



Clinical Performance of Two Automated Immunoassays, EliA CTD Screen and QUANTA Flash CTD Screen Plus, for Antinuclear Antibody Screening

Sumi Yoon , M.D.¹, Hee-Won Moon , M.D., Ph.D.², Hanah Kim , M.D., Ph.D.², Mina Hur , M.D., Ph.D.², and Yeo-Min Yun , M.D., Ph.D.²

¹Department of Laboratory Medicine, Chung-Ang University College of Medicine, Seoul, Korea; ²Department of Laboratory Medicine, Konkuk University School of Medicine, Seoul, Korea

Background: Recently, two fully automated immunoassays for antinuclear antibody (ANA) screening were introduced: EliA CTD Screen (Thermo Fisher Scientific, Freiburg, Germany) and QUANTA Flash CTD Screen Plus (Inova Diagnostics, San Diego, USA). We evaluated their clinical performance in comparison with the indirect immunofluorescence assay (IIFA) and analyzed samples with discrepant results.

Methods: In total, 406 serum samples (206 from patients undergoing routine checkups and 200 from rheumatology clinic patients) were assayed using EliA, QUANTA Flash, and IIFA. We evaluated assay concordance and agreement and confirmed the presence of anti-extractable nuclear antigen (ENA) antibodies in samples with discrepant automated immunoassay and IIFA results. Additionally, we compared the clinical performance of each assay in diagnosing ANA-associated rheumatic disease (AARD) and adjusted the cut-off values.

Results: In rheumatology clinic samples, the concordance and agreement were 91.5% and strong between EliA and QUANTA Flash, 79.0% and weak between EliA and IIFA, and 80.5% and moderate between QUANTA Flash and IIFA, respectively. In automated immunoassay-positive, IIFA-negative samples (N=15), all anti-ENA antibodies detected (6/15) were anti-Sjögren's syndrome antigen A/Ro (Ro60) antibodies. The automated immunoassays and IIFA showed high accuracy for diagnosing AARD, and adjusted cut-off values improved their sensitivities (EliA with 0.56 ratio, 82.9% sensitivity; QUANTA Flash with 9.7 chemiluminescent units, 87.8% sensitivity).

Conclusions: The two automated immunoassays showed reliable performance compared with IIFA and can be efficiently used with the IIFA in clinical immunology laboratories. Clinical cut-off values can be adjusted according to the workflow in each laboratory.

Key Words: EliA CTD Screen, QUANTA Flash CTD Screen Plus, Antinuclear antibody, Indirect immunofluorescence assay, Anti-extractable nuclear antigen antibody, Performance

Received: December 9, 2020

Revision received: February 26, 2021

Accepted: July 6, 2021

Corresponding author:

Hee-Won Moon, M.D., Ph.D.
Department of Laboratory Medicine,
Konkuk University School of Medicine,
Konkuk University Medical Center,
120-1 Neungdong-ro, Gwangjin-gu,
Seoul 05030, Korea
Tel: +82-2-2030-5583
Fax: +82-2-2030-5587
E-mail: hannasis@hanmail.net



© Korean Society for Laboratory Medicine

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<https://creativecommons.org/licenses/by-nc/4.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Antinuclear antibody (ANA) is a useful biomarker for the diagnosis of ANA-associated rheumatic diseases (AARDs), such as

systemic lupus erythematosus (SLE), systemic sclerosis (SSc), mixed connective tissue disease (MCTD), primary Sjögren's syndrome (SjS), and polymyositis/dermatomyositis (PM/DM) [1-4]. The most recent European League Against Rheumatism/Ameri-

can College of Rheumatology classification criteria require at least one positive ANA assay result to diagnose SLE [5]. ANA screening is less useful for the diagnosis of other autoimmune rheumatic diseases, such as rheumatoid arthritis [1-3]. ANA can be detected in various other diseases, including liver diseases, thyroid diseases, infectious diseases, and malignancies, and even in apparently healthy individuals [2, 3, 6].

The indirect immunofluorescence assay (IIFA), which was introduced in 1950, is still the gold-standard method for ANA screening [7]. The IIFA uses human epidermoid laryngeal carcinoma cells (HEp-2 or HEp-2000 cells), which serve as substrates presenting more than 100 autoantibodies [3, 4]. The overall sensitivity of the IIFA varies depending on the AARD: it is high for SLE and SSc, but relatively low for SjS and PM/DM [1]. The IIFA exhibits high false positivity in healthy individuals and patients with non-rheumatic diseases [4, 7]. Further, it is labor-intensive, and the determination of results is subjective, making standardization difficult [3]. In a recent survey, the American Association of Medical Laboratory Immunologists investigated several IIFA patterns that laboratory professionals found difficult to read [8]. With the development of novel technologies, automated ANA screening has become possible, and some of the limitations of IIFA have been addressed [9-11].

Recently, two fully automated immunoassays for ANA screening were introduced: EliA CTD Screen (Thermo Fisher Scientific, Freiburg, Germany) and QUANTA Flash CTD Screen Plus (Inova Diagnostics, San Diego, USA). Previous studies have compared

these automated immunoassays with IIFA alone [3, 4, 12-14]. To our knowledge, no studies have evaluated samples with discrepant automated immunoassay and IIFA results by confirming the presence of anti-extractable nuclear antigen (ENA) antibodies. In this study, we evaluated the clinical performance of the EliA and QUANTA Flash for ANA screening compared with the reference method, IIFA, and samples with discrepant results were analyzed.

MATERIALS AND METHODS

Study population

We assayed serum samples obtained at Konkuk University Medical Center (KUMC), Seoul, Korea, between December 2018 and January 2019. The study protocol was approved by the KUMC Institutional Review Board (KUH1200079). Informed consent was not required as the study used residual samples left over after requested assays. In total, 406 samples were collected. Routine samples (N=206; 83 females and 123 males; median age [range], 51 years [17-79 years]) were obtained from patients who visited KUMC for a routine medical check-up. Rheumatology clinic samples (N=200; 168 females and 32 males; 48 years [17-82 years]) were obtained from patients for whom an ANA assay was requested. Serum samples were prepared from whole blood samples and were stored at -70°C until use. The data were analyzed anonymously. The study population is presented in Table 1.

Table 1. Study population and positivity of EliA, QUANTA Flash, and IIFA in samples obtained from patients undergoing routine checkups and those from rheumatology clinic patients (N=406)

Sample details	EliA-positive (> 1.0 ratio) N (%)	QUANTA Flash-positive (≥20.0 CU) N (%)	IIFA-positive (≥ 1 : 80) N (%)
Patients undergoing routine checkups (N=206)	10 (4.9)	17 (8.3)	-
Rheumatology clinic patients (N=200)	97 (48.5)	104 (52.0)	119 (59.5)
AARD (N=109)	83 (76.1)	90 (82.6)	96 (88.1)
SLE (N=72)	52 (72.2)	58 (80.6)	62 (86.1)
SSc (N=11)	10 (90.9)	10 (90.9)	11 (100.0)
MCTD (N=6)	6 (100.0)	6 (100.0)	6 (100.0)
SjS (N=19)	15 (78.9)	16 (84.2)	16 (84.2)
PM/DM (N=1)	1 (100.0)	1 (100.0)	1 (100.0)
CTD (N=14)	6 (42.9)	5 (35.7)	8 (57.1)
RA (N=14)	2 (14.3)	2 (14.3)	3 (21.4)
Non-rheumatic disease (N=63)	5 (7.9)	6 (9.5)	12 (19.0)

Abbreviations: AARD, antinuclear antibody-associated rheumatic disease; CTD, connective tissue disease; CU, chemiluminescent units; IIFA, indirect immunofluorescence assay; MCTD, mixed connective tissue disease; N, number of samples; PM/DM, polymyositis/dermatomyositis; RA, rheumatoid arthritis; SjS, primary Sjögren's syndrome; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

Assays

All the samples were assayed using EliA and QUANTA Flash. EliA is a fluorescence enzyme immunoassay (FEIA) run on the Phadia 250 system (Thermo Fisher Scientific). EliA detects autoantibodies against 14 antigens: centromere (CENP-B), double-stranded (ds)DNA, Jo-1, Mi-2, proliferating cell nuclear antigen (PCNA), polymyositis (PM)-Scl, ribosomal-P, RNA Pol III, Scl-70, Sm, Sjögren's syndrome antigen A (SS-A)/Ro (Ro52 and Ro60), SS-B/La, U1-RNP (RNP-70, A, and C), and fibrillarin. The results of EliA were interpreted as positive, equivocal, or negative according to the cut-off values specified by the manufacturer (>1.0 ratio, positive; 0.7 – 1.0 ratio, equivocal; <0.7 ratio, negative) [15]. QUANTA Flash is a chemiluminescence immunoassay (CIA) that is run on the BIO-FLASH system (Inova Diagnostics). QUANTA Flash detects autoantibodies against 15 antigens: centromere (CENP-A and -B), dsDNA, Jo-1, Mi-2, PCNA, PM-Scl, ribosomal-P, RNA Pol III, Scl-70, Sm, SS-A/Ro (Ro52 and Ro60), SS-B/La, U1-RNP (RNP-70, A, and C), Ku, and Th/To. QUANTA Flash results were interpreted as positive or negative according to the cut-off values specified by the manufacturer (≥ 20.0 chemiluminescent units [CU], positive; <20.0 CU, negative) [12]. The units of EliA and QUANTA Flash cannot be compared because these assays have different principles and unit conversion calculations [12, 15].

IIFA was performed for the rheumatology clinic samples using the Kallestad HEp-2 Cell Line Substrate (Bio-Rad Laboratories, Hercules, CA, USA) on a PhD Ix system (Bio-Rad Laboratories). A trained and experienced immunology expert interpreted the IIFA results using an immunofluorescence microscope at $200\times$. IIFA results were interpreted by dividing the grades of fluorescence intensity at serum dilutions of 1:40 and additional dilutions according to the manufacturer's instructions. We interpreted $\geq 1:80$ as positive and $<1:80$ as negative [5].

For confirmation, an anti-ENA antibody assay was performed using EUROLINE ANA Profile 3 (EUROIMMUN, Lübeck, Germany) for samples that were positive in any of the three ANA screening assays. EUROLINE is a line immunoassay (LIA) that is run on the EUROBlotMaster system (EUROIMMUN). EUROLINE detects autoantibodies against 14 antigens: AMA M2, CENP-B, dsDNA, histones, Jo-1, nRNP, nucleosomes, PCNA, PM-Scl, ribosomal-P, Scl-70, Sm, SS-A/Ro (Ro52 and Ro60), and SS-B/La. EUROLINE results were interpreted according to the signal intensity specified by the manufacturer (>50 , strong positive; 11 – 50 , positive; 6 – 10 , borderline; 0 – 5 , negative) [16]. All assays were performed according to the manufacturers' instructions.

Statistical analysis

The positivity of EliA and QUANTA Flash in the routine and rheumatology clinic samples was compared using McNemar's Chi-squared test for paired proportions. The concordance and agreement among ANA screening assays were evaluated in total samples as well as in routine and rheumatology clinic samples separately. The concordance was calculated by dividing the total number of samples with the same results in multiple assays by the total number of samples [17]. The agreement was calculated using Cohen's kappa (κ) with 95% confidence intervals (CIs), which was interpreted as follows: ≤ 0.20 , none; 0.21 – 0.39 , minimal; 0.40 – 0.59 , weak; 0.60 – 0.79 , moderate; 0.80 – 0.90 , strong; and >0.90 , nearly perfect [18].

The clinical performance of the three ANA screening assays for diagnosing AARDs, which was evaluated based on the clinical diagnosis determined by medical chart review, was compared using ROC curves from the rheumatology clinic samples. Samples from patients diagnosed as having AARDs, including SLE, SSc, MCTD, SjS, and PM/DM, were classified as the AARD group, and samples with any other diagnosis were classified as the non-AARD group. Additionally, the clinical performance of EliA and QUANTA Flash in diagnosing AARDs was evaluated in all samples. The areas under the ROC curve (AUCs) with 95% CIs were calculated and interpreted as follows: $AUC=0.5$, non-informative; $AUC=0.5$ – 0.7 , less accurate; $AUC=0.7$ – 0.9 , moderately accurate; $AUC=0.9$ – 1.0 , highly accurate; and $AUC=1.0$, perfect [19]. The sensitivity and specificity of manufacturers' cut-off values were evaluated, and adjusted cut-off values for EliA and QUANTA Flash were calculated using the Youden index to optimize combined sensitivity and specificity. $P<0.05$ was considered statistically significant. Statistical analyses were performed using MedCalc Statistical Software version 19.5.1 (MedCalc Software, Ostend, Belgium).

RESULTS

The positivity of EliA and QUANTA Flash was 4.9% and 8.3%, respectively, in the routine samples and 48.5% and 52.0%, respectively, in the rheumatology clinic samples. The positivity of IIFA was 59.5% in the rheumatology clinic samples. In the rheumatology clinic samples, the positivity of EliA, QUANTA Flash, and IIFA was higher in the AARD group than in the non-AARD group (Table 1). QUANTA Flash showed slightly, albeit not significantly, higher positivity than EliA in both the routine and rheumatology clinic samples ($P=0.165$, routine samples; $P=0.485$, rheumatology clinic samples). The concordance and agreement

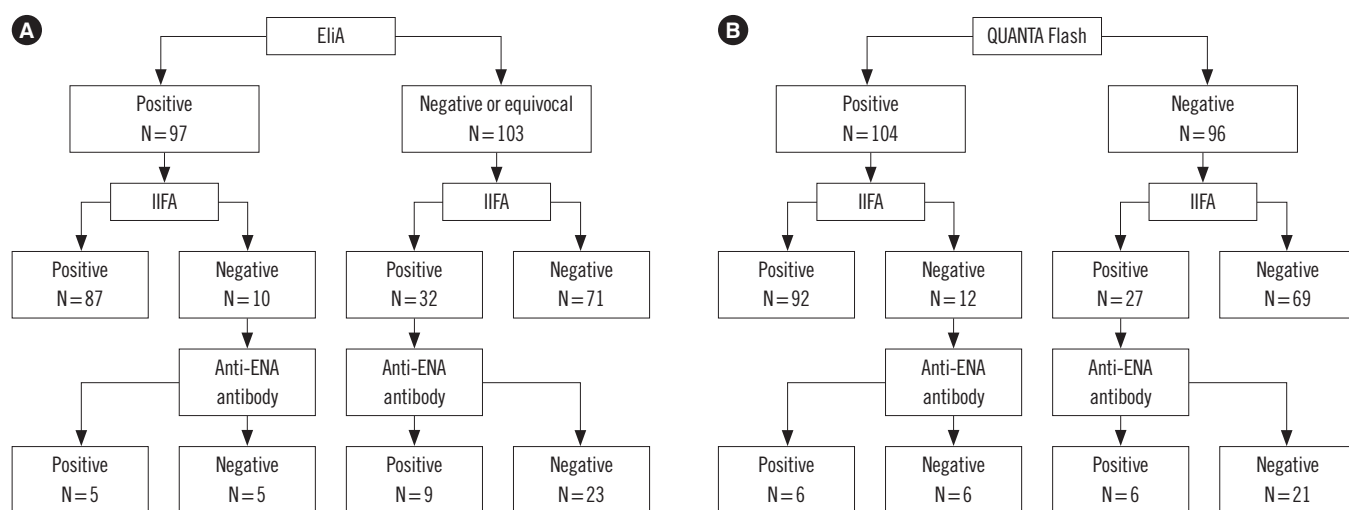


Fig. 1. Flow charts showing the assay results in samples from rheumatology clinic patients (N=200). (A) EliA, IIFA, and anti-ENA antibody assay results. (B) QUANTA Flash, IIFA, and anti-ENA antibody assay results.

Abbreviations: ENA, extractable nuclear antigen; IIFA, indirect immunofluorescence assay.

between EliA and QUANTA Flash were 92.6% and strong ($\kappa=0.82$; 95% CI, 0.76–0.88), respectively, in total samples, and 93.7% and weak ($\kappa=0.49$; 95% CI, 0.25–0.72), respectively, in the routine samples. In the rheumatology samples, the concordance and agreement were 91.5% and strong ($\kappa=0.83$; 95% CI, 0.75–0.91) between EliA and QUANTA Flash, 79.0% and weak ($\kappa=0.58$; 95% CI, 0.47–0.69) between EliA and IIFA, and 80.5% and moderate ($\kappa=0.61$; 95% CI, 0.50–0.72) between QUANTA Flash and IIFA, respectively.

One hundred fifty-four samples tested positive on at least one ANA screening assay. There were 42 samples with discrepant EliA and IIFA results, and 39 samples with discrepant QUANTA Flash and IIFA results (Fig. 1). The positivity of the anti-ENA antibody assay was 50.0% in automated immunoassay-positive, IIFA-negative samples (5/10 EliA-positive, IIFA-negative samples; 6/12 QUANTA Flash-positive, IIFA-negative samples). The positivity of the anti-ENA antibody assay was 28.1% (9/32) in EliA-negative, IIFA-positive samples (Fig. 1A), and 22.2% (6/27) in QUANTA Flash-negative, IIFA-positive samples (Fig. 1B). The results of IIFA and anti-ENA antibody assay for samples with discrepant results are presented in Table 2. The anti-ENA antibodies detected in the EliA- or QUANTA Flash-positive, IIFA-negative samples were all anti-SS-A/Ro (Ro60) antibodies (N=6). The anti-ENA antibodies detected in EliA-negative, IIFA-positive samples were antibodies against histones, Jo-1, nRNP, nucleosomes, Scl-70, and SS-A/Ro (Ro60), whereas those detected in QUANTA Flash-negative, IIFA-positive samples were antibodies against dsDNA, histones, Jo-1, nRNP, nucleosomes, ribosomal-

P, Scl-70, and Sm. Except for one patient (detection of anti-Jo-1 antibody) who was diagnosed as having SSc, all patients with EliA- or QUANTA Flash-negative, IIFA-positive results were diagnosed as having SLE.

EliA and QUANTA Flash showed a high accuracy (AUC=0.917 and 0.911, respectively), and IIFA showed a moderate accuracy (AUC=0.862) in diagnosing AARDs using rheumatology clinic samples (Table 3). The difference in AUCs between EliA and IIFA was statistically significant ($P=0.033$); however, the differences in AUCs between EliA and QUANTA Flash and between QUANTA Flash and IIFA were not significant ($P=0.714$ and $P=0.058$, respectively). Using manufacturers' cut-off values, the sensitivities of EliA, QUANTA Flash, and IIFA were 72.4%, 77.2%, and 84.6%, respectively. Using adjusted cut-off values for EliA and QUANTA Flash, their sensitivities were improved (82.9% and 87.8%, respectively). EliA and QUANTA Flash showed similar sensitivity and specificity in total samples (Table 3). The difference in AUCs between EliA and QUANTA Flash was not significant ($P=0.308$).

DISCUSSION

We evaluated the clinical performance of EliA and QUANTA Flash and analyzed samples with discrepant automated immunoassay and IIFA results for the first time. The present study provided baseline data for the clinical application of EliA, QUANTA Flash, and IIFA in patients undergoing routine checkups and in rheumatology clinic patients. The concordance and agreement be-

Table 2. EliA, QUANTA Flash, IIFA, and anti-ENA antibody assay results in samples with discrepant automated immunoassay and IIFA results

Case number	Diagnosis	EliA*	QUANTA Flash [†]	IIFA [‡]	Anti-ENA antibodies
1	SLE	(P) 7.5	(P) >200.0	(N) 1 : 40	SS-A (Ro60)
2	SLE	(P) 7.3	(P) 125.5	(N) 1 : 40	SS-A (Ro60)
3	CTD	(N) 0.4	(P) >200.0	(N) Weak	SS-A (Ro60)
4	SjS	(P) 1.5	(P) 29.5	(N) 1 : 40	SS-A (Ro60)
5	SLE	(N) 0.4	(N) 8.1	(P) 1 : 640	Nucleosome
6	SLE	(N) 0.5	(N) 9.8	(P) 1 : 320	Nucleosome
7	SLE	(P) 8.0	(P) 49.1	(N) Weak	Histone, SS-A (Ro60)
8	SLE	(P) 5.7	(P) 111.4	(N) 1 : 40	Ribosomal-P, SS-A (Ro60)
9	SLE	(P) 4.7	(N) 17.7	(P) 1 : 160	dsDNA, nRNP, ribosomal-P, Sm
10	SLE	(N) 0.9	(N) 8.7	(P) 1 : 320	Histone
11	SLE	(N) 0.7	(N) 11.0	(P) 1 : 80	Scl-70
12	SLE	(N) 0.6	(N) 11.7	(P) 1 : 1,280	ND
13	SLE	(N) 0.4	(N) 10.8	(P) 1 : 320	ND
14	SLE	(N) 0.3	(N) 15.4	(P) 1 : 80	ND
15	SLE	(N) 0.1	(N) 10.3	(P) 1 : 80	ND
16	SLE, SjS	(N) 0.1	(N) 8.8	(P) 1 : 80	ND
17	CTD	(N) 0.4	(N) 2.6	(P) 1 : 320	ND
18	CTD	(N) 0.2	(N) 5.7	(P) 1 : 80	ND
19	Unclassified CTD	(N) 0.2	(N) 8.4	(P) 1 : 160	ND
20	Collagen disease	(N) 0.1	(N) 3.9	(P) 1 : 320	ND
21	RA	(N) 0.1	(N) 8.1	(P) >1 : 1,280	ND
22	RA	(N) 0.1	(N) 7.4	(P) 1 : 160	ND
23	SjS	(N) 0.4	(N) 4.1	(P) 1 : 80	ND
24	SSc	(N) 0.3	(N) 5.5	(P) 1 : 320	ND
25	Arthralgia	(N) 0.3	(N) 6.6	(P) 1 : 80	ND
26	Arthrosis	(N) 0.2	(N) 3.8	(P) 1 : 80	ND
27	Cough	(N) 0.1	(N) 4.5	(P) 1 : 160	ND
28	Dry mouth	(N) 0.2	(N) 7.5	(P) 1 : 160	ND
29	Hemoptysis	(N) 0.2	(N) 6.9	(P) 1 : 80	ND
30	ILD	(N) 0.2	(N) 3.8	(P) 1 : 80	ND
31	Oral ulcer	(N) 0.2	(N) 3.0	(P) 1 : 160	ND
32	R/O FFA	(N) 0.2	(N) 4.2	(P) 1 : 160	ND
33	SLE	(N) 0.2	(P) 24.1	(N) Weak	ND
34	SLE	(N) 0.9	(P) 24.1	(N) 1 : 40	ND
35	Arthrosis	(N) 0.1	(P) 44.7	(N) 1 : 40	ND
36	Rash	(N) 0.2	(P) 131.7	(N) Negative	ND
37	CTD	(P) 1.1	(N) 13.6	(N) Weak	ND
38	OA	(P) 1.7	(N) 7.8	(N) 1 : 40	ND
39	SLE	(P) 1.7	(N) 12.7	(N) 1 : 40	ND
40	RA	(P) 1.1	(P) 24.6	(N) 1 : 40	ND
41	Thrombocytopenia	(P) 1.2	(P) 51.2	(N) 1 : 40	ND
42	CTD	(P) 1.1	(N) 6.8	(P) 1 : 80	ND

*EliA: >1.0 ratio, positive; 0.7–1.0 ratio, equivocal; <0.7 ratio, negative. [†]QUANTA Flash: ≥20.0 CU, positive; <20.0 CU, negative. [‡]IIFA: ≥1:80, positive; <1:80, negative.

Abbreviations: CTD, connective tissue disease; CU, chemiluminescent units; ENA, extractable nuclear antigen; FFA, Frontal fibrosing alopecia; IIFA, indirect immunofluorescence assay; ILD, interstitial lung disease; N, negative; ND, not detected; OA, osteoarthritis; P, positive; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SjS, Sjögren's syndrome; SSc, systemic sclerosis; R/O, rule out; SjS, primary Sjögren's syndrome; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

Table 3. Clinical performances of EliA, QUANTA Flash, and IIFA for diagnosing AARDs

Rheumatology clinic patients (N=200)				
	AUC (95% CI)	Cut-off value	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
EliA	0.917 (0.870-0.952)	> 1.0 ratio (manufacturer)	72.4 (63.6–80.0)	90.9 (82.2–96.3)
		> 0.56 ratio (adjusted)	82.9 (75.1–89.1)	90.9 (82.2–96.3)
QUANTA Flash	0.911 (0.863-0.947)	≥20.0 CU (manufacturer)	77.2 (68.8–84.3)	89.6 (80.6–95.4)
		>9.7 CU (adjusted)	87.8 (80.7–93.0)	85.7 (75.9–92.6)
IIFA	0.862 (0.806-0.906)	≥ 1 : 80 titer	84.6 (76.9–90.4)	80.5 (69.9–88.7)
Total samples (N=406)				
	AUC (95% CI)	Cut-off value	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
EliA	0.930 (0.900-0.953)	> 1.0 ratio (manufacturer)	71.5 (62.7–79.3)	94.0 (90.6–96.5)
		> 0.52 ratio (adjusted)	83.7 (76.0–89.8)	91.2 (87.2–94.2)
QUANTA Flash	0.915 (0.883-0.940)	≥20.0 CU (manufacturer)	76.4 (67.9–83.6)	91.2 (87.2–94.2)
		>9.9 CU (adjusted)	87.0 (80.7–93.0)	83.0 (78.1–87.2)

Abbreviations: AARD, antinuclear antibody-associated rheumatic disease; AUC, area under the ROC curve; CI, confidence interval; CU, chemiluminescent units; IIFA, indirect immunofluorescence assay.

tween QUANTA Flash and IIFA were similar to those between EliA and IIFA. In a previous study, opposite results were obtained [3]. Therefore, our results alone do not suggest that QUANTA Flash is more suitable than EliA as a replacement for IIFA.

Automated immunoassays reportedly are less sensitive than IIFA for detecting ANA [20]. In this study, however, the anti-ENA antibody assay positivity was higher in automated immunoassay-positive, IIFA-negative samples than in automated immunoassay-negative, IIFA-positive samples. Moreover, the anti-ENA antibodies detected by automated immunoassays and IIFA differed. All automated immunoassay-positive, IIFA-negative samples were anti-SS-A/Ro (Ro60) antibody-positive. This indicates that automated immunoassays can detect the anti-SS-A/Ro antibody, which may be missed by the IIFA. These results are in line with those of previous studies in which solid phase assays, such as FEIA and LIA, detected the anti-SS-A/Ro antibody, which was not detected by IIFA [21, 22]. According to international recommendations for ANA screening, patients should be assayed for specific anti-ENA antibodies, such as SS-A/Ro, even if the IIFA result is negative, in cases of high clinical suspicion [7]. EliA and QUANTA Flash may be suitable for ANA screening of patients with clinically suspected anti-SS-A/Ro antibody.

The anti-ENA antibodies detected in automated immunoassay-negative, IIFA-positive samples were variable. These antibodies have been reported to be associated with SLE [23-25]. In the present study, all patients with automated immunoassay-negative, IIFA-positive results were diagnosed as having SLE, except for one patient. The ANA titer reported in IIFA is not con-

sistently associated with disease severity; but higher ANA titers are more clinically significant, as healthy individuals generally have low ANA titers [26]. For these reasons, automated immunoassays should be used in combination with the IIFA in patients who are clinically suspected of having AARDs, especially, SLE.

Both EliA and QUANTA Flash were highly accurate in diagnosing AARDs. Although the AUCs of EliA and QUANTA Flash were as high as that of IIFA in this study, the manufacturers' cut-off values showed lower sensitivity than the adjusted cut-off values. The cut-off values can be adjusted to optimize combined sensitivity and specificity according to the assay purpose. Enzyme immunoassay pre-screening followed by IIFA reportedly reduces the overall time compared with screening by IIFA alone [27]. For ANA screening, cut-off values with high sensitivity, adjusted to nearly half of the manufacturers' cut-off values in this study, can be used. This study showed a tendency toward higher specificity for EliA and higher sensitivity for QUANTA Flash; however, as in previous studies, the differences between EliA and QUANTA Flash were not statistically significant [3, 4].

This study had several limitations. First, although we included the rheumatology clinic samples, the number of abnormal samples from patients diagnosed as having AARDs was relatively small; further studies with large numbers of abnormal samples are needed. Second, there were differences in antigen composition between EliA, QUANTA Flash, and the anti-ENA antibody assay. We did not perform further confirmatory assays for specific antibodies that were missed by EUROLINE. Third, we did not perform separate ROC curve analyses for individual diseases,

while previous studies have evaluated the clinical performance for not only AARDs, but also individual diseases [3, 4]. Disease-specific performance of the two automated immunoassays would be required to identify clinically suspected patients. Last, in patients with SLE, ANA can be detected several years before clinical symptoms appear [28]. As ANA screening using EliA and QUANTA Flash was performed without patient identification, it was not possible to follow up patients with ANA screening among those undergoing routine checkups.

In conclusion, EliA and QUANTA Flash showed reliable performance compared with the IIFA, and adjusting the cut-off values improved their sensitivities. These automated immunoassays may be used in combination with the IIFA in clinical immunology laboratories with a high ANA screening workload. The clinical cut-off values can be adjusted according to the workflow in each laboratory.

ACKNOWLEDGEMENTS

Thermo Fisher Scientific and Inova Diagnostics provided assay kits. They did not have any role in the study design or data analysis.

AUTHOR CONTRIBUTIONS

Yoon S collected the samples, analyzed and interpreted the data, and wrote the draft; Moon HW conceived the study and finalized the draft; Kim H, Hur M, and Yun YM discussed the data and reviewed the manuscript. All authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

RESEARCH FUNDING

None declared.

ORCID

Sumi Yoon <https://orcid.org/0000-0001-7529-1613>
Hee-Won Moon <https://orcid.org/0000-0001-9509-6073>
Hanah Kim <https://orcid.org/0000-0002-3266-638X>

Mina Hur <https://orcid.org/0000-0002-4429-9978>

Yeo-Min Yun <https://orcid.org/0000-0002-5485-8331>

REFERENCES

- Solomon DH, Kavanaugh AJ, Schur PH; American College of Rheumatology Ad Hoc Committee on Immunologic Testing Guidelines. Evidence-based guidelines for the use of immunologic tests: antinuclear antibody testing. *Arthritis Rheum* 2002;47:434-44.
- Mahler M, Meroni PL, Bossuyt X, Fritzler MJ. Current concepts and future directions for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. *J Immunol Res* 2014;2014:315179.
- van der Pol P, Bakker-Jonges LE, Kuijpers JHSAM, Schreurs MWJ. Analytical and clinical comparison of two fully automated immunoassay systems for the detection of autoantibodies to extractable nuclear antigens. *Clin Chim Acta* 2018;476:154-9.
- Claessens J, Belmondo T, De Langhe E, Westhovens R, Poesen K, Hüs S, et al. Solid phase assays versus automated indirect immunofluorescence for detection of antinuclear antibodies. *Autoimmun Rev* 2018;17:533-40.
- Aringer M, Costenbader K, Daikh D, Brinks R, Mosca M, Ramsey-Goldman R, et al. 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. *Arthritis Rheumatol* 2019;71:1400-12.
- Bauer A and Habior A. Detection of autoantibodies against nucleoporin p62 in sera of patients with primary biliary cholangitis. *Ann Lab Med* 2019;39:291-8.
- Agmon-Levin N, Damoiseaux J, Kallenberg C, Sack U, Witte T, Herold M, et al. International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. *Ann Rheum Dis* 2014;73:17-23.
- Peterson LK, Tebo AE, Wener MH, Cople SS, Fritzler MJ. Assessment of antinuclear antibodies by indirect immunofluorescence assay: report from a survey by the American Association of Medical Laboratory Immunologists. *Clin Chem Lab Med* 2020;58:1489-97.
- Yoo IY, Oh JW, Cha HS, Koh EM, Kang ES. Performance of an automated fluorescence antinuclear antibody image analyzer. *Ann Lab Med* 2017;37:240-7.
- Showman O, Gilburd B, Barzilai O, Shinar E, Larida B, Zandman-Goddard G, et al. Evaluation of the BioPlex 2200 ANA screen: analysis of 510 healthy subjects: incidence of natural/predictive autoantibodies. *Ann N Y Acad Sci* 2005;1050:380-8.
- Showman O, Gilburd B, Zandman-Goddard G, Yehiely A, Langevitz P, Shoenfeld Y. Multiplexed AtheNA multi-lyte immunoassay for ANA screening in autoimmune diseases. *Autoimmunity* 2005;38:105-9.
- Bentow C, Lakos G, Rosenblum R, Bryant C, Seaman A, Mahler M. Clinical performance evaluation of a novel, automated chemiluminescent immunoassay, QUANTA Flash CTD Screen Plus. *Immunol Res* 2015;61:110-6.
- Jeong S, Yang H, Hwang H. Evaluation of an automated connective tissue disease screening assay in Korean patients with systemic rheumatic diseases. *PLoS One* 2017;12:e0173597.
- Jeong S, Hwang H, Roh J, Shim JE, Kim J, Kim GT, et al. Evaluation of an automated screening assay, compared to indirect immunofluorescence, an extractable nuclear antigen assay, and a line immunoassay in a large cohort of Asian patients with antinuclear antibody-associated rheumatoid diseases: a multicenter retrospective study. *J Immunol Res* 2018;2018:9094217.
- Parker JC and Bunn CC. Sensitivity of the Phadia EliA connective tissue

- disease screen for less common disease-specific autoantibodies. *J Clin Pathol* 2011;64:631-3.
16. Aksu G, Gulez N, Azarsiz E, Karaca N, Kutukçuler N. Determination of cut-off titers and agreement between immunofluorescence and immunoblotting methods for detecting antinuclear antibodies in children. *J Clin Lab Anal* 2010;24:230-6.
 17. Garbati M, Yusuph H, Kagu M, Moses A. Diagnosis of pulmonary tuberculosis: utility of serology and Mantoux reaction in a resource-limited setting. *Internet J Infect Dis* 2009;8.
 18. McHugh ML. Interrater reliability: the kappa statistic. *Biochem Med* 2012;22:276-82.
 19. Greiner M, Pfeiffer D, Smith RD. Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. *Prev Vet Med* 2000;45:23-41.
 20. Op De Beeck K, Vermeersch P, Verschueren P, Westhovens R, Mariën G, Blockmans D, et al. Detection of antinuclear antibodies by indirect immunofluorescence and by solid phase assay. *Autoimmun Rev* 2011;10:801-8.
 21. Hoffman IE, Peene I, Veys EM, De Keyser F. Detection of specific antinuclear reactivities in patients with negative anti-nuclear antibody immunofluorescence screening tests. *Clin Chem* 2002;48:2171-6.
 22. Bossuyt X and Luyckx A. Antibodies to extractable nuclear antigens in antinuclear antibody-negative samples. *Clin Chem* 2005;51:2426-7.
 23. Egner W. The use of laboratory tests in the diagnosis of SLE. *J Clin Pathol* 2000;53:424-32.
 24. Gussin HA, Ignat GP, Varga J, Teodorescu M. Anti-topoisomerase I (anti-Scl-70) antibodies in patients with systemic lupus erythematosus. *Arthritis Rheum* 2001;44:376-83.
 25. González C, Garcia-Berrocal B, Herráez O, Navajo JA, González-Buitrago JM. Anti-nucleosome, anti-chromatin, anti-dsDNA and anti-histone antibody reactivity in systemic lupus erythematosus. *Clin Chem Lab Med* 2004;42:266-72.
 26. Satoh M, Vázquez-Del Mercado M, Chan EK. Clinical interpretation of antinuclear antibody tests in systemic rheumatic diseases. *Mod Rheumatol* 2009;19:219-28.
 27. Kern P, Kron M, Hiesche K. Measurement of antinuclear antibodies: assessment of different test systems. *Clin Diagn Lab Immunol* 2000;7:72-8.
 28. O'Sullivan M, McLean-Tooke A, Loh RK. Antinuclear antibody test. *Aust Fam Physician* 2013;42:718-21.