Fully Automated Indirect Immunofluorescence Assay versus Fully Automated Solid-Phase Assay for Anti-Nuclear Antibody Detection: A Meta-Analysis of Adjusted Indirect Comparison

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Abstract

Abstract Objective To analyze the performance of automated indirect immunofluorescence (Automated-IIF) versus solid-phase assays for antinuclear antibody (ANA) detection using meta-analysis and adjusted indirect comparison methods with manual indirect immunofluorescence (manual-IIF) as the common reference standard. Methods A computerized search was conducted for diagnostic studies related to Automated-IIF and solid-phase assays for ANA detection published in PubMed, EMbase, Web of Science, The Cochrane Library, Chinese Biomedical Literature Database (CBM), China National Knowledge Infrastructure (CNKI), and Wanfang Database from inception to March 2022. Two investigators independently screened the literature and extracted basic data according to predefined inclusion and exclusion criteria, and evaluated the quality of the studies using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool. Meta-analysis was performed using Meta-Disc 1.4 and Stata 12.0 software to calculate pooled sensitivity, pooled specificity, pooled positive likelihood ratio, pooled negative likelihood ratio, and pooled diagnostic odds ratio. Summary receiver operating characteristic (SROC) curves were plotted using RevMan 5.3 software. The relative diagnostic odds ratio (RDOR) results for the indirect comparison between Automated-IIF and solid-phase assays for ANA detection were implemented through R software, with adjusted indirect comparison forest plots displaying the RDOR values and their 95% confidence intervals (CI) for the two testing methods, and Deek' s funnel plot asymmetry test was used to assess potential publication bias. Results A total of 23 studies involving 20,608 cases were finally included. Heterogeneity tests indicated significant heterogeneity not caused by threshold effects, and a random-effects model was adopted for meta-analysis. Meta-analysis results for Automated-IIF in ANA

detection: pooled sensitivity was 0.86 (95% CI 0.85-0.87), pooled specificity was 0.90 (95% CI 0.90-0.91), pooled positive likelihood ratio was 10.47 (95% CI 5.63-19.47), pooled negative likelihood ratio was 0.06 (95% CI 0.03-0.12), pooled diagnostic odds ratio (DOR) was 172.36 (95% CI 66.47-446.94), and the area under the SROC curve (AUC) was 0.974. Meta-analysis results for solid-phase assays in ANA detection: pooled sensitivity was 0.43 (95% CI 0.42-0.45), pooled specificity was 0.94 (95% CI 0.93-0.94), pooled positive likelihood ratio was 6.48 (95% CI 4.19-10.01), pooled negative likelihood ratio was 0.45 (95% CI 0.38-0.54), pooled DOR was 14.86 (95% CI 8.88-24.88), and AUC was 0.863. Indirect comparison results demonstrated that the accuracy of Automated-IIF for ANA detection was significantly higher than that of solid-phase assays. Conclusion When the demand for ANA testing increases in clinical laboratories, Automated-IIF can be recommended as an alternative method for ANA screening.

Full Text

An Adjusted Indirect Comparison Meta-Analysis of Automated Indirect Immunofluorescence Versus Fully Automated Solid Phase Immunoassays for ANA Detection

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Abstract

Objective: This study employed meta-analysis and adjusted indirect comparison methods, using manual indirect immunofluorescence (manual-IIF) as the common reference standard, to evaluate the diagnostic performance of automated indirect immunofluorescence (automated-IIF) and fully automated solid-phase assays for antinuclear antibody (ANA) detection.

Methods: A systematic literature search was conducted in PubMed, EMbase, Web of Science, The Cochrane Library, Chinese Biomedical Literature Database (CBM), China National Knowledge Infrastructure (CNKI), and WANFANG electronic databases from inception to March 2022. Diagnostic studies related to automated IIF and solid-phase assays for ANA detection were retrieved. Two investigators independently screened literature and extracted data according to predefined inclusion and exclusion criteria. Study quality was assessed using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool. Meta-Disc 1.4 and Stata 12.0 software were used for meta-analysis to calculate pooled sensitivity, pooled specificity, pooled positive likelihood ratio, pooled negative likelihood ratio, and pooled diagnostic odds ratio. RevMan 5.3 software was used to generate summary receiver operating characteristic (SROC) curves. R software was employed to calculate the relative diagnostic odds ratio (RDOR)

for indirect comparison between automated-IIF and solid-phase assays, with forest plots displaying RDOR values and 95% confidence intervals (CI). Deek' s funnel plot asymmetry test was used to assess publication bias.

Results: Twenty-three studies involving 20,608 subjects were included. Heterogeneity testing indicated significant heterogeneity not caused by threshold effects, warranting use of a random-effects model for meta-analysis. For automated-IIF, the pooled sensitivity was 0.86 (95% CI 0.85-0.87), pooled specificity 0.90 (95% CI 0.90-0.91), pooled positive likelihood ratio 10.47 (95% CI 5.63-19.47), pooled negative likelihood ratio 0.06 (95% CI 0.03-0.12), pooled diagnostic odds ratio (DOR) 172.36 (95% CI 66.47-446.94), and SROC AUC 0.974. For solid-phase assays, the pooled sensitivity was 0.43 (95% CI 0.42-0.45), pooled specificity 0.94 (95% CI 0.93-0.94), pooled positive likelihood ratio 6.48 (95% CI 4.19-10.01), pooled negative likelihood ratio 0.45 (95% CI 0.38-0.54), pooled DOR 14.86 (95% CI 8.88-24.88), and AUC 0.863. Indirect comparison results demonstrated that automated-IIF had significantly higher accuracy than solid-phase assays for ANA detection.

Conclusions: Automated-IIF can be recommended as an alternative screening method for ANA detection in clinical laboratories facing increased testing demands.

Keywords: Adjusted indirect comparison; Indirect immunofluorescence; Meta-analysis; Antinuclear antibody; Solid-phase immunoassay

Introduction

Antinuclear antibody (ANA) testing is widely used for screening systemic autoimmune rheumatic diseases (SARDs). Indirect immunofluorescence using HEp-2 cells as the antigen substrate is the preferred method for ANA detection and has been incorporated into classification criteria for various autoimmune diseases (AID), playing an important role in the diagnosis, treatment, and prognostic evaluation of ANA-associated rheumatic diseases (AARD). Manual indirect immunofluorescence (manual-IIF) is currently recommended as the gold standard method for ANA detection. However, this method presents several challenges in clinical laboratory practice: (1) increased workload due to time-consuming and continuous testing procedures, particularly when clinical testing demands surge dramatically; and (2) subjective interpretation of fluorescence patterns through visual microscopic observation, which is susceptible to inter-observer variability.

The advent of automated fluorescence slide processing systems has addressed these manual processing limitations by automating the entire workflow from sample dilution and dispensing to washing, thereby achieving automation and standardization of sample pretreatment and testing procedures. In recent years, automated digital reading systems have continuously evolved, enabling auto-

mated capture, storage, and interpretation of ANA fluorescence slides through corresponding software systems. With the rapid development of automated fluorescence slide processing and automated interpretation systems, automated indirect immunofluorescence technology (automated-IIF) has emerged and is now widely used in numerous laboratories.

Despite its advantages, automated-IIF still has certain limitations, while other ANA detection technologies continue to emerge and develop. Fully automated solid-phase assays have evolved for screening specific autoantibodies and can overcome some limitations of manual-IIF, serving as an alternative method. Among solid-phase assays, fluorescence enzyme immunoassay (FEIA) is widely applied—a method based on enzyme-linked immunosorbent assay (ELISA) technology that coats multiple human recombinant antigens on a solid-phase carrier. Commercial automated FEIA systems such as Phadia (Thermo Fisher) enable qualitative ANA analysis. Another automated solid-phase method is chemiluminescent immunoassay (CLIA), with commercial systems like LIAISON (DiaSorin) coating multiple purified recombinant antigens and HEp-2 cell nuclear extracts on magnetic particles for qualitative detection of clinically relevant autoantibodies, and BIO-FLASH (INOVA) primarily for detecting antibodies against extractable nuclear antigens (ENAs). Compared with traditional manual-IIF, solid-phase assays have gained widespread application in high-throughput laboratories due to their diverse and well-defined antigen panels, offering greater convenience, speed, and operational ease in ANA screening. This study evaluated the accuracy of these two widely used ANA screening methods—automated-IIF and solid-phase assays—through adjusted indirect comparison using manual-IIF as the common reference standard.

Methods

1.1 Literature Search Strategy

A comprehensive computer-based literature search was conducted in PubMed, EMbase, Web of Science, The Cochrane Library, Chinese Biomedical Literature Database (CBM), China National Knowledge Infrastructure (CNKI), and WANFANG electronic databases from inception to March 2022, with no language restrictions (Chinese and English only). Chinese search terms included: “抗核抗体” (antinuclear antibody), “间接免疫荧光法” (indirect immunofluorescence), “全自动间接免疫荧光分析系统” (automated indirect immunofluorescence analysis system), “荧光酶免疫分析技术” (fluorescence enzyme immunoassay), and “化学发光免疫分析” (chemiluminescent immunoassay). English search terms included: “Antinuclear antibodies,” “immunofluorescence,” “Automated indirect immunofluorescence,” “fluorescence enzyme immunoassay,” and “chemiluminescent immunoassay.”

1.2 Inclusion and Exclusion Criteria

Inclusion criteria: (1) Studies evaluating ANA detection accuracy using traditional manual-IIF as the gold standard; (2) Testing methods where all samples were simultaneously tested with both manual-IIF and either automated-IIF or automated solid-phase assays, with manual-IIF procedures performed manually and results interpreted visually via fluorescence microscopy, automated-IIF processes including sample dilution and result interpretation completed automatically by instrumentation, and automated solid-phase assays including FEIA and CLIA; (3) Outcome measures including pooled sensitivity, pooled specificity, pooled positive likelihood ratio, pooled negative likelihood ratio, pooled diagnostic odds ratio, SROC curve construction with area under curve (AUC) calculation, and RDOR for adjusted indirect comparison between automated-IIF and solid-phase assays.

Exclusion criteria: (1) Studies using non-patient peripheral blood samples (e.g., quality control sera); (2) Studies comparing accuracy of various detection methods (automated-IIF, solid-phase assays, ELISA, multiplex immunoassay, line immunoassays) without using traditional manual-IIF as gold standard; (3) Multi-center studies without specified IIF reagent manufacturers or instruments, making it impossible to distinguish manual-IIF from automated-IIF; (4) Studies with erroneous or incomplete data preventing extraction of 2$×$2 tables; (5) Duplicate studies or publications from the same author or institution on the same population; (6) Non-Chinese or non-English studies.

1.3 Literature Screening and Data Extraction

Two authors independently screened literature and extracted data by reviewing each article. Disagreements regarding inclusion were resolved through discussion with a third and fourth author. Extracted data included: first author, publication year, total number of cases, patient population characteristics, manual-IIF screening titer, automated-IIF screening titer or cut-off values, automated-IIF instrumentation, solid-phase assay cut-off values, solid-phase assay methods and instruments, and 2$×$2 table data (true positives [TP], false positives [FP], false negatives [FN], true negatives [TN]). Included studies were evaluated for bias risk using the QUADAS-2 tool.

1.4 Quality Assessment Using QUADAS-2

The QUADAS-2 tool comprises four domains: (1) patient selection, (2) index test, (3) reference standard, and (4) flow and timing. Two authors independently assessed all studies, achieving good inter-rater consistency.

Statistical Analysis

Threshold effect analysis was performed for each diagnostic test by calculating the Spearman correlation coefficient between the logit of sensitivity and

logit of (1-specificity), with P<0.05 indicating threshold effect presence. Heterogeneity within each diagnostic test was analyzed using Meta-Disc 1.4 software by calculating Cochran' s Q statistic and I^2 value for diagnostic odds ratios. P>0.05 and I^2<50% indicated no statistical heterogeneity, warranting fixed-effects model; P<0.05 and I^2>50% indicated significant heterogeneity, requiring random-effects model for pooling sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and DOR. RevMan 5.3 generated SROC curves for both tests, with AUC calculated using Meta-Disc 1.4. R software "netmeta" package calculated RDOR for indirect comparison between automated-IIF and solid-phase assays. RDOR confidence intervals containing 1 indicated no statistically significant difference between tests. For A vs B comparison, RDOR>1 with CI excluding 1 favored A; RDOR<1 with CI excluding 1 favored B. Subgroup analysis explored heterogeneity sources. Stata 12.0 generated Deek' s funnel plots to assess publication bias, with P<0.05 indicating significant asymmetry.

Results

2.1 Literature Search Results

The initial search yielded 16,652 articles. After removing duplicates across databases, 10,327 articles remained. Full-text review against inclusion criteria, excluding case reports, clinical symptom analyses, reviews, and conference abstracts without full text, yielded 448 articles. Strict application of exclusion criteria resulted in final inclusion of 23 articles: 16 on automated-IIF and 7 on solid-phase assays, comprising 20,608 cases. Study characteristics are summarized in .

2.2 Quality Assessment and Bias Risk Evaluation

Using QUADAS-2, bias risk was evaluated as follows: $ $2 "yes" answers to signaling questions indicated low bias risk; $ $2 "no" answers indicated high bias risk; other combinations were rated "unclear." For applicability concerns in patient selection, studies with clearly diagnosed cases were rated low concern; those including "suspected" cases were high concern; unclear diagnosis was rated "unclear." For index test applicability, studies with clear instrumentation, methods, and kit sources were low concern; otherwise high concern. For reference standard applicability, studies with clinically diagnosed cases inconsistent with manual-IIF results were high concern; unmentioned cases were low concern.

Based on these criteria, one study had high bias risk in patient selection [31]; two studies included "suspected" cases rated as high applicability concern [13,22]. Two studies had high bias risk in index test evaluation [14,23]. All studies had clear instrumentation, methods, and kit sources, yielding low applicability concerns. Five studies had discrepancies between clinical diagnosis and manual-

IIF results, resulting in high applicability concerns for the reference standard. Results are shown in [Figure 1: see original paper].

FIGURE:1 Quality evaluation of included studies (Patient Selection, Index Test, Reference Standard, Flow and Timing; Risk of Bias and Applicability Concerns).

2.3 Meta-Analysis Results

(1) Accuracy assessment of automated-IIF and solid-phase assays for ANA detection: Sixteen studies on automated-IIF and seven on solid-phase assays were included. Spearman correlation coefficients were 0.010 (P=0.963) and 0.527 (P=0.117), respectively, indicating no threshold effect; random-effects models were applied. Significant heterogeneity not caused by threshold effects was present. For automated-IIF, pooled sensitivity was 0.86 [95% CI (0.85-0.87)], pooled specificity 0.90 (95% CI 0.90-0.91), pooled positive likelihood ratio 10.47 (95% CI 5.63-19.47), pooled negative likelihood ratio 0.06 (95% CI 0.03-0.12), pooled DOR 172.36 (95% CI 66.47-446.94), and AUC 0.974. For solid-phase assays, pooled sensitivity was 0.43 (95% CI 0.42-0.45), pooled specificity 0.94 (95% CI 0.93-0.94), pooled positive likelihood ratio 6.48 (95% CI 4.19-10.01), pooled negative likelihood ratio 0.45 (95% CI 0.38-0.54), pooled DOR 14.86 (95% CI 8.88-24.88), and AUC 0.863. The larger SROC AUC for automated-IIF indicated superior accuracy compared to solid-phase assays [Figure 2: see original paper].

FIGURE:2 Summary receiver operating characteristics (SROC) curve and coupled forest plots of sensitivity and specificity for automated-IIF and solid-phase assays.

(2) Adjusted indirect comparison RDOR results: Using manual-IIF as the common reference standard, adjusted indirect comparison showed automated-IIF vs solid-phase assay RDOR of 10.74 (95% CI 2.92-39.44) (RDOR>1, CI excluding 1), and solid-phase assay vs automated-IIF RDOR of 0.09 (95% CI 0.03-0.34) (RDOR<1, CI excluding 1), indicating statistically significantly higher accuracy for automated-IIF [Figure 3: see original paper].

FIGURE:3 Adjusted indirect comparison forest plots of RDOR between automated-IIF and solid-phase assays.

(3) Heterogeneity testing: For automated-IIF, Cochran' s Q=915.73 (P=0.0000), I^2=97.3%, indicating substantial heterogeneity. Subgroup analyses were performed based on: automated instrumentation (Aklides, EUROPattern, Zenit G-sight, Helios, NOVA View groups), cut-off values (explicit vs none), and manual-IIF screening titers (1:80, $ 1 : 100, 1 : 40 *dilution groups*). *The NOVA View subgroups showed markedly reduced heterogeneity* ($I^{\{2\}=0}${2}$=49.6%, P=0.094) compared to other subgroups [Figure 5: see original paper]. Cut-off value subgroup analysis did not reduce heterogeneity.

For solid-phase assays, Cochran' s Q=113.87 (P=0.0000), I^2=92.1%, indicat-

ing substantial heterogeneity. Subgroup analyses by detection method (CLIA vs EliA FEIA groups), automated instrumentation (DiaSorin LIAISON, Inova Diagnostics, Thermo Fisher groups), and manual-IIF screening titer (1:80 vs >1:80 dilution groups) did not substantially alter heterogeneity.

FIGURE:4 Subgroup analysis forest plot of DOR by automated instrumentation for automated-IIF.

FIGURE:5 Subgroup analysis by working cut-off dilution of manual-IIF for automated-IIF.

2.4 Sensitivity Analysis and Publication Bias

Sensitivity analysis was performed by sequentially removing studies with markedly different results or large sample sizes. Results remained stable for both diagnostic tests, indicating good robustness and credibility. Publication bias was assessed using linear regression Deek's funnel plots. For automated-IIF, slope coefficient P=0.17 (26 data points from 16 studies) showed no significant publication bias. For solid-phase assays, slope coefficient P=0.63 (10 data points from 7 studies) also indicated no significant publication bias [Figure 6: see original paper].

FIGURE:6 Deek' s funnel plot for automated-IIF and solid-phase assays for ANA detection.

Discussion

This study comprehensively compared automated-IIF and solid-phase assay accuracy for ANA detection using manual-IIF as the common reference standard. Initial meta-analysis of 23 studies showed automated-IIF pooled sensitivity of 0.86 (95% CI 0.85-0.87) was substantially higher than solid-phase assays (0.43, 95% CI 0.42-0.45), while pooled specificity was slightly lower (0.90 vs 0.94). Automated-IIF also demonstrated superior pooled positive likelihood ratio (10.47 vs 6.48), negative likelihood ratio (0.06 vs 0.45), and markedly higher DOR. SROC curves visually confirmed higher accuracy for automated-IIF, though without quantitative comparison. Therefore, we calculated RDOR values using manual-IIF as the common reference standard, with forest plots visualizing RDOR and 95% CI. Results showed statistically significant differences, confirming automated-IIF' s superior accuracy for ANA detection.

However, substantial heterogeneity existed among included studies. To explore sources, we performed subgroup analyses from an analytical perspective based on study characteristics. Six automated-IIF systems were included (NOVA View, Aklides, Zenit G-Sight, EUROPattern, Image Navigator, Helios) from different manufacturers, suggesting system differences might contribute to heterogeneity. Subgroup analysis by instrumentation supported this hypothesis. Additionally, studies used different manual-IIF screening titers (1:80, $ $1:100,

1:40). Heterogeneity was lower at 1:40 dilution compared to higher titers, indicating screening titer variation also contributed to heterogeneity. Despite limited study numbers, these findings identify factors potentially affecting overall diagnostic accuracy.

Automated-IIF represents an important step toward ANA detection standardization and automation, offering more flexible result interpretation. However, challenges remain, including variations in fluorescence slides from different manufacturers, differences in conjugated antibody properties, and variations among automated digital reading systems, all potentially causing significant differences in fluorescence staining patterns. Our results demonstrate automated-IIF' s superiority over solid-phase assays for qualitative (positive/negative) result interpretation, though identification of major ANA fluorescence patterns was not evaluated.

In conclusion, automated-IIF can serve as an alternative to traditional manual-IIF in clinical laboratories with rapidly increasing ANA testing demands. However, several limitations may affect meta-analysis results, including diverse patient and control populations, varying manual-IIF screening titers, and different reagents and instruments across studies. Further homogeneous studies are needed to comprehensively analyze how various factors (including cut-off values) affect diagnostic accuracy.

Conflict of Interest: All authors declare no conflicts of interest.

Author Contributions: Zhang Minjie and Yang Huanli designed the study, performed literature search, data extraction, statistical analysis, and manuscript writing. Zhang Minjie conducted literature search. Yang Huanli performed manuscript revision, study implementation, and data collection.

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Tables

TABLE:1 Characteristics of Included Studies

Study	Patient Population	Manual IIF Titer	Automated-IIF/Solid-phase Method (Instrument)	Titer/Cut-off
Egerer (a)[13]	Suspected systemic rheumatic disease	1:100	Automated-IIF (Helios)	PI>50
Egerer (b)[13]	Suspected rheumatic symptoms	1:100	Automated-IIF (Aklides)	90
Melegari (a)[14]	Random routine samples	1:160	Automated-IIF (Aklides)	LIU$ $100
Melegari (b)[14]	Known autoantibody-positive samples	1:160	Automated-IIF (EUROPattern)	FI<15
Voigt[15]	SLE(10), SSc(10), SjS(16), PM/DM(8), ANA-positive(12), DC(47), HBD(48), unclassified(200)	1:100	Automated-IIF (Zenit G-Sight)	FI>25
Bonroy[16]	SSc(141), SpA(38), OA(22), PMR(13), ANCA-associated vasculitis(5), HBD(100)	1:100	Automated-IIF (Aklides)	FI=1.8

Study	Patient Population	Manual IIF Titer	Automated-IIF/Solid-phase Method (Instrument)	Titer/Cut-off
Bizzaro (a)[17]	SLE(21), PMR(1), SSc(17), HCV(3), SjS(8), EBV infection(1), PM/DM(6), NS(1), UCTD(12), rhinopathy(1), MCTD(3), chronic urticaria(1), PBC(11), bladder cancer(1), fibromyalgia(1), A-NHBD(40)	1:100	Automated-IIF (EUROPattern)	20
Bizzaro (b)[17]	SLE(50), RA(44), SSc(35), SjS(19), PM(10), HBD(99), random controls(112)	1:100	Automated-IIF (Zenit G-Sight)	RCU
Bizzaro (c)[17]	ANA-positive AID(120), ANA-negative non-AID(78)	1:100	Automated-IIF (Nova View)	Automated-IIF (Image Navigator)
Bizzaro (d)[17]	SLE, SjS, SS, MCTD	1:100	Automated-IIF (Zenit G-Sight)	Automated-IIF (Nova View)

Study	Patient Population	Manual IIF Titer	Automated-IIF/Solid-phase Method (Instrument)	Titer/Cut-off
Bizzaro (e)[17]	Suspected autoimmune rheumatic disease	1:160	Automated-IIF (Nova View)	Automated-IIF (Nova View)
Bizzaro (f)[17]	Rheumatology(1470), Internal Medicine(836), Dermatology(380), Neurology(215), Pediatrics(187), Others(187)	1:160	Automated-IIF (Zenit G-Sight)	Automated-IIF (Zenit G-Sight)
Copple (a)[18]	ANA-positive AID(177), healthy controls(25)	>1:100	Automated-IIF (Nova View)	Automated-IIF (Nova View)
Copple (b)[18]	SLE(187), SjS(35), UCTD(13), myositis(10), Sharp disease(6), SSc(21), CREST syndrome(19), APS(10), PBC(10), RA(16), unclassified(273)	>1:100	Automated-IIF (Zenit G-Sight)	Automated-IIF (Zenit G-Sight)
Alsuwaidi[19]	SLE, SjS, SS, MCTD	1:100	Automated-IIF (“ICARE”)	FSI=70
Bertin[20]	Suspected systemic rheumatic disease	1:100	Automated-IIF (Nova View)	1:100

Study	Patient Population	Manual IIF Titer	Automated-IIF/Solid-phase Method (Instrument)	Titer/Cut-off
Daves (a)[21]	SLE(44), SSc(4), SjS(16), MCTD(4), PM/DM(4), remission AARD(9), undiagnosed AARD(9), RA(12), JIA(10), others(210)	1:160	Automated-IIF (Helios)	115
Daves (b)[21]	N-AID(147), malignancies(12), OSAID(31), SARD(133)	1:160	Automated-IIF (EUROPattern)	Automated-IIF (Helios)
Loock (a)[22]	SLE(72), SSc(11), MCTD(6), SjS(19), PM/DM(1), CTD(14), RA(14), NRD(63), unclassified(206)	$ $1:80	Automated-IIF (Aklides)	1 U/ml
Loock (b)[22]	SLE(10), SjS(5), SSc(1), OSAD(14), unclassified(21)	$ $1:80	Automated-IIF (Helios)	1 U/ml
Yoo[23]	SLE, SjS, SS, MCTD	$ $1:80	Automated-IIF (EUROPattern)	$ $20 CU
Ricchiuti[24]	SLE, SjS, SS, MCTD	1:160	CLIA (DiaSorin LIAISON)	EliA FEIA (Thermo Fisher)

Study	Patient Population	Manual IIF Titer	Automated-IIF/Solid-phase Method (Instrument)	Titer/Cut-off
Li Z[25]	SLE, SjS, SS, MCTD	1:160	CLIA (DiaSorin LIAISON)	EliA FEIA (Thermo Fisher)
Park[26]	SLE, SjS, SS, MCTD	1:100	CLIA (Inova Diagnostics)	EliA FEIA (Thermo Fisher)
Choi[5]	SLE, SjS, SS, MCTD	$ $1:160	CLIA (Inova Diagnostics)	EliA FEIA (Thermo Fisher)
徐红星[27]	SLE, SjS, SS, MCTD	1:160	Automated-IIF (EUROPattern)	EliA FEIA (Thermo Fisher)
Ghillani[28]	SLE, SjS, SS, MCTD	1:100	CLIA (DiaSorin LIAISON)	EliA FEIA (Thermo Fisher)
Callado[29]	SLE, SjS, SS, MCTD	1:100	CLIA (Inova Diagnostics)	EliA FEIA (Thermo Fisher)
Robier[30]	SLE, SjS, SS, MCTD	1:100	EliA FEIA (Thermo Fisher)	EliA FEIA (Thermo Fisher)
Bizzaro (a)[7]	SLE, SjS, SS, MCTD	1:100	EliA FEIA (Thermo Fisher)	EliA FEIA (Thermo Fisher)
Bizzaro (b)[7]	SLE, SjS, SS, MCTD	1:100	EliA FEIA (Thermo Fisher)	EliA FEIA (Thermo Fisher)
Van Hoovels L (a)[31]	SLE, SjS, SS, MCTD	$ $1:80	EliA FEIA (Thermo Fisher)	1 U/ml

Study	Patient Population	Manual IIF Titer	Automated-IIF/Solid-phase Method (Instrument)	Titer/Cut-off
Van Hoovels L (b)[31]	SLE, SjS, SS, MCTD	$ $1:80	EliA FEIA (Inova Diagnostics)	1 U/ml
González [32]	SLE, SjS, SS, MCTD	$ $1:80	EliA FEIA (Thermo Fisher)	EliA FEIA (Thermo Fisher)
Yoon (a)[33]	SLE, SjS, SS, MCTD	$ $1:80	EliA FEIA (Thermo Fisher)	EliA FEIA (Thermo Fisher)
Yoon (b)[33]	SLE, SjS, SS, MCTD	$ $1:80	EliA FEIA (Inova Diagnostics)	EliA FEIA (Thermo Fisher)

Abbreviations: ANA: antinuclear antibodies; ANCA: antineutrophil cytoplasmic antibodies; Automated-IIF: automated indirect immunofluorescence; EliA: enzyme-linked immunosorbent assay; FEIA: fluorescence enzyme immunoassay; CLIA: chemiluminescent immunoassay; PI: probability index; CU: chemiluminescent units; FI: fluorescence index; "ICARE" (Immunofluorescence for Computed Antinuclear antibody Rational Evaluation); LIU: light intensity units; RI: reactivity index; FSI: fluorescence signal intensity; AID: autoimmune disease; SLE: systemic lupus erythematosus; SjS: Sjögren' s syndrome; SSc: systemic sclerosis; OSAID: organ-specific autoimmune disease; A-NHBD: ANA-negative healthy blood donors; HCV: viral hepatitis; NS: nephrotic syndrome; CTD: connective tissue disease; UCTD: undifferentiated connective tissue disease; MCTD: mixed connective tissue disease; PBC: primary biliary cirrhosis; SpA: spondyloarthropathy; OA: osteoarthritis; PMR: polymyalgia rheumatica; HBD: healthy blood donors; RA: rheumatoid arthritis; PM: polymyositis; PM/DM: polymyositis/dermatomyositis; DC: disease controls; HC: healthy controls; SARD: systemic autoimmune rheumatic disease; N-AID: non-autoimmune disease; AARD: antinuclear antibody-associated rheumatic disease; NRD: non-rheumatic disease; APS: antiphospholipid syndrome; JIA: juvenile idiopathic arthritis.

Note: Figure translations are in progress. See original paper for figures.