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Performance Assessment of the Concepta Automated ANA Detection System in Routine Clinical Samples

Concepta Otomatik ANA Tespit Sisteminin Rutin Klinik Örneklerde Performans Değerlendirmesi

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ABSTRACT

Aim: Indirect immunofluorescence (IIF) on HEp-2 cells is the standard method for detecting anti-nuclear antibodies (ANA) due to its high sensitivity. Recently, several artificial intelligence-supported automated immunofluorescence systems have been developed to improve standardization and efficiency in ANA detection using the IIF method. This study aimed to evaluate the performance of the Concepta automated immunofluorescence system in routine clinical settings for ANA testing using the IIF method.
Material and Methods: A total of 1000 patient serum samples were analyzed using the Concepta automated system after preparation with the iPRO processor. The results were compared to manual evaluations conducted by two expert clinicians to assess the system's agreement, sensitivity, specificity, and accuracy in pattern recognition.
Results: The Concepta system demonstrated an overall agreement of 98.11% with manual evaluations for positive and negative discrimination, corresponding to a κ value of 0.958. The sensitivity and specificity were found to be 99.08% and 97.61%, respectively, with positive and negative predictive values of 87.96% and 99.83%. High concordance rates were observed for homogeneous (95.7%), centromere (92.3%), and nucleolar (92.1%) patterns, while lower rates were noted for speckled (60%) and cytoplasmic (44.4%) patterns.
Conclusion: The Concepta automated system demonstrated very high accuracy in ANA positive and negative discrimination, comparable to other automated systems. Despite some limitations in recognizing dense fine speckled and mixed patterns, it proved particularly effective in distinguishing between positive and negative results. These findings suggest that the Concepta system is a promising new alternative in the field of ANA testing.
Keywords: Autoantibodies; antinuclear autoantibodies; immunofluorescence microscopy.

ÖZ

Amaç: Anti-nükleer antikorların (ANA) tanısında HEp-2 hücrelerinin kullanıldığı indirekt immünofloresans (IIF) testi, yüksek duyarlılığı nedeniyle standart yöntemdir. Son yıllarda, IIF yöntemi ile ANA tespitinde standardizasyonu ve verimliliği artırmak amacıyla yapay zeka destekli birçok otomatik immünofloresans sistemi geliştirilmiştir. Bu çalışmanın amacı, otomatik immünofloresans sistemi olan Concepta'nın, IIF yöntemi kullanılarak ANA testinde rutin klinik ortamlardaki performansını değerlendirmektir.
Gereç ve Yöntemler: iPRO işlemcisiyle hazırlanan toplam 1000 hasta serum örneği, Concepta otomatik sistemi kullanılarak analiz edildi. Sonuçlar, iki uzman klinisyen tarafından yapılan manuel değerlendirmelerle karşılaştırılarak sistemin uyumu, duyarlılığı, özgüllüğü ve patern tanımlama doğruluğu değerlendirildi.
Bulgular: Concepta sistemi, pozitif ve negatif ayrımda manuel değerlendirmelerle %98,11 genel uyum gösterdi ve κ değeri 0,958 olarak hesaplandı. Duyarlılık ve özgüllük sırasıyla %99,08 ve %97,61 olarak belirlenirken, pozitif prediktif değerleri ve negatif prediktif değerleri %87,96 ve %99,83 bulundu. Homojen (%95,7), sentromer (%92,3) ve nükleoler (%92,1) paternlerde yüksek uyum oranları gözlemlenirken, benekli (%60) ve sitoplazmik (%44,4) paternlerde uyum daha düşüktü.
Sonuç: Concepta otomatik sistemi, ANA pozitif ve negatif ayrımında diğer otomatik sistemlerle kıyaslanabilir oldukça yüksek bir doğruluk oranı sergiledi. Concepta sisteminin yoğun ince benekli ve mix paternlerin tanınmasında bazı sınırlılıkları bulunsa da özellikle pozitif ve negatif sonuçlar arasında ayrım yapmada gösterdiği başarı dikkat çekmektedir. Bu sonuçlar, Concepta sisteminin ANA testi alanında umut verici yeni bir alternatif olduğunu göstermektedir.
Anahtar kelimeler: Otoantikorlar; antinükleer antikor testi; immünfloressan mikroskobi.

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INTRODUCTION

Autoimmune diseases can occur through various mechanisms. One of these mechanisms is the production of autoantibodies, by which the immune system attacks an individual's structural antigens and creates a pathological response (1). Autoantibodies that target nuclear and cytoplasmic antigens, commonly known as anti-nuclear antibodies (ANA), are crucial diagnostic markers for systemic autoimmune rheumatic diseases. These diseases include systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, Sjögren's syndrome, idiopathic inflammatory myopathies, and systemic vasculitis (2,3).

Indirect immunofluorescence (IIF) on HEp-2 cells remains the favored approach for ANA screening. The remarkable sensitivity of ANA assessment using IIF enables the detection of over 50 antibodies. Consequently, this method is an indispensable tool for identifying systemic autoantibodies during the initial stages of diagnostic procedures (4).

In the ANA test, 30 different classes have been defined based on image differences (5). The International Consensus on Anti-nuclear Antibody Patterns has a web portal (www.anapatterns.org) that includes the classification and image examples (6). Furthermore, the variability of IIF is significantly influenced by biological factors (such as sample preparation, antigen expression, and disease heterogeneity) and non-biological factors (including laboratory procedures, observer subjectivity, and CAD system algorithms), which poses a challenge to the standardization of IIF (7,8). The results may differ when specialized physicians evaluate IIF images. The specialist physician can examine the image with manual microscopes, but commercial automated products that include a decision support system in their structure can also perform image analysis in their structure (3). Advancements in technology have led to the development of automated IIF systems. These systems utilize artificial intelligence to process and recognize digitized fluorescent images automatically. Using a standardized approach, patterns are classified to facilitate computer-aided diagnosis, including automatic positive and negative discrimination and pattern interpretation. In these systems, high-resolution digital images captured using precision cameras and integrated automatic microscopes are analyzed by computer-aided systems. Numerous automated systems are currently available (9). Computer-aided systems and artificial intelligence are becoming increasingly present in our lives. In the IIF evaluation, automated systems are becoming increasingly crucial in immunology laboratories.

We evaluated the performance of a system that is an automatic system recently used in Türkiye. In this context, this study analyzed the performance of the automated system in ANA IIF testing. The evaluation focused on the ability of the systems to distinguish between positive and negative results and to identify various anti-nuclear antibody patterns.

To achieve this goal, we studied 1000 routine patient samples that were sent to the laboratory and evaluated the results. To evaluate the performance of automated systems in real life, we planned the study on routine patients, not on a predefined patient group.

MATERIAL AND METHODS

Between November 2023 and February 2024, 1000 serum samples that were sent to the laboratory to analyze ANA patterns using IIF were studied. For ANA detection, iPRO (BioSystems, Barcelona, Spain) was used for sample preparation, and Concepta (BioSystems, San Giovanni Valdarno, Italy) for automated evaluation. iPRO is a fully automated IIF processor. Samples are loaded to the processor with racks. The system will scan barcodes automatically. Test assignments can be programmed manually or sent by the laboratory information system. According to the programmed worklist, necessary dilutions are prepared automatically, samples can be unloaded from the device, and another group of samples can be loaded. At the session's end, laboratory professionals must pick the slides up, dispense mounting medium, and close coverslips. After closing the coverslips, slides are placed in the Concepta automated microscope and evaluation device. Concepta is an automated in vitro diagnostic system that reads, displays, and archives slides containing IIF assays from patient serum or plasma samples. The system provides positive/uncertain/negative results for the HEp-2 test that must be confirmed by expert laboratory personnel or physicians and provides aid for pattern identification through the automatic recognition of the HEp-2 patterns: Homogeneous, speckled, centromere, nuclear dots, nucleolar, cytoplasmic, and cytoplasmic AMA-like. Concepta only identifies a single pattern. In mixed patterns, it identifies only one pattern. Although it provides a value based on fluorescence intensity, it cannot determine the titer based on this intensity.

All samples finalized with Concepta were evaluated by two experienced experts separately on the system screen. Internal quality controls, both positive and negative, were performed in each study. In addition, our laboratory participates in two external quality assurance programs (Institute for Quality Assurance Lübeck and UK NEQAS). This research was approved by the Ethics Committee of Başakşehir Çam and Sakura City Hospital with approval date/number: 2024-62.

Statistical Analysis

IBM SPSS version 27 statistical program for Mac was used for statistical evaluation, and descriptive information was shown using number and percentage distributions. The percentages of agreement and κ coefficients were calculated to determine the agreement between the automated system and the manual expert evaluation.

RESULTS

A total of 1000 serum samples sent to the immunology laboratory for routine ANA testing were analyzed. 47 samples were excluded from the study due to repetition, dilution requirements, or inter-observer variability in pattern identification during manual evaluation.

Of the 953 samples analyzed by the expert physicians, 326 were positive, and 627 were negative. The percentage of agreement between Concepta and the expert assessment in the positive-negative discrimination is 98%, indicating a near-perfect match (Table 1).

Concepta's analytical sensitivity and specificity were 99.08% and 97.61%, respectively, while the positive and negative predictive values were 87.96% and 99.83%, respectively (Table 2). Of the 326 positive samples, 29 had

Table 1. Compatibility between Concepta and expert assessment

		Concepta		Total	Agreement (%)	κ value (95% CI)
		Positive (%)	Negative (%)			
Expert	**Positive**	323	3	326 (34.21)	323/326 (99.08)	
	Negative	15	612	627 (65.79)	612/627 (97.61)	0.958 (0.939-0.977)
	Total	338 (35.47)	615 (64.53)	953	935/953 (98.11)	

Table 2. Performance parameters of Concepta

Test	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Concepta	99.08 (97.33-99.81)	97.61 (96.08-98.65)	87.96 (81.59-92.34)	99.83 (99.49-99.95)

PPV: positive predictive value, NPV: negative predictive value, CI: confidence interval

more than one pattern. The compatibility results of the 326 positive sample results were shown in Table 3.

Concepta identifies seven patterns (homogeneous, speckled, centromere, nuclear dots, nucleolar, cytoplasmic, and cytoplasmic AMA-like) but not dense fine speckled and nuclear membrane. It identified 52 of 63 samples with dense fine speckled as homogeneous (Figure 1).

Among the patterns that Concepta can identify, the largest group it fails to recognize is the speckled pattern. Among the 100 specimens that specialists identified as speckled, Concepta correctly classified 60, while 40 were misclassified.

DISCUSSION

Initially, the ANA test was primarily requested by rheumatologists. Due to its association with various diseases, the ANA test is in increasing demand from physicians of different specialties, going beyond rheumatology (10). Studies conducted with groups diagnosed with autoimmune diseases do not adequately reflect daily laboratory practices. When these groups are selected and studied, a group with higher ANA positivity is selected compared to the routine patient group. At the same time, ANA positivity in this group will be at higher titers than in the routine patient group. Since ANA can also be found in healthy individuals, this often leads to uncertain results. Studies involving routine patient groups are likely to yield more uncertain results. Consequently, research conducted with routine patient samples more accurately reflects actual laboratory conditions. 47 samples were excluded (duplicate samples, requiring dilution, and samples where two physicians identified different patterns during manual assessment). There may be differences in judgment between the experts, so samples where both experts disagreed were not evaluated (11). Among the studies on ANA IIF, there were no data on the Concepta system. However, our study revealed a 98.11% agreement between Concepta and expert assessment, with a close to perfect

Table 3. Agreement between expert evaluation and Concepta in pattern identification

Pattern	Expert (n)	Concepta (n)	Concordance rate (%)
Cytoplasmic reticular/AMA like	12	8	66.7
Homogeneous	47	45	95.7
Speckled	100	60	60
Nucleolare	38	35	92.1
Nuclear dots	5	3	60
Nuclear membrane	3	0	-
Dense fine speckled	63	0	-
Centromere	13	12	92.3
Cytoplasmic	9	4	44.4
Multiple patterns	29	0	-
Other	7		
Total	326	167	

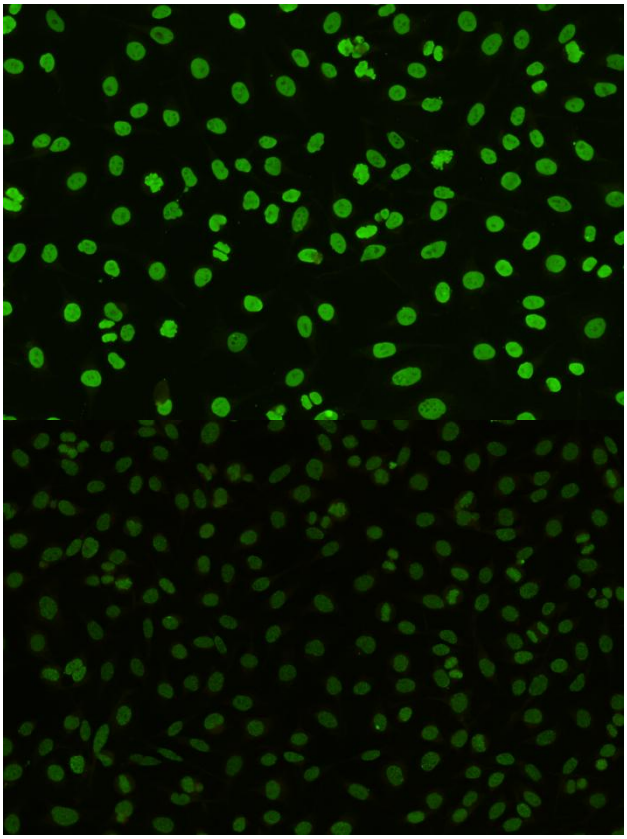


Figure 1. BioSystems ANA IIF pattern image examples, **A)** homogeneous, **B)** dense fine speckled

match (κ value 0.958). In 3 studies conducted with different commercial automated systems, the κ value was found to be 0.97, 0.932, and 0.860 (12-14). These findings are significant as they present a new alternative to automated systems.

The sensitivity and specificity of Concepta were 99.08% and 97.61%, respectively. In a study by Loock et al (15) in which two different automated systems were evaluated with routine samples coming to the laboratory, sensitivity was satisfying (89%/87% for Aklides/Helios), and the specificity was relatively low (59%/54% for Aklides/Helios). In two different studies with another automated system, EUROPattern, analytical sensitivity and specificity varied, with sensitivity between 94.3% and 98.95% and specificity between 88.2% and 98.4% (12-14). In another study with two different automated systems, Nova View/Helios's sensitivity was 96.7%/95.8%, and its specificity was 91%/93.5% (16).

When the pattern identification results are analyzed, Concepta and conventional IIF pattern recognition results match 51.2% (167/326) of the samples. Concepta could identify homogeneous, speckled, centromere, nuclear dots, nucleolar, cytoplasmic, and cytoplasmic AMA-like patterns. However, it struggled with mixed patterns as it could only provide a single result, leading to incomplete classifications. The Concepta identification algorithm does not include the dense fine speckled pattern. When the unidentified specimens were analyzed, it was found that the majority belonged to the dense fine speckled pattern. In particular, Concepta was identified as homogeneous in 52 out of 63 dense fine speckled samples. One sample was classified as nucleolar, two as negative, and two as speckled. The remaining six specimens could not be identified. Out of 1,000 patient samples processed by the device, experts identified 326 positive cases. The Concepta classification algorithm identifies various patterns, including homogeneous, speckled, centromere, nuclear dots, nucleolar, cytoplasmic, and cytoplasmic AMA-like patterns (Table 4). Concepta was unable to identify 112 samples due to various patterns such as dense fine speckled, nuclear membrane, and mixed patterns. As a result, it could only identify 224 out of the 326 positive samples. When its success in these patterns was analyzed, it was found that it correctly identified 167 out of 224 samples. Concepta concordance rates differed across pattern types in the following sequence: homogeneous (95.7%), centromere (92.3%), nucleolar (92.1%), cytoplasmic reticular /AMA-like (66.7%), nuclear dot (60%), speckled (60%), and cytoplasmic (44.4%). As no study was conducted with Concepta, no comparison could be made.

In the study by Park et al. (13), the EUROPattern concordance rates varied between different pattern types in the following order: cytoplasmic and nuclear dot (100%), centromere (87.5%), speckled (79.3%), homogeneous (62.5%), nucleolar (60.0%), nuclear membrane and mitotic (0.0%) patterns. van Beers et al. (11) found the relative sensitivity as homogenous (93.6%), speckled (87.3%), nucleolar (91.9%), centromere (93.8%), nuclear spots (80.0%), nuclear membrane (100%), and cytoplasmic (86.4%). Another study observed correct pattern recognition in 94.6% of sera with a single pattern. The effectiveness of automated recognition for various patterns differed (12): cytoplasmic pattern, nucleolar pattern (100%), speckled pattern (97.2%), homogeneous pattern (91.6%), nuclear dots pattern (75%), centromeres pattern (60.7%). Our study's Concepta results demonstrated higher concordance rates for centromere, homogeneous, and nucleolar patterns than others. However, lower concordance rates were observed for cytoplasmic, nuclear dot, and speckled patterns. Concepta identified only 60 out of 100 speckled patterns correctly.

Among the 40 specimens that Concepta failed to identify, 24 were incorrectly classified, while 16 could not be evaluated at all. Other studies have also shown that identifying the speckled pattern is complex (17-20). Therefore, low identification rates in speckled can be considered normal.

There are automated systems that only distinguish between positive and negative results, as well as systems that can identify various patterns. The number and types of patterns recognized by these systems differ (Table 4). Pattern recognition algorithms may experience difficulties in identification as the number of patterns they can recognize increases. Homogeneous, speckled, and dense fine speckled patterns share similarities, making them challenging to distinguish, particularly for AI-enabled systems (15,21). An algorithm designed to identify only homogeneous and speckled patterns will classify homogeneous patterns more accurately, as dense fine speckles cannot be easily confused with them. However, as our study shows, this algorithm may mistakenly classify dense fine speckled patterns as homogeneous. Therefore, it is advisable to evaluate each system based on its unique characteristics and limitations.

The present study was conducted using routine patient samples rather than samples from patients with autoimmune rheumatologic diseases. ANA positivity is more

Table 4. Patterns identified by automated systems*

System	Patterns
EUROPattern	Homogeneous, speckled, dense fine speckled, nucleolar, centromere, nuclear dots, nuclear membrane, cytoplasmic reticular / AMA like and cytoplasmic
NOVA View	Homogeneous, speckled, fine speckled, coarse speckled, dense fine speckled centromere, nucleolar and nuclear dots
Helios	Homogeneous, speckled, centromere, nuclear membrane, nuclear dots, nucleolar, and cytoplasmic
Zenit G-Sight	Homogeneous, nucleolar, speckled, centromere, and mitochondrial patterns
Aklides	Homogeneous, speckled, nucleolar, centromere, nuclear dots, and cytoplasmic
Image Navigator	Positive/negative (no pattern distinction)
Concepta	Homogeneous, speckled, nucleolar, centromere, nuclear dots, cytoplasmic reticular / AMA like and cytoplasmic

* Adapted from Bizzaro et al. (3) and Tebo AE (22)

clearly observed in studies that focus on patient groups with these autoimmune conditions. The design of our study, which involves routine patient groups, may influence the performance of the device due to the presence of more intermediate values.

CONCLUSION

This study highlights the performance and limitations of the Concepta automated system in the detection and pattern recognition of ANA using IIF. The system demonstrated high sensitivity and specificity in distinguishing between positive and negative results, achieving a 98.1% agreement with expert evaluations. Concepta's inability to identify the dense fine speckled pattern we frequently encounter can be considered a disadvantage. However, its pattern recognition capability was limited, especially for homogeneous, speckled, centromere, nuclear dots, nucleolar, cytoplasmic, and cytoplasmic AMA-like patterns. Concepta, presented as a new alternative among automated systems, is an important tool for its success in distinguishing both positive and negative results.

Ethics Committee Approval: The study was approved by the Scientific Research Ethics Committee of Başakşehir Çam and Sakura City Hospital (18.12.2024, 62).

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