE (n)	non-SLE (n)
C1+	E1+	15	2
C1+	E1-	10	3
C1-	E1+	4	3
C1-	E1-	6	2
C1+	E2+	12	1
C1+	E2-	13	4
C1-	E2+	9	5
C1-	E2-	1	0
C2+	E1+	4	0
C2+	E1-	1	0
C2-	E1+	15	5
C2-	E1-	15	5
C2+	E2+	1	0
C2+	E2-	4	0
C2-	E2+	20	6
C2-	E2-	10	4
C3+	E1+	15	1
C3+	E1-	8	0
C3-	E1+	4	4
C3-	E1-	8	5
C3+	E2+	12	0
C3+	E2-	11	1
C3-	E2+	9	6
C3-	E2-	3	3

CLIFT = *Crithidia luciliae* indirect immunofluorescence test; EIA = enzyme immunoassay; SLE = systemic lupus erythematosus

kits and the importance of anti-dsDNA kits for the diagnosis when examining sera from patients having three SLE criteria. It might be advisable to use more than one method for anti-dsDNA testing[15].

In conclusion, kit C3 is selected as the first assay for anti-dsDNA detection. Kit E2 is used as an adjunct when kit C3 shows negative result and clinical information still indicates SLE. The present data suggested that the availability of two methods in the laboratories for anti-dsDNA detection is more advantageous.

What is already known on this topic?

Previous studies demonstrated sensitivities and specificities of CLIFT and EIA kits.

What this study adds?

CLIFT and EIA were combined. These commercial kits have been never compared. Clinicians should be aware of discrepancy between kits.

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Potential conflicts of interest

None.

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การประเมินชุดตรวจเชิงพาณิชย์ 5 ชนิด สำหรับตรวจหา anti-dsDNA antibodies: วิธี Crithidia luciliae indirect immunofluorescence test 3 ชนิด และ วิธี enzyme immunoassay 2 ชนิด

รณชัย วิริยะทวีกุล, จารุดา กอบกิจเจริญ, จินตนา ใจเย็น, ศิริพร ก้องเกรียงเดช, สุมาลี พจน์ประสาท

วัตถุประสงค์: *การตรวจหา anti-dsDNA antibodies มีหลายวิธี ปัจจุบันวิธีที่นิยมใช้ ได้แก่ Crithidia luciliae indirect immunofluorescence test (CLIFT) และ enzyme immunoassay (EIA) ซึ่งมีชุดตรวจเชิงพาณิชย์มากมายให้เลือกใช้ การศึกษานี้มีวัตถุประสงค์เพื่อประเมิน diagnostic performance ของชุดตรวจ CLIFT 3 ชนิด ชุดตรวจ EIA 2 ชนิด และชุดตรวจ 2 วิธีควบคู่กัน*

วัสดุและวิธีการ: *ซีรัม 139 ราย ที่ส่งมาตรวจหา anti-dsDNA antibodies ถูกนำมาใช้ประเมินชุดตรวจ CLIFT 3 ชนิด (C1, C2 และ C3) และชุดตรวจ EIA 2 ชนิด (E1 และ E2) ความไวและความจำเพาะคำนวณจากวิธีมาตรฐาน (gold standard method) ได้แก่ ผลที่ตรงกันของชุดตรวจทั้ง 5 ชนิด และใช้การวินิจฉัยโรคเป็นวิธีมาตรฐานเมื่อชุดตรวจ 5 ชนิด ให้ผลไม่ตรงกัน*

ผลการศึกษา: *มีซีรัม 94 ราย (67.6%) ที่ชุดตรวจทั้ง 5 ชนิด ให้ผลตรงกันหมด และซีรัม 45 ราย (32.4%) ให้ผลไม่ตรงกัน ซึ่งในจำนวนซีรัมที่ให้ผลไม่ตรงกันนี้ มี 35 ราย เป็น SLE ความไวและความจำเพาะของชุดตรวจแต่ละชนิดเป็นดังนี้: C1 82.1% และ 94%, C2 46.4% และ 100%, C3 78.6% และ 98.8%, E1 71.4% และ 94% และ E2 75% และ 93.8% ตามลำดับ ชุดตรวจ C3 ให้ผลรวมของความไวและความจำเพาะสูงที่สุด (177.4%) ความไวและความจำเพาะของชุดตรวจวิธี CLIFT และ EIA ควบคู่กันเป็นดังนี้: C1 + E1 89.3% และ 90.4%, C1 + E2 98.2% และ 87.9%, C2 + E1 73.2% และ 94%, C2 + E2 82.1% และ 92.8%, C3 + E1 85.7% และ 94% และ C3 + E2 94.6% และ 91.6% ตามลำดับ การตรวจควบคู่กันของชุดตรวจ C3 และ E2 ให้ผลรวมของความไวและความจำเพาะสูงที่สุด (186.2%)*

สรุป: *ชุดตรวจ C3 เป็นชุดตรวจที่จะเลือกใช้เป็นตัวแรกในการตรวจหา anti-dsDNA antibodies ชุดตรวจ EIA ให้ความไวและความจำเพาะต่ำกว่าชุดตรวจ CLIFT 2 ชนิด จึงไม่ควรใช้ชุดตรวจ EIA เป็นตัวแรก เมื่อใช้ชุดตรวจวิธี CLIFT และ EIA ควบคู่กันทำให้ความไวสูงขึ้น ซึ่งชุดตรวจ E2 ช่วยให้ตรวจพบ SLE ได้มากกว่าชุดตรวจ E1*