

Comparison of Three Commercial *Crithidia luciliae* Immunofluorescence Test (CLIFT) Kits for Anti-dsDNA Detection

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ABSTRACT

The *Crithidia luciliae* immunofluorescence test (CLIFT) is widely used in clinical laboratories for anti-double stranded DNA (anti-dsDNA) detection. It has comparable sensitivity and specificity to the standard method, Farr assay, but employs simpler and safer techniques. A number of CLIFT kits are commercially available. We evaluated the sensitivities and specificities of three commercial CLIFT kits, including IMTEC, Hemagen and Euroimmun. The IMTEC kit detected IgG, IgM and IgA classes, while the Hemagen and Euroimmun kits detected only the IgG class. Farr assays were performed when three kits gave discrepant results. The gold standard methods were the consensus results of all three kits together with the Farr assay. Out of one-hundred and thirty sera, 111 sera (85%) gave concordant results by all three CLIFT kits and 19 sera (15%) gave discrepant results. Sensitivities and specificities of the IMTEC, Hemagen and Euroimmun kits were 97.4% and 94.5%, 66.7% and 100%, and 89.7% and 94.5%, respectively. The size of the IMTEC *C. luciliae* was smaller than those of the other two kits, leading to more difficulty in slide reading. Factors affecting the kit's performance may include substrate preparation and immunoglobulin class detection.

Keywords: *Crithidia luciliae*, immunofluorescence test

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INTRODUCTION

Anti-double stranded DNA (anti-dsDNA) antibody is a serological marker of systemic lupus erythematosus (SLE). It is one of eleven diagnostic criteria for SLE according to the American College of Rheumatology (ACR).¹ It also correlates with disease activity, especially lupus nephritis, serving as a marker of disease exacerbation and treatment monitoring.^{2,3}

There are a variety of techniques for determination of anti-dsDNA; for example Farr radioimmunoassay, *Crithidia luciliae* immunofluorescence test (CLIFT), enzyme immunoassay (EIA), automated immunofluorescence immunoassay using *Escherichia coli* as antigen

(EliA) and line immunoassay. The Farr assay has been the standard method of anti-dsDNA detection, having high specificity, reasonable sensitivity and good correlation to disease activity. However, it requires the use of radioactive material which is not suitable for clinical laboratories.

The CLIFT is an indirect immunofluorescence assay using the hemoflagellate *Crithidia luciliae* as a substrate. *C. luciliae* possesses the kinetoplast, a giant mitochondrion containing concentrated double-stranded DNA and lacking other nuclear antigens. It has sensitivity and specificity comparable to Farr assay, but it is safer and easier to perform.⁴⁻⁶ It employs more complicated techniques than EIA, although it has higher specificity to SLE. It detects medium to high avidity antibodies which are more associated with the disease, whereas EIA detects both low and high avidity antibodies, which gives frequent false positive results.^{6,7} Therefore, the CLIFT is well accepted and widely used in clinical laboratories at present. Currently, there are a number of CLIFT kits commercially available.

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The objective of this study was to evaluate the sensitivities and specificities of three commercial CLIFT kits, in order to select the kit providing the best performance for our clinical laboratory.

MATERIALS AND METHODS

Sera requested for anti-dsDNA test during June-November 2008 were randomly selected, regardless of clinical diagnosis and antinuclear antibody (ANA) result. Sera showing high level of hemolysis, lipemia or icterus were excluded.

Three commercial CLIFT kits were supplied by the following manufacturers; IMTEC Immundiagnostika, Inc. (Zepernick, Germany), Hemagen Diagnostics, Inc. (Columbia, Maryland) and Euroimmun AG, Inc. (Luebeck, Germany). There were some differences in manufacturers' protocols (Table 1). The tests were appropriately performed according to the manufacturers' instructions. Briefly, sera were diluted to the dilution according to each kit's instruction and incubated with *C. luciliae* on the slides. The slides were washed and FITC-labeled anti-human globulin was applied. The FITC-labeled anti-human globulin of Hemagen and Euroimmun kit detected IgG class, while that of the IMTEC kit detected IgG, IgM and IgA classes. After washing, the slides were visualized under a fluorescent microscope. The presence of apple-green fluorescent staining on the kinetoplast of *C. luciliae* was considered positive for anti-dsDNA. In the routine practice of our laboratory, sera are screened with starting dilution and only sera giving positive results are further titrated to obtain quantitative results. Therefore, in this study we compared the kits by focusing on starting dilution, not the titer.

If three kits showed concordant results (all positive or all negative), no further test was required. If three kits showed discrepant results, sera were tested by Farr assay. Farr assay was performed using Euroimmun anti-dsDNA RIA kit (Euroimmun AG, Inc. Lubeck, Germany). Briefly, sera were incubated with radioactive-labeled dsDNA. Antigen-antibody complexes were precipitated by ammonium sulfate solution and the radioactivity was measured.

The gold standard methods were the consensus results of all three kits together with the Farr assay. The sensitivities and specificities for each kit were calculated and the sums of sensitivities and specificities were also determined to demonstrate which kit gave the best performance.

This study obtained ethical approval of the Siriraj Institutional Review Board (Protocol number Si 598/2009).

RESULTS

One hundred and thirty sera sent to the Clinical Pathology Laboratory at Siriraj Hospital for anti-dsDNA test were investigated. One hundred and eleven samples (85.4%) had concordant results from all three CLIFT kits and 19 samples (14.6%) had discrepant results. Of those 19 samples, 13 (68.4%) were positive by Farr assay

TABLE 1. Differences in manufacturers' protocols.

	FITC Conjugate	Starting Dilution
IMTEC	IgG, IgM, IgA	1:41
Hemagen	IgG	1:10
Euroimmun	IgG	1:10

and 6 (31.6%) were negative by Farr assay (Table 2). The IMTEC kit provided the highest sensitivity and good specificity, while the Hemagen kit provided the best specificity but poor sensitivity (Table 3). The IMTEC kit yielded the highest value of the sum of sensitivity and specificity (191.9%), followed by the Euroimmun kit (184.2%) and Hemagen kit (166.7%).

When comparing the images of *C. luciliae* under fluorescent microscope, the size from the IMTEC *C. luciliae* kit was smaller than those of the other two kits, leading to more difficulty in slide examination. The numbers of the organisms per one field were comparable among the three kits (Fig 1).

DISCUSSION

The CLIFT was introduced in 1975 for the detection of anti-native DNA antibodies.⁴ Because of its simplicity and comparable sensitivity and specificity to Farr assay, it has been developed as a commercial kit and is widely used in clinical laboratories. It detects medium to high avidity antibodies, while the Farr assay detects only high avidity antibodies which are specific for SLE.⁷⁻⁹ Therefore, the Farr assay has a higher specificity and better correlation to disease activity. For this reason, the present study used the Farr assay as the gold standard method when the three commercial CLIFT kits gave discrepant results.

This study demonstrated the difference in sensitivities and specificities of three commercial CLIFT kits, as well as, the discrepancies among results of the kits. This may be explained by two factors.

The first, was the preparation of *C. luciliae* substrate. The presence of histone in the kinetoplast could lead to false positive CLIFT. There was evidence that sera containing anti-histone antibodies showed reactivity on *C. luciliae* kinetoplast.¹⁰⁻¹² In addition, the kinetoplast probably reacted with anti-nucleosome antibodies, because nucleosomes are structured from histone and DNA.

TABLE 2. Number of sera giving positive or negative results by CLIFT kits and Farr assay.

CLIFT	N*	Farr positive	Farr negative
IMTEC + Hemagen + Euroimmun +	26	N/A	N/A
IMTEC - Hemagen - Euroimmun -	85	N/A	N/A
IMTEC + Hemagen + Euroimmun -	0	N/A	N/A
IMTEC + Hemagen - Euroimmun +	12	8	4
IMTEC + Hemagen - Euroimmun -	5	4	1
IMTEC - Hemagen + Euroimmun +	0	N/A	N/A
IMTEC - Hemagen + Euroimmun -	0	N/A	N/A
IMTEC - Hemagen - Euroimmun +	2	1	1

* N = Number of sera in each category of CLIFT results.
+ = positive and - = negative.

TABLE 3. Sensitivities and specificities of IMTEC, Hemagen and Euroimmun kits.

	Sensitivity (95%CI), %	Specificity (95%CI), %
IMTEC	97.4 (84.9-99.9)	94.5 (87.1-98)
Hemagen	66.7 (49.7-80.4)	100 (95-100)
Euroimmun	89.7 (74.8-96.7)	94.5 (87.1-98)

Anti-nucleosome antibodies are defined as antibodies that react with the portion of histone exposed in nucleosome/chromatin, the structure of DNA found in nucleosome/chromatin, or an epitope comprised of the native histone-DNA complex.^{9,13} From the authors' experience, it was found that some sera showing positive reaction on the kinetoplast contained anti-nucleosome antibodies detected by Immunoblot assay. Treating *C. luciliae* substrate with HCL could eliminate histone from the kinetoplast.¹⁰⁻¹² Furthermore, the growth cycle of *C. luciliae* influenced histone appearance in the kinetoplast.¹² Therefore, the preparation of *C. luciliae* substrate, including culture, harvest, and fixation conditions, by different manufacturers can cause variation in CLIFT results.

Second, immunoglobulin (Ig) class detection possibly affected the test performance. The IMTEC kit using polyvalent conjugate provided the highest sensitivity, despite high screening dilution. The other two kits using monovalent conjugate (only IgG class) had lower sensitivities. The IMTEC kit and the Euroimmun kit yielded equal specificity, demonstrating that the detection of various Ig classes increased sensitivity without decreasing specificity. However, clinical association of the detection of Ig classes, other than IgG, has not been described due to a limitation of clinical data.

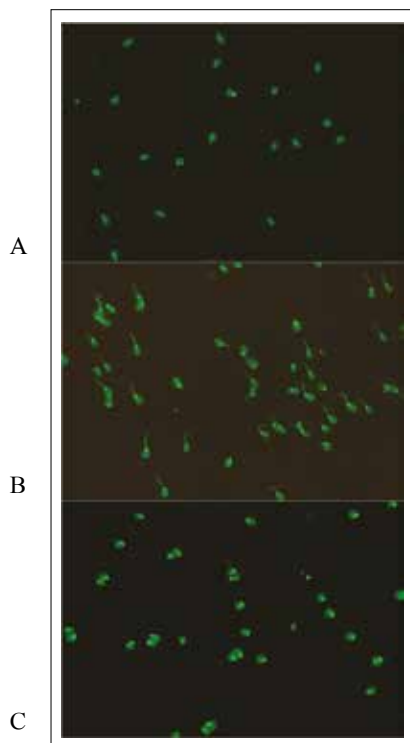


Fig 1. Indirect immunofluorescence reactions of positive control sera with *Crithidia luciliae* (x400). (A) IMTEC. (B) Hemagen. (C) Euroimmun.

Other investigators reported discrepancies among five different commercial CLIFT kits.¹⁴ They found a variation in antibody titer and staining pattern on *C. luciliae*. This agrees with the present study in that the differences in substrate preparation by different manufacturers could affect the kit's performance.

In conclusion, among tests with the same methodology, different kits produced from different manufacturers may give discrepancies in test results. These can be influenced by various factors, for instance, substrate preparation and immunoglobulin class detection. For clinical laboratories, the selection of commercial kit requires an evaluation of a kit's performance and consideration of discrepancies among manufacturers. Based on the evidence of the difference among kits in positive/negative results and antibody titers, using the same commercial kit is recommended for monitoring disease activity.

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