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Evaluation of the automated indirect immunofluorescence test for anti-dsDNA antibodies

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ABSTRACT

Background: Anti-dsDNA antibody is a specific antibody in systemic lupus erythematosus (SLE). Indirect immunofluorescence test (IIFT) is a highly specific method in detecting anti-dsDNA antibody. The application of automated system has gained better consistency than manual operation. This study detected anti-dsDNA antibodies using EUROPattern Computer-aided immunofluorescence microscopy (EPA), and evaluated the performance of the automated system.

Methods: The sera of 96 patients with suspected SLE and 102 control patients were examined using IIFT. The consistency between the EPA and manual reading was analyzed.

Results: Analysis of 198 samples showed that the overall consistency of the negative/positive results between the EPA and manual reading was 94.95%. Based on the manual reading results, the sensitivity and specificity of EPA were 95.70% and 94.29%, respectively. The analysis of 57 samples with non-specific fluorescence showed that the overall consistency of the negative/positive results was 96.49%. The analysis of the antibody titer of 89 positive samples showed that the consistency between the EPA and manual reading was 97.75%.

Conclusion: EPA was consistent with the manual reading with regard to qualitative reading and antibody titer. With low-exposure function, EPA could read samples with non-specific fluorescence. EPA was superior to manual reading in automation and standardization.

1. Introduction

Anti-double stranded DNA (dsDNA) antibodies are mainly seen in patients with systemic lupus erythematosus (SLE) and are highly specific to SLE [1]. These antibodies have been considered a diagnostic criterion of SLE since 1982 [2,3]. Therefore, they are very important for the determination of disease activity and assessment of efficacy. The positive rate of anti-dsDNA antibodies is 20%–90%, as the methodologies and disease activities differ. It has been reported that anti-dsDNA antibodies are involved in the kidney damage observed in SLE patients [4], thus effective and accurate detection is important for the diagnosis and monitoring of SLE.

Many detection methods are available for anti-dsDNA antibodies, including indirect immunofluorescence test (IIFT) with Crithidia luciliae as the antigen (CLIFT), immunoblotting and enzyme-linked immunosorbent assay (ELISA). The sensitivity and specificity of the different detection methods differs [5,6]. CLIFT shows high specificity [7,8], thus it is currently widely used in clinical detection. The characteristics of IIFT pose some limitations to manual reading. For example, wrong procedures and unskilled manual preparation of fluorescence slides may affect the final quality of the images. Furthermore, lack of standardized analysis of IIFT reading, especially discrimination of the critical value and determination of titer, and low objectivity of the results are not beneficial for the recognition of the quality control and results. Additionally, manual operation is time consuming and the reading is not efficient. An automatic system could reduce variation, and improve efficiency and quality. Our laboratory began to use automatic operation and reading equipment to detect antinuclear antibody (ANA); the automatic reading was consistent with the manual reading. Previous reports have shown that the automated IIFT platform based on

Abbreviations: ANA, antinuclear antibody; ANCA, anti-neutrophil cytoplasmic antibody; Anti-dsDNA, Anti-double stranded DNA; CLIFT, *Crithidia luciliae* immunofluorescence test; ELISA, enzyme-linked immunosorbent assay; EPA, EUROPattern Computer-aided immunofluorescence microscopy; IIFT, Indirect immunofluorescence test; PLA2R, anti-phospholipase A2 receptor; SLE, systemic lupus erythematosus

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Computer-aided immunofluorescence microscopy (EUROPattern) was consistent in the detection of ANA with regard to negative/positive results, fluorescence pattern and antibody titer. Moreover, an automatic system is superior to manual reading in terms of automation, standardization and saving human resources, thus could improve the laboratory diagnosis of autoimmune diseases. Many studies and clinical practices have shown this system could be applied to the automatic reading of the IIFT of auto-antibodies [9–12].

To establish automated operation of IIFT for the detection of anti-dsDNA antibodies without the effect of artificial errors to improve working efficiency, we prepared fluorescence slides using Sprinter XL automated IFT/ELISA analyzer (EUROIMMUN AG, Lübeck, Germany). Using consistent fluorescence slides, we compared the EUROPattern Computer-aided immunofluorescence microscopy (EPA) and manual reading, analyzed the consistency, sensitivity and specificity of qualitative results and antibody titer, and provided evidence for evaluating the clinical value of an automated reading system.

2. Materials and methods

2.1. Subjects

The present study included 96 SLE patients who were found positive for anti-dsDNA antibodies in Wuhan Tongji Hospital from March to September 2018. There were 17 males, 79 females, and the mean age was 36 years (9–82). The healthy control group included 102 samples, for which the IIFT of ANA was negative.

This study was approved by the ethical committee of Tongji hospital, Tongji Medical College, Huazhong University of Science and Technology. All subjects gave written informed consent.

2.2. Methods

2.2.1. Sample preparation

After 8 h of fasting, 2 ml of venous blood were collected into a test tube for serum separation, which was then stored in at −20 °C until use. The serum was thawed and mixed before measurement.

2.2.2. Slide preparation

IIFT with Crithidia luciliae as the antigen (specific anti-human IgG conjugate kit, EUROIMMUN AG, Lübeck, Germany) was used to detect anti-dsDNA antibodies. Sprinter XL automated IFT/ELISA analyzer (EUROIMMUN AG, Lübeck, Germany) was used in accordance with the manufacturer's instructions. The initial titer of the serum was 1:10. The prepared slides were mounted with glycerol until use. After first detection, the positive sera were diluted 1:10, 1:32, 1:100, 1:320 and 1:1000 for second detection, to determine the final titer.

2.2.3. Reading

The prepared slides were read by both EPA and manual reading. EPA read negative/positive results and further calculated the titer of positive results automatically (generally, 500 samples were loaded once, the reading time was 18 s per sample, images of all samples were taken and stored on a hard drive). For manual reading, two experienced laboratory technicians who were qualified to make laboratory reports, reviewed and confirmed the images, and examined the fluorescence staining of Crithidia luciliae. Absence of specific fluorescence in the cinetonucleus was considered negative, while presence of specific fluorescence in the cinetonucleus was considered positive. All the above mentioned equipment and reagents were purchased from EUROIMMUN.

2.3. Statistical analysis

SPSS 17.0 software was used for statistical analysis. Categorical data was analyzed by chi-square test. Spearman correlation analysis was also performed. $P < .05$ indicates significant difference. Kappa analysis was performed to analyze consistency: $K \leq 0.4$ indicates low consistency, $0.4 < K \leq 0.6$ indicates moderate consistency, $0.6 < K \leq 0.8$ indicates high consistency, $K > 0.8$ indicates perfect consistency.

Table 1
Negative/positive results by EPA and manual reading.

$n = 198$		Manual reading		Total
		Positive	Negative	
EPA	Positive	89	6	95
	Negative	4	99	103
	Total	93	105	198

3. Results

3.1. Consistency analysis of the negative/positive results by EPA and manual reading

As shown in Table 1, manual reading determined 93 positive samples and 105 negative samples, while EPA determined 95 positive samples and 103 negative samples. In total there were 198 samples, 89 samples were positive and 99 samples were negative by both methods. Four samples that were determined positive by the manual reading were determined negative by EPA, while six samples that were determined negative by the manual reading were determined positive by EPA. The sensitivity and specificity of EPA was 95.70% and 94.29%, respectively. Based on the manual reading results, the overall consistency of the negative/positive results between EPA and manual reading was 94.95%. The statistical analysis results were $K = 0.899$ and $P < .05$. The Spearman correlation analysis results were $r = 0.899$ and $P < .05$. The results of both methods positively correlated.

3.2. Correlation analysis of the non-specific fluorescence read by EPA and manual reading

As shown in Table 2 and Fig. 1, 57 samples with non-specific fluorescence in Crithidia luciliae were seen among the 198 samples. The manual reading used the eyepiece to read the slides, while EPA read the slides automatically by low exposure. In total, 49 samples were positive and six samples were negative by both methods; one sample that was determined positive by the manual reading was determined negative by EPA, while one sample that was determined negative by the manual reading was determined positive by EPA. The sensitivity and specificity of EPA was 98.00% and 85.71%, respectively. Based on the manual reading results, the overall consistency of the negative/positive results between the EPA and manual reading was 96.49%. The statistical analysis results were $K = 0.837$ and $P < .05$. The Spearman correlation analysis results were $r = 0.837$ and $P < .05$. The results of both methods positively correlated.

3.3. Comparison of the antibody titer determined by the EPA and manual reading

As shown in Tables 1 and 3, 89 samples were determined positive by

Table 2
Non-specific fluorescence samples read by EPA and manual reading.

$n = 57$		Manual reading		Total
		Positive	Negative	
EPA	Positive	49	1	50
	Negative	1	6	7
	Total	50	7	57

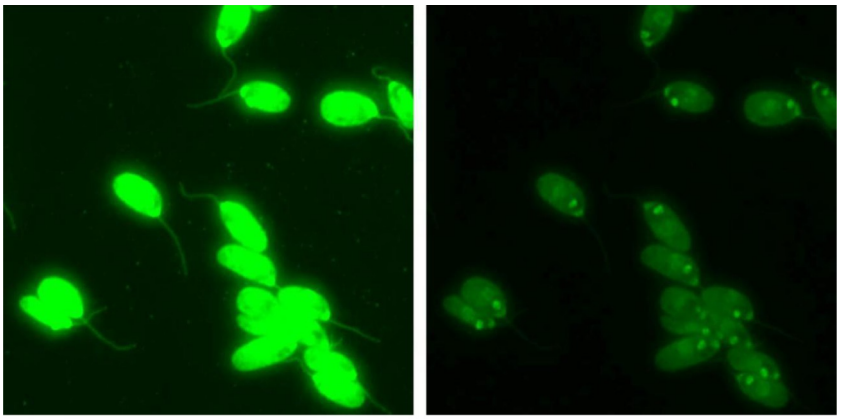

Image of positive normal exposure Image of positive low exposure

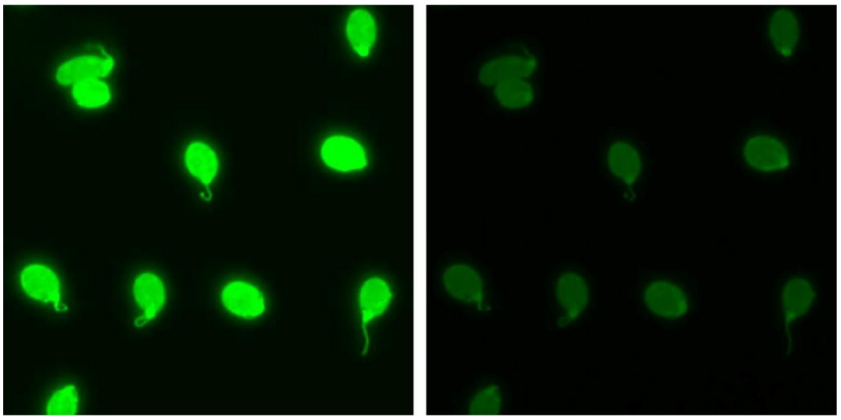

Image of negative normal exposure Image of negative low exposure

Fig. 1. Non-specific fluorescence of Crithidia luciliae.

Table 3.Table 3
Distribution of the antibody titer by EPA and manual reading.

Antibody titer		Manual reading					
		1:10	1:32	1:100	1:320	1:1000	> 1:1000
EPA	1:10						
	1:32		4	1			
	1:100	1	12	11			
	1:320		1	4	16		
	1:1000				11	10	1
	> 1:1000					1	16
Consistency		97.75%					

Note: Deviation of ± 1 titer was considered consistent.

both manual reading and EPA. Based on the manual reading results, 87 samples were read positive by EPA with correct determination of antibody titer; the consistency of positive antibody titer was 97.75%.

4. Discussion

Standardization and automation of IIFT are very important in the diagnosis of autoimmune diseases, to ensure an objective antibody detection procedure. The loading, washing and incubation of the fluorescence slides were performed automatically, with some procedures read automatically, such as the anti-dsDNA antibody, ANA and anti-neutrophil cytoplasmic antibody (ANCA). Anti-dsDNA antibody is the marker antibody for SLE diagnosis. IIFT, ELISA and immunoblotting are common detection methods for anti-dsDNA antibodies, where IIFT is straightforward and specific compared with other methods [13–16]. Although IIFT is irreplaceable for the detection of anti-dsDNA antibodies, its standardization and automation lag behind other immunological techniques. Visual reading is performed in most laboratories in China. It requires skill and experience, and is vulnerable to subjective factors. Furthermore, the difference between intra-laboratory and inter-laboratory reading is significant, resulting in standardization failure, thus reciprocal recognition of inter-laboratory results is difficult.

The present study used a new standardized fluorescence detection platform (Sprinter XL automated indirect immunofluorescence analyzer + EUROPattern Computer-aided immunofluorescence microscopy + EUROLabOffice experiment management software + reagents) and a two-dimensional code-scanning module. Sprinter XL scanned and read the two-dimensional code of specific fluorescence slides, identified the locations of fluorescence and generated the experimental distribution information, which communicated with EUROPattern to provide the information. EUROPattern loaded the fluorescence slides randomly, recognized fluorescence automatically, and matched samples, images and results automatically. Finally, paperless office and automated processes were achieved, possible errors due to manual operation were reduced, and reliability of the results was achieved. Additionally, the EUROLabOffice experiment management software combined the detection results of the serum samples with different dilutions and generated the final results.

As reported by the previously studies, the coincidence rate between EPA automated system and manual reading was > 93% [17,18], and 96.0% for a different automated system [19]. The present study showed that the overall consistency of EPA and manual reading was 94.95%, which indicated high consistency of the negative/positive results.

The present study used the new function of taking images with low exposure by EPA. After the first image was taken automatically, if the brightness was too high, EPA decreased the exposure of the camera automatically and took another image, which would be used for the determination of the karyotype and negative/positive determination. In previous detection of anti-dsDNA antibodies, some samples with non-specific fluorescence may have been determined positive. In strongly positive ANA samples, a higher titer made it difficult to determine the karyotype. The function of low-exposure image in EUROPattern increased the accuracy of these samples. Among the 57 samples with non-specific fluorescence, the negative/positive results of 55 samples were completely consistent between the EPA and manual reading, with an overall consistency of 96.49% (K = 0.837), and the sensitivity and specificity of the EPA reading were 98.00% and 85.71%, respectively. This indicated high consistency of the negative/positive results of samples with non-specific fluorescence between the EPA and manual reading. In the initially diluted samples, the samples were determined by low-exposure images, which indicated whether serial dilution of samples was needed. Accurate and effective screening required serial dilution of positive samples and fewer serial dilutions of negative samples owing to the interference of non-specific fluorescence.

The present study found inconsistency between the EPA and manual reading in certain cases. Ten out of 198 negative/positive results were different; four samples that were determined positive by the manual reading were determined negative by EPA, while six samples that were determined negative by the manual reading were determined positive by EPA. In the determination of antibody titer, two out of 89 samples were inconsistent, as the EPA result was higher than that of the manual reading by 2 titers.

In the determination of negative/positive results, six samples that were determined negative by the manual reading were determined positive by EPA. These false positive were primarily caused by an intensive fluorescence of the basal bodies and inconsistent insect adhesion. Four samples that were determined positive by the manual reading were determined negative by EPA, which caused by an insufficient number of Crithidia luciliae with positive cinetonucleus staining and with the critical value of fluorescence intensity. In the determination of antibody titer, two samples were inconsistent, as the nucleus staining brightness was heterogeneous and the fluorescence intensity was of critical value. The discordance phenomenon has also been reported in previous studies [17,18]. The final results were confirmed by expert analysis of the data (or images) saved by the EPA system, which would effectively avoid false positives and false negatives and guarantee the accuracy of the results.

The EPA automation system has several advantages. Firstly, it could reduce the chance of human errors. Secondly, the system has new function of low-exposure imaging which could improve the consistency of samples with non-specific fluorescence. Thirdly, it provides substantial labor savings and good concordance with technologist IIFT microscopy, thus increasing standardization, laboratory efficiency, and removing subjectivity [20]. However, the automation equipment itself is very expensive which might limit its wide application in common laboratories.

In conclusion, the consistency between EPA and manual reading was good for negative/positive results and antibody titer. Currently, this system is predominantly used in the automatic reading of ANA (Hep-2 as antigen), anti-dsDNA antibodies (Crithidia luciliae as antigen), ANCA (Granulocytes as antigen) and anti-phospholipase A2 receptor (PLA2R) antibodies (transfected cells as antigen) [11]. More antigens, reagents and fluorescence modes were expected to achieve standardized reading and clinical application, such as the tissue sections of antikeratin antibodies and anti-smooth muscle antibodies, respiratory pathogens and Epstein-Barr virus. Yet, this technique requires further improvements. In the future, accumulation and update of databases and algorithms, discovery of more functions as well as increased consistency of automatic reading and manual reading will promote the automation and standardization of IIFT.

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