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ORIGINAL ARTICLE

Comparison of Anti-dsDNA Evaluation with 20x and 40x Objectives in Automated Systems to Expert Agreement

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SUMMARY

Background: **In this study, we investigated the effect of using a 40x objective instead of the recommended 20x objective in the computer-assisted automated evaluation of anti-dsDNA antibodies using the *Crithidia luciliae* indirect immunofluorescence (CLIF) test. By using a 40x objective, we aimed to increase the accuracy and specificity of the CLIF test by improving image clarity and enabling easier interpretation by the expert physician.**
Methods: **Anti-dsDNA tests of 156 positive and 40 negative samples were evaluated using the automated EUROPattern system at 20x and 40x magnifications. The results were compared with the assessments of experienced physicians. Statistical analysis included chi-squared and kappa agreement tests. Sensitivity, specificity, and accuracy metrics were calculated for both objectives.**
Results: **When evaluated with the 20x objective, the system achieved a sensitivity of 100%, specificity of 29.85%, and accuracy of 50.26%, with a kappa coefficient of 0.199 (95% CI: 0.129 - 0.267). With the 40x objective, sensitivity was 94.55%, specificity was 90.30%, and accuracy was 91.53%, with a kappa coefficient of 0.805 (95% CI: 0.714 - 0.895). The agreement between the automated system and the expert evaluations significantly improved with the 40x objective.**
Conclusions: **Using a 40x microscope objective enhances the compatibility between automated systems and expert evaluations, providing clearer and larger images. This adjustment reduces false positives, increases accuracy, and facilitates decision-making for specialists, supporting the adoption of 40x objectives for routine laboratory use.**
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KEYWORDS

Crithidia luciliae, indirect immunofluorescence, anti-dsDNA antibodies, automated systems

INTRODUCTION

Systemic lupus erythematosus (SLE) is a severe autoimmune rheumatic disease that presents with various clinical manifestations. Anti-dsDNA antibodies are a key diagnostic criterion for SLE, according to the American College of Rheumatology (ACR) [1]. Anti-dsDNA is an important serological marker of SLE. It is used to determine disease activity and renal involvement [2]. Anti-dsDNA positivity rate varies between 20% and 90% [3]. Enzyme-linked immunosorbent assays (ELISA), Farr radioimmunoassay (RIA), and *Crithidia luciliae* indirect immunofluorescence (CLIF) test can be used to detect

anti-dsDNA [4]. Out of these methods, ELISA has the highest sensitivity but lower specificity. Therefore, primary test results obtained by ELISA usually must be confirmed by a second test such as Farr immunoassay and/or CLIF [5]. The CLIF test has a high specificity (≥ 90%) and is therefore frequently used for confirmation [4]. This test uses the flagellated protozoan *C. luciliae*, a tightly packed dsDNA network within large mitochondria, to detect anti-dsDNA antibodies [6]. Although indirect immunofluorescence tests are evaluated by manual microscopes, computer-aided automated identification systems have recently been used due to subjective interpretation and lack of standardization. In these systems, which are also used in more complicated tests such as ANA detection and pattern identification compared to the CLIF test, the final decision is left to the laboratory specialist, and these systems work as decision support systems. In addition to helping to ensure standardization, these systems offer the advantage of keeping the past results of the patients in memory [7]. Furthermore, in some commercial manufacturers, CLIFT and ANA IFA results can be evaluated together if the patient's order is available. This provides an important advantage in accurately evaluating patient results [8].

Our laboratory analyzes anti-dsDNA using the CLIF method in the computer-aided automated system EUROPattern (Euroimmun AG, Lubeck, Germany). The EUROPattern system in the laboratory has lenses with different magnifications in the microscope for image detection. The company recommends using a 20x objective magnification in automated evaluation, but evaluation can also be performed with a 40x magnification objective if the expert requests it. In anti-dsDNA evaluation, it reads and evaluates at 20x objective with company recommendation. The computerized system interprets the results as positive or negative. The final decision is left to the specialized physician to evaluate and approve the images taken by the automated system on the computer screen or, if necessary, by looking at the image with the microscope within the device. The specialized physician can evaluate the positive and negative results given by the automated system and, if necessary, change these results to the opposite.

The protozoan *C. luciliae*, which is used in the anti-dsDNA test by indirect immunofluorescence method, has small cells, and the kinetoplast structure used in the evaluation of the test in the microscope is challenging to distinguish with a 20x objective. Although the computer system interprets the results as positive/negative, the final decision is left to the expert physician, and decision-making on the system computer screen becomes difficult, because the image is small with 20x objective. When evaluating many images, it is necessary to enlarge the image on the computer screen. In our 4 years of experience, it has been observed that the automated system has difficulty with positive samples, while it separates negative samples easily. This situation was found to be similar in other studies, and its sensitivity was found to be higher than its specificity [9,10]. The 40x objective to be used instead of the 20x objective will provide ease of evaluation to the specialist physician in making the final decision. In addition, false positivity is thought to decrease in the automated system. The study aimed to investigate the impact of using a 40x objective instead of the recommended 20x objective in the computer-assisted automated evaluation of dsDNA antibodies using the CLIF in the commercial automated EUROPattern system.

MATERIALS AND METHODS

Between October 1, 2024, and January 1, 2025, the study included 195 anti-dsDNA test results. These tests were analyzed using a 20x objective lens with the automated immunofluorescence evaluation system EUROPattern (Euroimmun AG, Lubeck, Germany), employing the indirect immunofluorescence method. The evaluation was conducted using the Eurolab Office software, which was connected to the system. Samples that required dilution were excluded from the study.

The samples analyzed with the 20x objective lens were stored at -80 degrees Celsius. Subsequently, the company was contacted to adjust the device software for 40x evaluation. The samples were then re-examined and re-evaluated using a 40x objective magnification. Two experienced physicians conducted the evaluations. Anti-dsDNA titers of ≥ 1:10 were considered positive.

Our laboratory is enrolled in the external quality assurance program provided by UK NEQAS.

Statistics

Data analysis was performed using SPSS 26 software. Continuous data is presented as mean and standard deviation, while categorical data is shown as numbers and percentages. Chi-squared tests and kappa agreement analysis were utilized for statistical evaluation.

RESULTS

A total of six specimens were not evaluated due to discrepancies between the 20x and 40x expert evaluations. One hundred eighty-nine samples, which yielded consistent results across both lenses, were assessed.

When using a 20x objective, the sensitivity was found to be 100%, while the specificity was 29.85%, resulting in an overall accuracy of 50.26%. The automated system correctly identified all positive samples, but it classified 94 samples, which the expert had deemed negative, as positive. The κ value of 0.19 indicates poor agreement.

In contrast, when evaluated at 40x magnification, the results were as follows: sensitivity was 94.55%, specificity was 90.30%, and overall accuracy was 91.53%. In this case, three samples that were evaluated as positive by the expert were classified as negative by the auto-

Table 1. Comparison of expert assessment and EUROPattern assessment with 40x microscope objective.

		40 x EUROPattern	
		Positive	Negative
Expert	Positive	52	3
	Negative	13	121

Sensitivity 94.55%.
Specificity 90.30%.
Accuracy 91.53%.
κ value 0.805 (95% CI: 0.714 - 0.895).

Table 2. Comparison of expert assessment and EUROPattern assessment with 20x microscope objective.

		20 x EUROPattern	
		Positive	Negative
Expert	Positive	55	0
	Negative	94	40

Sensitivity 100%.
Specificity 29.85%.
Accuracy 50.26%.
κ value 0.199 (95% CI: 0.129 - 0.267).

mated system. The automated system exhibited better agreement with the negative samples, with only three positive samples misclassified as negative. The overall agreement yielded a κ value of 0.80, indicating strong agreement.

DISCUSSION

Automated system pre-screen dsDNA assessments allow physicians to focus on identifying positive cases. In addition to conducting batch screenings for positive and negative results, these systems serve as a second reader, supporting physician judgment and reducing error rates. This enhances the efficiency and reliability of the assessment process [11]. The results are evaluated through the Eurolab Office, which is linked to the EUROPattern system. In this process, cases can be reviewed and approved individually, or groups classified as negative by the system can be collectively reviewed and approved. This approach allows for more efficient use of the system. However, the very small size of the *C. luciliae* in 20x images, particularly during batch screening, makes evaluation challenging.

The *C. luciliae* IIF test demonstrates high specificity [12]. *C. luciliae* is a protozoan that has several key structures, including a nucleus, kinetoplast, and basal body. The kinetoplast contains a condensed mass of double-stranded DNA (dsDNA), and its fluorescence is the primary indicator for a positive test result. However, evaluating the IIF can be challenging due to fluorescence occurring in structures other than the kinetoplast in *C. luciliae* cells [5,11]. The EUROPattern Suite employs a 20x lens rather than a 40x lens, allowing for the evaluation of more cells in a single image [8]. This study did not aim to establish the sensitivity and specificity of EUROPattern in routine patient samples; instead, it explored the feasibility of using a 40x objective lens as an alternative to the recommended 20x objective. Sample selection did not rely on routine patient samples. For this study, 156 positive and 40 negative samples were analyzed while using the 20x microscope objective. Therefore, comparisons of sensitivity and specificity with other studies may not be valid. In our evaluation using the 20x objective, we found a sensitivity of 100%, a specificity of 29.85%, and an accuracy of 50.26%. In contrast, when utilizing a 40x microscope objective, we recorded a sensitivity of 94.55%, a specificity of 90.30%, and an accuracy of 91.53%.

Figure 1 displays images of the same sample taken with 20x and 40x microscope objectives. The dsDNA IIF test for this patient, who had a negative ANA result, was assessed as positive by the system when using the 20x objective, but negative when using the 40x objective. In the expert review, both assessments were recorded as negative.

The results using the 40x microscope objective showed greater agreement with expert assessments compared to those from the 20x objective. In one of the two studies that followed company recommendations for using a 20x microscope objective to evaluate dsDNA with the EUROPattern, the EUROPattern demonstrated a sensitivity of 95.7% and a specificity of 94.3%. This resulted in an overall agreement rate of 94.95% for negative and positive results when compared to manual reading [10]. In the second study, the system's relative sensitivity and specificity were recorded at 94.1% and 93.2%, respectively, with an agreement rate of 93.3% between EUROPattern analysis and visual reading [9]. In a study conducted with a 40x microscope objective, the system achieved a sensitivity of 100%, a specificity of 96.8%, and an accuracy of 97.2% [5]. Additionally, another automated system called NOVA Lite, which also utilized a 40x microscope objective, found an agreement rate of 98.4% between expert assessments and instrument results (kappa = 0.94, 95% CI: 96.0 - 99.6%) [13]. Overall, the 40x microscope objective provided better agreement than the 20x objective.

Six samples were excluded due to discrepancies between the 20x and 40x expert evaluations. Standardizing immunofluorescence evaluation is challenging because many biological, technical, and operator-related variables can influence the results [14]. Additionally, differences may arise in the outcomes of the same sample within the same laboratory using the same device [15]. Furthermore, images of the same sample from two different studies will not be completely identical. We

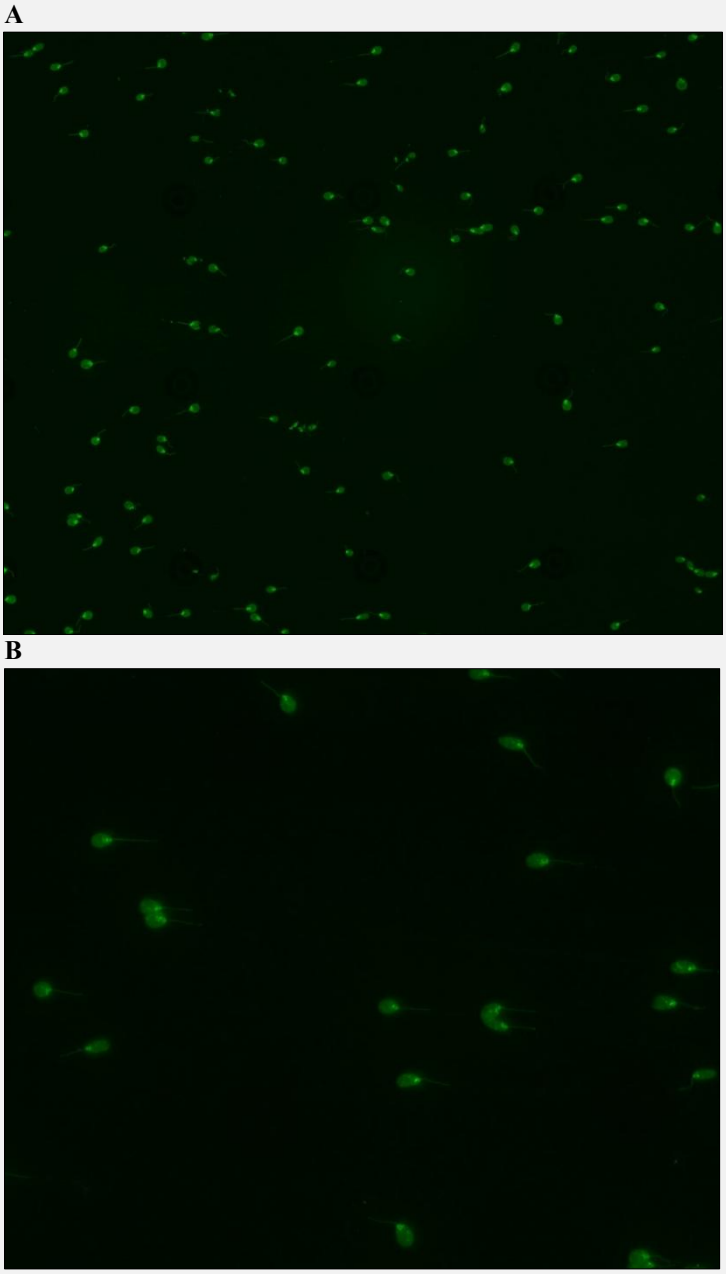

Figure 1. Image of the same sample viewed under 20x and 40x microscopic magnifications with EUROPattern.

A) The 20x image was automatically classified as positive by the EUROPattern system, but the expert evaluated it as negative.
B) The 40x image was automatically classified as negative by the EUROPattern system and was also deemed negative by the expert.

did not compare the total positive and negative results based on the evaluation outcomes of the 20x and 40x samples, even though they were the same samples. Instead, we focused on the compliance of the EUROPattern with expert evaluations for both the 20x and 40x samples.

EUROPattern employs a four-stage algorithm for detecting dsDNA. In the first stage, images from both the green and red channels are analyzed. The sharpness of these images is evaluated, and any out-of-focus images are identified. During the second stage, each cell is examined for its shape characteristics and any potential defects. Cells that are defective or non-fluorescent are eliminated from consideration. In the third stage, the re-

maining cells are classified as positive or negative based on their fluorescence signals and intensities. In the fourth stage, images are labeled as positive or negative according to a specified percentage of positive cells. Additionally, the antibody titer is estimated based on the brightness of fluorescence [5]. It is worth noting that the images taken with a 40x objective lens tend to be larger, which may lead to better alignment with expert assessments in EUROPattern's evaluation.

Using the 40x objective from EUROPattern can enhance both system and expert agreement by providing larger and clearer images during the evaluation process. This improvement can lead to higher accuracy rates, particularly when assessing positive samples. Therefore, guiding users to choose the 40x microscope objective will increase system efficiency and simplify the evaluation process.

Limitation

This study used the EUROpattern system, and the findings may be less applicable to laboratories using alternative methods or systems.

Another important point is that the samples could not be evaluated using 20x and 40x lenses simultaneously after preparation. We informed Euroimmun application specialists that we would like to evaluate the same sample with different objectives in succession. However, we were informed that this was not possible with the software. Because the sample slides are square-coded, the software cannot re-evaluate a sample scanned at 20x when switching to 40x. While we were able to perform consecutive reads in EUROPattern, it is important to note that these conditions are not entirely equivalent, as fluorescence signal fading may occur during the reads. Therefore, for this study, the samples were stored at -80°C, and new samples were prepared for the second run.

Ethical Approval Statement:

This study was approved by the Ethics Committee of Cam and Sakura City Hospital (date/number: 25.09. 2024 - 182).

Source of Funds:

Only the dsDNA IFA kit was donated by Euroimmun Türkiye.

Declaration of Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence their work.

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