Short Communication

Diagnostic Validation of Fully Automated ANA Detection by Indirect Immunofluorescence: An Initiative towards Standardization

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Abstract
Objective: This study entailed the diagnostic validation of an automated system utilized for the detection of antinuclear antibodies (ANA) through the indirect immunofluorescence method, juxtaposed with the traditional manual approach for ANA detection. The study was undertaken in the Department of Chemical Pathology at Chughtai Laboratory, Lahore, spanning a period of three months, from December 2018 to February 2019.

Methods: A total of 132 patient samples were gathered for the purpose of conducting this validation study. The manual detection of ANA was performed by using slides coated with HEp-2 cells. An LED fluorescence microscope was used for the interpretation of results by a trained technologist as well as by a pathologist. After manual detection of ANA, the same specimens were evaluated by a fully automated ANA-IIF detection system which has inbuilt characteristics of slide processing, microscopy, image capturing and displaying specific ANA patterns. The results were interpreted as positive for those samples having sample titers more than 1:80. The same criteria of positivity were used for both manual and automatic systems.

Results: A total of 132 specimens underwent evaluation through both manual and automated ANA indirect immunofluorescence (IIF) detection systems. The concordance between these two methodologies exhibited an overall agreement of 95.7%, with a positive agreement of 97.5% and a negative agreement of 94.9%. Cohen’s kappa value stood at 91.7% (ranging from 83.4% to 98.9%), indicating a robust correlation between the two detection systems. The diagnostic sensitivity of the fully automated IIF methodology demonstrated 89.38% (with a 95% confidence interval of 77.24% to 96.13%) and the diagnostic specificity of 95.22% (with a 95% confidence interval of 87.88% to 98.76%).

Conclusion: The fully automated showed strong correlation with manual ANA detection methodology based on the principle of indirect immunofluorescence.

Keywords: indirect immunofluorescence, automated, manual, anti-nuclear antibodies
1 INTRODUCTION

The primary and pivotal role of the immune system is to discern and differentiate between endogenous (“self”) and exogenous (“non-self”) antigens. Autoimmune disorders are diseases which develop as a result of alteration in regulatory and control functions of immune system. Many rheumatologic and non-rheumatologic autoimmune diseases involve testing for anti-nuclear antibodies and cytoplasmic antibodies to reach a final diagnostic evaluation.[5,6]. The anti-nuclear antibodies detection is one of the most sensitive tests for the evaluation of autoimmune disorders and various methodologies are used for this detection, such as manual and automated indirect immunofluorescence (IIF) and enzyme-linked immunosassay. The most effective approach for anti-nuclear antibodies (ANA) screening is conducted through the utilization of indirect IIF on human epithelial cells[7]. The identification process of autoantibodies is done through the analysis of their characteristic fluorescence patterns[8], alongside the determination of their titers. The conventional method of ANA testing was characterized by its time-intensive nature, substantial demands for labor and susceptibility to investigator-related bias owing to the subjectivity inherent in visual interpretation using LED microscopes. Given the escalating demand from clinicians to diagnose autoimmune disorders, the healthcare sector necessitated the implementation of automation and standardization in the evaluation of ANA detection via IIF. To address this demand, a limited number of commercial platforms have been launched to the market, incorporating automated motorized camera microscopes and digital image analysis software.[8-12]

In the present study, we conducted an evaluation of the Helios automatic ANA detection system in comparison to the manual method, focusing specifically on the visual interpretation of IIF results. This evaluation encompassed aspects such as the classification of results as positive or negative, as well as the recognition of specific pattern manifestations.

2 MATERIALS AND METHODS

This diagnostic validation study was undertaken in the Department of Chemical Pathology at Chughtai Laboratory in Lahore. The study spanned a duration of three months, commencing from December 2018 and concluding in February 2019.

A total of 132 patient samples were collected for this validation study. The manual detection of ANA was performed by using slides coated with HEP-2 cells. A LED fluorescence microscope was used to interpret results by a trained technologist as well as by a pathologist. After manual detection of ANA, a fully automated ANA-IIF detection system was used to evaluate the same specimens which had inbuilt characteristics of slides processing, microscopy, image capturing and displaying specific ANA patterns. Positive ANA-IIF results were classified, basing on the prevailing ANA pattern observed at the highest dilution exhibiting a positive outcome. These patterns encompassed homogeneous, speckled, nucleolar, centromere, mitochondrial, or nuclear dots.

2.1 Statistics

All gathered data were input and analyzed with SPSS. The concordance between the two methods was compared and the Cohen Kappa value was calculated. The diagnostic sensitivity and specificity of the methods were also determined.

3 RESULTS

A total of 132 specimens were evaluated by manual and automated ANA IIF detection systems. The concordance between this two methodologies revealed an overall agreement of 95.7%, with a positive agreement of 97.5% and a negative agreement of 94.9%. The kappa value of Cohen amounted to 91.7% (ranging from 83.4% to 98.9%), underscoring a robust correlation between the two detection systems. The fully automated IIF methodology demonstrated a diagnostic sensitivity of 89.38% (with a 95% confidence interval ranging from 77.24% to 96.13%) and a diagnostic specificity of 95.22% (with a 95% confidence interval ranging from 87.88% to 98.76%). The incidence of different patterns recognized by two methods were succinctly summarized in Table 1, with the predominant pattern observed as homogenous. It was noteworthy that a confirmatory test for this pattern was the dsDNA assay (as Table 1).

4 DISCUSSION

The utilization of cutting-edge technology in automated ANA detection systems was a major step towards the standardization of this test across different detecting platforms. The precision and accuracy of analytical assays were of crucial concerns to clinicians as intra- and inter-laboratory variations may lead to erroneous diagnoses. Significant variations could be attributed to methodological factors, encompassing variations in microscopy techniques, test kits, reagents, and the methodology for incubation. These variations might also be impacted by the level of expertise and experience possessed by the laboratory’s technical staff.[13]

Currently, at least six commercial automated ANA detection systems were available with variable sensitivity
and specificity. These diagnostic systems relied on the utilization of diverse hardware modules, which were integrated with advanced software algorithms for mathematical pattern recognition. This integration facilitated the achievement of a completely automated image analysis and evaluation process for the detection of ANA[14]. Machine learning models were also being developed for automated pattern recognition[15]. Nevertheless, it was imperative to validate the clinical diagnostic efficacy of these systems through clinical investigations, supplementing the existing body of analytical research. Moreover, these emerging methodologies could be enhanced by a heightened capacity to accurately identify complex mixed fluorescent patterns or comparatively uncommon fluorescent profiles[16].

In our study, the automated IIF method demonstrated a diagnostic sensitivity of 89.38% (with a 95% confidence interval of 77.24% to 96.13%) and a diagnostic specificity of 95.22% (with a 95% confidence interval of 87.88% to 98.76%). A recent study was conducted to compare various automation systems for ANA detection, concluding that when subjected to the same samples for analysis, all the systems collectively exhibited a total sensitivity rate of 96.7% and a specificity rate of 89.9%[4]. The outcomes of this study aligned with the aforementioned study’s findings, as well as with other reports which assessed the performance of our automated interpretation system and similar systems. Notably, the automated interpretation system utilized in our study reliably distinguished between positive and negative results[17].

5 CONCLUSION
The implementation of the automated system yields had resulted in a significant reduction in labor requirements, coupled with a commendable level of agreement when compared to technologist-performed ANA IIF microscopy. This convergence had enhanced the standardization of diagnostic processes, elevated laboratory efficiency, and mitigated inherent subjectivity. The outcomes further underscored the system’s enhanced reliability, translating into a heightened level of operational efficiency and performance robustness.

Table 1. Pattern Recognition by Manual and Automated Method

<table>
<thead>
<tr>
<th>Pattern Recognition</th>
<th>Manual IIF Method</th>
<th>Automated IIF Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>84</td>
<td>85</td>
</tr>
<tr>
<td>Homogenous</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>Speckled</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>Nucleolar</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Cytoplasmic granules</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Nucleoplasm dots</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total cases</td>
<td>132</td>
<td>132</td>
</tr>
</tbody>
</table>

Acknowledgements
Not applicable.

Conflicts of Interest
The authors declared no conflict of interest.

Author Contribution
Zubair M conceived, designed, conducted data collection, and authored the manuscript, being responsible for the research’s integrity. Rasool Z performed statistical analysis and edited the manuscript.

Abbreviation List
ANA, Antinuclear antibodies
IIF, Indirect immunofluorescence

References


