

# Comparison of antinuclear antibody profiles obtained using line immunoassay and fluorescence enzyme immunoassay

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## Abstract

**Objective:** LIA-ANA-Profile-17S is a multiplex line immunoassay that simultaneously detects 17 antinuclear antibodies (ANAs) against extractable nuclear antigens (ENAs). We evaluated the utility of LIA-ANA-Profile-17S as a supplement to ANA indirect immunofluorescence (IIF) and EliA ENA (a fluorescence enzyme immunoassay) for diagnosis of ANA-associated rheumatic diseases.

**Methods:** Sera were collected from 245 patients referred for an ANA IIF test. LIA-ANA-Profile-17S results were compared with those of EliA ENA. The kappa coefficients, agreement rates, and diagnostic performance of these tests were assessed for systemic lupus erythematosus (SLE) and Sjögren's syndrome (SjS).

**Results:** We observed almost perfect interassay agreement for antibodies against Ro52/Ro60, CENP-B, and Scl-70 ( $\kappa = 0.91, 0.97, \text{ and } 1.00$ , respectively); strong agreement for anti-SS-B/La antibody ( $\kappa = 0.81$ ); and relatively low agreement for other antibodies, including those against dsDNA, Sm, RNP, and Jo-1. For SLE diagnosis, LIA-ANA-Profile-17S showed lower sensitivity and similar specificity compared with EliA ENA. The sensitivity and specificity of these two assays were similar for SjS diagnosis.

**Conclusions:** The specificity of LIA-ANA-Profile-17S was enhanced when combined with ANA IIF and was comparable with that of EliA ENA. LIA-ANA-Profile-17S showed relatively good agreement with EliA ENA. In combination with ANA IIF, these assays showed enhanced diagnostic performance.

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## Keywords

LIA-ANA-Profile-17S, EliA ENA, line immunoassay, antinuclear antibody, diagnostic, systemic lupus erythematosus, Sjögren's syndrome

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## Introduction

Antinuclear antibodies (ANAs) are diagnostic hallmarks of autoimmune diseases. ANAs are used to diagnose and monitor disease activity as well as to evaluate prognosis.<sup>1</sup> Diseases specifically related to ANAs are known as ANA-associated rheumatic diseases (AARDs) and include systemic lupus erythematosus (SLE), Sjögren's syndrome (SjS), systemic sclerosis (SSc), and mixed connective tissue disease (MCTD). Specific antibodies included in the diagnostic criteria for AARDs include those against double-stranded DNA (dsDNA) and the Smith (Sm) antigen for SLE,<sup>2</sup> those against Ro60 and SS-B/La for SjS,<sup>3</sup> those against Scl-70 and RNA polymerase III for SSc,<sup>4</sup> and those against U1-ribonucleoprotein (RNP) for MCTD.<sup>5</sup>

ANA profile investigation is critical for AARD diagnosis. Autoantibody specificities are usually assessed by examining patterns of ANA immunofluorescence. Conventionally, the ANA test is performed by indirect immunofluorescence (IIF) and the ANA profile of extractable nuclear antigen (ENA) specificities is determined using automated fluorescence enzyme immunoassay (FEIA), enzyme-linked immunosorbent assay, or line immunoassay (LIA).

IIF using HEp-2 cells is a common assay used for ANA screening owing to its high sensitivity.<sup>1</sup> Because antibody titers and immunofluorescence patterns can be evaluated using this assay, ANA IIF may be used as a screening test.<sup>1,6</sup> However, further tests are often necessary to confirm antibody

specificity. FEIA is widely used to evaluate antibody specificity and quantitatively measure antibody titers with high specificity.<sup>7-9</sup> The LIA is used to measure multiple antibodies automatically and concurrently with high speed and is therefore useful for simultaneously screening and typing various antibodies.

This study aimed to evaluate and compare the diagnostic performance of LIA (LIA-ANA-Profile-17S, Shenzhen YHLO Biotech, Shenzhen, China) and FEIA (EliA, Phadia AB, Uppsala, Sweden) as assays for determining ENA specificity in patients with AARDs. We assessed the concordance rates between the two methods and their performance for clinical diagnosis.

## Materials and methods

### Study population

Sera were randomly collected and tested using ANA IIF. Sera were obtained from AARD patients (SLE, SjS, SSc, and MCTD) as well as non-AARD patients (rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, leukopenia, anemia, hepatitis, dry eye, and dry mouth).

This study was approved by the independent Institutional Review Board of Severance Hospital, Seoul, Korea (IRB No: 4-2017-0761). The requirement for informed consent was waived because of the retrospective nature of the study provided that all patient data were anonymized and that the study involved samples that

were already available rather than prospectively collected. The study complied with all relevant national regulations and institutional policies and was conducted in accordance with the principles laid out in the Helsinki Declaration (as revised in 2013).

### Assays

LIA-ANA-Profile-17S (Shenzhen YHLO Biotech, Shenzhen, China) is a LIA that facilitates multiplex detection of human IgG antibodies. The assay measures antibody binding to 17 antinuclear antigens: dsDNA, nucleosomes, histones, Smith (Sm)D1, U1-small nuclear RNP (snRNP), ribosomal p protein (RPP)/P0, proliferating cell nuclear antigen (PCNA), Ro52, Ro60, SS-B/La, centromere protein (CENP)-B, Scl70, Jo-1, PM-Scl, antimitochondrial antibody (AMA)-M2, Mi-2, and Ku. These antigens were marked on a strip with three positive control lines (low, medium, and high). Intensity was graded by densitometry. The intensity value of the low control line was used as the positive cut-off value. The results of each antigen band were interpreted as negative or positive based on the low control line.

EliA ENA assays (Phadia AB, Uppsala, Sweden) were used as a comparative method. These assays facilitated the quantitative measurement of IgG antibodies against ENAs including dsDNA, U1-snRNP, CENP-B, Ro52/Ro60, SS-B/La, SmD3, Scl-70, and Jo-1. Each EliA Kit contained wells coated with different antigens. Intensity values of >10 international units (IU)/mL for dsDNA; >7 IU/mL for U1-snRNP; and >5 IU/mL for CENP-B, Ro52/Ro60, SS-B/La, SmD3, Scl-70, and Jo-1 were interpreted as positive. The assays were performed according to the manufacturer's instructions.

The performance of the two assays in detecting the nine antibody specificities detectable by EliA ENA was compared.

Antibodies against Ro52 and Ro60 could not be detected separately by the EliA ENA because a single well in the kit was coated with both antigens. Consequently, anti-Ro52 and anti-Ro60 antibodies were assessed as a single group.

ANA IIF using HEp-2 cells [NOVA Lite 4',6-diamidino-2-phenylindole (DAPI) ANA Kit, Inova Diagnostics, San Diego, CA, USA] was performed according to the manufacturer's specifications. Light-emitting diodes with wavelengths of 400 nm (DAPI) and 490 nm (fluorescein isothiocyanate) were used. The screening dilution was 1:80. The slides were evaluated using NOVA View software (version 3.6.0.1). Two specialists evaluated slides independently and assessed the presence and patterns of fluorescence.

### Statistics

Cohen's kappa coefficient was used to evaluate inter-assay agreement. Values of <0.20, 0.21–0.39, 0.40–0.59, 0.60–0.79, 0.80–0.90, and >0.91 were interpreted as no, minimal, weak, moderate, strong, and almost perfect agreement, respectively. Positive (negative) agreement rates were assessed using the proportions of the test method results showing positive (negative) results among samples testing positive (negative) using the comparator method. Assay sensitivity and specificity were assessed based on clinical diagnosis. The total agreement rate was calculated as the number of agreements divided by the total number of cases. Distributions of the two assays in different disease groups were compared using the Chi-square test and Fisher's exact test. Values of  $P < 0.05$  were considered statistically significant. Statistical analyses were performed using R software 4.03 (R Foundation for Statistical Computing, Vienna, Austria).

## Results

### Study population

A total of 245 patient sera (200 positive, 45 negative) were tested using ANA IIF. Among these 245 samples, 191 showed positive results and the remaining 54 showed negative results in ANA IIF following re-evaluation by two specialists. Overall, the study population had a median age of 55.0 years (interquartile range: 39.0–66.0 years) and consisted of 187 (76.3%) women. Of the 68 (27.8%) AARD patients, 42 (17.1%) presented with SLE, 30 (12.2%) with SjS, one (0.4%) with SSc, and one (0.4%) with MCTD. Among the SLE and SjS patients, six (8.3%) showed overlap with the clinical manifestations of both SLE and SjS. Of the remaining 177 non-AARD patients, eight were diagnosed with rheumatoid arthritis.

### Characteristics and results of the two assays

The characteristics of the two assays are summarized in Table 1 and the positive detection rates of LIA-ANA-Profile-17S and EliA ENA among ANA IIF-positive samples are shown in Table 2. Anti-Ro52 and Anti-Ro60 antibodies were the most frequently detected specific autoantibodies in ANA profiles, and were detected in 74 (38.7%) and 69 (36.1%) of samples,

respectively. Using LIA-ANA-Profile-17S, 43 samples were positive for both Ro52 and Ro60. LIA-ANA-Profile-17S and EliA ENA both showed that one (0.5%) sample was positive for Scl-70 and that three (1.6%) samples and 1 (0.5%) sample were positive for Jo-1, respectively. No significant differences in the positive detection rates for these antigens, except for Sm and RNP, was observed between the two assays using ANA IIF-positive samples. For the anti-Sm antibody used in SLE diagnosis, the LIA-ANA-Profile-17S showed lower positive rates among ANA-positive samples [6 (3.1%) vs. 22 (11.5%)] and among all samples [6 (2.4%) vs. 22 (9.0%)] compared with EliA ENA. The positive detection rates of LIA-ANA-Profile-17S for anti-Sm and anti-RNP antibodies were significantly lower than those of EliA ENA ( $P=0.002$  and  $P=0.019$ , respectively). However, no significant difference in the total positive detection rate for all nine autoantibodies was observed between the two assays [100 (52.4%) vs. 114 (59.7%),  $P=0.149$ ]. In addition, no significant difference was observed between the two assays for ANA IIF-negative samples [48 (88.9%) vs. 53 (98.1%),  $P=0.113$ ]. The eight autoantibodies that were not detected by EliA ENA included antibodies against nucleosomes, histones, RPP/P0, PCNA, PM-Scl, AMA-M2, Mi-2, and Ku. Antibodies against nucleosomes, histones, RPP/P0, PCNA,

**Table 1.** Characteristics of LIA-ANA-Profile-17S and EliA ENA.

	LIA-ANA-Profile-17S	EliA ENA
Number of specificities	17 antigens in one test run	Eight antigens separately
Principle	LIA	FEIA
Sample volume	10 $\mu$ L	9–20 $\mu$ L (different for each assay)
Time to result	<3 hours	<2 hours
Automated instrument	Tenfly Phoenix (automatic western blot analyzer)	Phadia™ 250 immunoassay analyzer

LIA, line immunoassay; ANA, antinuclear antibody; ENA, extractable nuclear antigen; FEIA, fluorescence enzyme immunoassay.

**Table 2.** Positive detection rates of LIA-ANA-Profile-17S and EliA ENA according to ANA IIF results.

	LIA-ANA-Profile-17S-positive, n (%)	EliA ENA-positive, n (%)	P-value <sup>b</sup>
ANA IIF-positive, n = 191			
Positive <sup>a</sup>	100 (52.4)	114 (59.7)	0.149
dsDNA	33 (17.3)	31 (16.2)	0.784
Sm	6 (3.1)	22 (11.5)	0.002
U1-snRNP	19 (9.9)	35 (18.3)	0.019
Ro52/Ro60	74 (38.7)	69 (36.1)	0.597
Ro52	56 (29.3)		
Ro60	61 (31.9)		
SS-B/La	17 (8.9)	22 (11.5)	0.398
CENP-B	16 (8.4)	17 (8.9)	0.856
Scl-70	1 (0.5)	1 (0.5)	1.000
Jo-1	3 (1.6)	1 (0.5)	0.623
Positive	110 (57.6)	114 (59.7)	0.321
ANA IIF-negative, n = 54			
Negative <sup>a</sup>	48 (88.9)	53 (98.1)	0.113

<sup>a</sup>Results for only the nine antibodies in common between the two assays are presented.

<sup>b</sup>P-values were calculated using the Chi-square and Fisher's exact tests.

LIA, line immunoassay; ANA, antinuclear antibody; ENA, extractable nuclear antigen; IIF, indirect immunofluorescence; dsDNA, double-stranded DNA; Sm, Smith; snRNP, small nuclear ribonucleoprotein; CENP-B, centromere protein-B.

and AMA-M2 were detected in six (3.1%), 20 (10.5%), 11 (5.8%), two (1.0%), and 13 (6.8%) samples, respectively. LIA-ANA-Profile-17S showed negative results for antibodies against PM-Scl, Mi-2, and Ku.

### Agreement between LIA-ANA-Profile-17S and EliA ENA

The agreement rates and kappa values for all autoantibody specificities comparing LIA-ANA-Profile-17S with EliA ENA are summarized in Table 3. LIA-ANA-Profile-17S and EliA ENA showed overall high agreement rates (83.7%–100.0%) for the nine tested autoimmune antibodies. The positive agreement rates between the two assays for antibodies against dsDNA, Sm, and RNP were lower than those of other antibodies. Minimal agreement was observed for antibodies against dsDNA and Sm (kappa = 0.32 and 0.26, respectively), whereas weak agreement was observed for antibodies against RNP and Jo-1 (kappa = 0.57 and 0.40, respectively).

Ro52/Ro60, CENP-B, and Scl-70 showed almost perfect agreement (kappa = 0.91, 0.97, and 1.00, respectively) whereas SS-B/La showed strong agreement (kappa = 0.81).

### Diagnostic performance

The diagnostic performance of the two assays is summarized in Table 4. For AARD diagnosis, the sensitivity and specificity of LIA-ANA-Profile-17S was 92.6% and 68.9%, respectively, whereas the sensitivity and specificity of LIA EliA ENA was 97.1% and 72.3%, respectively. The sensitivity and specificity of ANA IIF was 98.5% and 44.1%, respectively. The sensitivity and specificity of LIA-ANA-Profile-17S was 42.9% and 89.2%, respectively, for SLE diagnosis, and 93.3% and 83.3%, respectively, for SjS diagnosis. The sensitivity and specificity of EliA ENA was 61.9% and 90.1%, respectively, for SLE diagnosis and 100.0% and 81.4%, respectively, for SjS diagnosis.

**Table 3.** Agreement rates and kappa coefficients of the LIA-ANA-Profile-17S and EliA ENA assays.

Antibody	EliA ENA	LIA-ANA-Profile-17S		Positive Kappa (95% CI)	Negative	Total
		Positive	Negative			
dsDNA	Positive	14	17	45.2	89.3	83.7
	Negative	23	191	0.32 (0.16–0.48)		
Sm	Positive	4	18	18.2	99.1	91.8
	Negative	2	221	0.26 (0.04–0.47)		
RNP	Positive	17	18	48.6	98.6	91.4
	Negative	3	207	0.57 (0.41–0.73)		
R052/Ro60	Positive	68	2	97.1	96	96.3
	Negative	7	168	0.91 (0.86–0.97)		
SS-B/La	Positive	16	6	72.7	99.6	97.1
	Negative	1	222	0.81 (0.67–0.95)		
CENP-B	Positive	16	1	94.1	100	99.6
	Negative	0	228	0.97 (0.90–1.00)		
Scl-70	Positive	1	0	100	100	100
	Negative	0	244	1.00 (1.00–1.00)		
Jo-1	Positive	1	0	100	98.8	98.8
	Negative	3	241	0.40 (–0.15 to 0.94)		

LIA, line immunoassay; ANA, antinuclear antibody; ENA, extractable nuclear antigen; 95% CI, 95% confidence interval dsDNA, double-stranded DNA; Sm, Smith; RNP, ribonucleoprotein; CENP-B, centromere protein-B.

### Discordant results

The ANA IIF patterns and disease groups for samples showing discordant results between LIA-ANA-Profile-17S and EliA ENA are shown in Table 5. Of the 40 discrepant samples for anti-dsDNA antibodies, 17 were LIA-ANA-Profile-17S-positive and EliA ENA-negative, while 23 were LIA-ANA-Profile-17S-negative and EliA ENA-positive. Among these 17 and 23 samples, 13 (56.6%) and 6 (35.3%), respectively, showed a homogeneous ANA IIF pattern. Of the 20 discrepant samples for anti-Sm antibodies, two were LIA-ANA-Profile-17S-positive and EliA ENA-negative while 18 were LIA-ANA-Profile-17S-negative and EliA ENA-positive. Among these 2 and 18 samples, 1 (50.0%) and 4 (22.2%), respectively, showed a coarse speckled ANA IIF pattern. Of the nine discrepant samples for anti-Ro52/Ro60 antibodies, seven were LIA-ANA-Profile-17S-positive and EliA

ENA-negative while two were LIA-ANA-Profile-17S-negative and EliA ENA-positive. Among these seven and two samples, six (85.7%) and two (100.0%), respectively, showed a fine speckled ANA IIF pattern. Of the seven discrepant samples for anti-SS-B/La antibodies, one was LIA-ANA-Profile-17S-positive and EliA ENA-negative, while six were LIA-ANA-Profile-17S-negative and EliA ENA-positive. All seven discrepant samples for the SS-B/La samples showed a coarse speckled ANA IIF pattern.

### Discussion

To confirm the specificities of anti-ENA antibodies, it is more efficient to use ANA IIF-positive samples than to directly identify anti-ENA antibodies without prior ANA IIF screening.<sup>10</sup> In this study, concordance rates and diagnostic utility in identifying anti-ENA specificities were compared for

**Table 4.** Diagnostic performance of the LIA-ANA-Profiles-17S and EIA ENA assays.

Disease group	AARD		SLE (n=42)		SJS (n=30)	
	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
Diagnostic antibodies			anti-dsDNA, anti-Sm		anti-R052/Ro60, anti-SS-B/La	
Parameter	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
LIA-ANA-Profiles-17S	92.6 (83.7–97.6)	68.9 (61.5–75.7)	42.9 (27.7–59.0)	89.2 (84.1–93.1)	100.0 (88.4–100.0)	79.1 (73.0–84.3)
EIA ENA	97.1 (89.8–99.6)	72.3 (65.1–78.8)	61.9 (45.6–76.4)	90.1 (85.2–93.9)	100.0 (88.4–100.0)	81.4 (75.5–86.4)
ANA IIF	98.5 (92.1–100.0)	29.9 (23.3–37.3)	97.6 (87.4–99.9)	26.1 (20.2–32.7)	100.0 (88.4–100.0)	25.1 (19.5–31.5)
LIA-ANA-Profiles-17S-positive and ANA-positive	91.2 (81.8–96.7)	72.9 (65.7–79.3)	42.9 (27.7–59.0)	91.1 (86.3–94.7)	100.0 (88.4–100.0)	79.5 (73.5–84.7)
EIA ENA-positive and ANA-positive	95.6 (87.6–99.1)	72.3 (65.1–78.8)	61.9 (45.6–76.4)	90.1 (86.3–94.7)	100.0 (88.4–100.0)	81.9 (76.0–86.8)
LIA-ANA-Profiles-17S-positive, EIA ENA-positive, and ANA-positive	91.2 (81.8–96.7)	80.8 (74.2–86.3)	31.0 (17.6–47.1)	98.0 (95.0–99.5)	100.0 (88.4–100.0)	82.8 (77.1–87.6)

LIA, line immunoassay; ANA, antinuclear antibody; ENA, extractable nuclear antigen; 95% CI, 95% confidence interval; AARD, ANA-associated rheumatic disease; SLE, systemic lupus erythematosus; SJS, Sjogren's syndrome; dsDNA, double stranded DNA; Sm, Smith; IIF, indirect immunofluorescence.

**Table 5.** ANA IIF patterns and clinical diagnosis related to antibody specificity in discordant samples.

Antibodies	LIA-ANA-Profile-17S	EliA ENA	n	ANA IIF patterns	n (%)	Diagnosis	n (%)
dsDNA	Positive	Negative	23	Homo	13 (56.6)	SLE	6 (26.1)
	Negative	Positive	17		6 (35.3)		8 (47.1)
Sm	Positive	Negative	2	Coarse speckled	1 (50.0)	SLE	0 (0.0)
	Negative	Positive	18		4 (22.2)		10 (55.6)
RNP	Positive	Negative	3	Coarse speckled	0 (0.0)	MCTD	0 (0.0)
	Negative	Positive	18		0 (0.0)		0 (0.0)
R052/Ro60	Positive	Negative	7	Fine speckled	6 (85.7)	SjS	0 (0.0)
	Negative	Positive	2		2 (100.0)		0 (0.0)
SS-B/La	Positive	Negative	1	Fine speckled	1 (100.0)	SjS	0 (0.0)
	Negative	Positive	6		6 (100.0)		3 (50.0)
CENP-B	Positive	Negative	0	Centromere	–	SSc	–
	Negative	Positive	1		1 (100.0)		0 (0.0)
Jo-1	Positive	Negative	3	Cytoplasmic	1 (33.3)	AARD	0 (0.0)
	Negative	Positive	0		–		–

ANA, antinuclear antibody; IIF, indirect immunofluorescence; LIA, line immunoassay; ENA, extractable nuclear antigen; dsDNA, double stranded DNA; Sm, Smith; RNP, ribonucleoprotein; CENP-B, centromere protein-B; SLE, systemic lupus erythematosus; MCTD, mixed connective tissue disorder; SjS, Sjögren's syndrome; SSc, systemic sclerosis; AARD, ANA-associated rheumatic disease.

the LIA-ANA-Profile-17S and EliA ENA assays. The results of the two assays in combination with ANA IIF were compared. This allowed us to evaluate the efficiency of current clinical laboratory processes for autoantibody identification in ANA IIF-positive samples.

Among ANA IIF-positive samples and excluding antibodies against Sm and RNP, the positive detection rates of the two assays in each disease group showed no significant differences. Thus, identifying the assay with the highest sensitivity was challenging. However, the positive detection rates for anti-Sm antibodies differed significantly between the two assays, probably because LIA-ANA-Profile-17S uses the SmD1 antigen and EliA ENA uses the SmD3 antigen. However, a previous study reported no significant differences in detection rates for SmD1 and SmD3.<sup>11</sup> Among SLE patients, the dsDNA- and Sm-positive rates of LIA-ANA-Profile-17S were 40.5% [higher than the result of a previous study

(26.7%)] and 11.9% [similar to the result of a previous study (11.7%)], respectively.<sup>12</sup> Among the 54 ANA-negative specimens, the negative detection rates of the nine antibodies showed no significant differences between the two assays [48 (88.9%) vs. 53 (98.1%),  $P=0.113$ ]. A dense fine speckled ANA IIF pattern was observed for 25 ANA-positive samples. Among these 25 samples, two positive samples were identified using both LIA-ANA-Profile-17S and EliA ENA, indicating a weak relationship between DFS pattern and autoantibody detection. The DFS pattern is associated with healthy individuals, signifying a low correlation with autoimmune diseases.<sup>6</sup>

A high agreement rate for antibodies against Scl-70 and Jo-1 (100.0% and 98.8%, respectively) was observed between the two assays. However, this could not be evaluated accurately because of the low numbers of positive cases ( $n=1$  and  $n=4$ , respectively). Negative results for all antibodies common to the two assays showed



high agreement (89.3%–100.0%). Kappa coefficients take into account the rate of discordance in all cases by calculating the positive and negative occurrence.<sup>13</sup> Thus, the kappa coefficient was lower for antibodies against dsDNA, Sm, and RNP than for other antibodies because of the lower matched positive rates. Higher positive detection rates were observed for Ro52/Ro60 than for other antibodies, and strong agreement was evident between the two assays (kappa = 0.91).

Because identification of specific antibodies is necessary for AARD diagnosis,<sup>2-4</sup> sensitivity and specificity were assessed for the following antibodies used for clinical diagnosis: anti-dsDNA, anti-Sm and anti-Ro52/Ro60 (in EliA ENA) for SLE, and anti-Ro60 (in LIA-ANA-Profile-17s) and anti-SS-B/La for SjS. The specificity of EliA ENA in this study was lower than that reported in a previous study (72.3% vs. 90.7%).<sup>14</sup> However, when considering each individual disease separately, EliA ENA showed high specificity. The specificity of LIA-ANA-Profile-17S was slightly lower than that of EliA ENA. However, LIA-ANA-Profile-17S showed low sensitivity for SLE diagnosis because Sm-positive rates were low. Additionally, false-negative results could have occurred because of antibody titer changes resulting from changes in disease activity.<sup>15</sup> Because this study evaluated the diagnostic potential of these assays based on retrospective medical record review, some specimens could not be acquired at the time of diagnosis or may not accurately reflect disease activity, thereby contributing to lower sensitivity. Similar specificity for SjS diagnosis was observed between the two assays. Higher sensitivity was observed for all AARDs considered together than for individual diseases, probably because autoimmune antibodies that are not included in the diagnostic criteria could be detected using these assays. Diagnostic performance was considered along with

ANA IIF positivity, because ANA IIF screening was also conducted as a part of the diagnostic process. Both assays showed increased specificity with concurrent testing compared with individual evaluations. However, the specificity of both assays did not differ greatly when combined with ANA IIF. Thus, the performance of LIA-ANA-Profile-17S and EliA ENA was similar for SLE and SjS diagnosis. Our data provide a better portrayal of the clinical situation where ANA identification is conducted together with ANA IIF. Diagnostic performance for SSc and MCTD could not be evaluated owing to the extremely low prevalence of these diseases in our sample.

In our discordant samples, ANA IIF patterns and diagnoses associated with each antibody specificity were compared based on the clinical process of antibody identification using EliA ENA after the ANA IIF test. For each antibody, the probabilities of displaying the relevant patterns were not significantly different for LIA-ANA-Profile-17S-positive/EliA ENA-negative and LIA-ANA-Profile-17S-negative/EliA ENA-positive samples ( $P=0.184$ ,  $P=0.447$ ,  $P=0.491$ , and  $P=1.000$  for dsDNA, Sm, Ro52/Ro60, and SS-B/La, respectively). Because the preparation and extraction processes of the antigens used for each test differed, inconsistencies in test results may occur. In two cases where a PCNA-like pattern was observed by ANA IIF, PCNA could not be detected using LIA-ANA-Profile-17S. Moreover, in two other cases where the PCNA band was confirmed by LIA-ANA-Profile-17S, the PCNA-like pattern was not observed by ANA IIF. The epitope of the PCNA used in LIA may be cryptic in Hep-2 substrates and vice versa. Most of the inconsistent results were observed in samples with a weakly positive band intensity of 1+ or a lower number of antibodies detected by LIA-ANA-Profile-17S. Additional work will be required to establish an appropriate cut-off value using a sufficient number of

samples with defined clinical diagnoses for which specific antibodies have been identified.

This study had several limitations. EliA ENA can concurrently assess nine antibody specificities against ENAs (antibodies against Ro52 and Ro60 are assessed together), whereas LIA-ANA-Profile-17S can analyze 17 antibodies. Thus, the diagnostic performance of the two assays was compared for only the nine common antibodies. Moreover, LIA-ANA-Profile-17S detects antibodies simultaneously, whereas EliA ENA investigates antibodies individually, leading to a limitation of the ENA kit for detecting uncommon antibodies. Of the 11 samples that were Ro52-positive and Ro60-negative by LIA-ANA-Profile-17S, nine were positive for Ro52/Ro60 in EliA ENA. Because EliA ENA detects antibodies against Ro60 and Ro52 together, it is difficult to distinguish between these two antibodies using this assay. By contrast, the positive bands of the two antibodies could be differentiated by LIA-ANA-Profile-17S. Therefore, this assay could be helpful for accurate antibody detection and patient diagnosis. Another limitation was the small sample size and the lack of disease subgroups. Because the prevalence of AARDs, excluding SLE and SjS, was rather low, it was difficult to evaluate the usefulness of the assay for detailed diagnosis. Furthermore, the diagnostic performance of these tests for other AARDs may not be clinically satisfactory. Finally, no additional tests were used for determining antibody specificity in discordant cases; thus, ANA IIF patterns were evaluated to complement this weakness.

Further studies of more samples in various disease subgroups are necessary to achieve a more accurate understanding. If ANA IIF and EliA ENA results are discrepant, LIA-ANA-Profile-17S may provide some assistance in making clinical decisions. Thus, simultaneous detection of

ENA specificities using LIA-ANA-Profile-17S may be useful for identification of ANA profiles in clinical laboratories.

## Conclusion

In this study, LIA-ANA-Profile-17S was compared with EliA ENA for autoimmune rheumatic disease diagnosis. The LIA-ANA-Profile-17S results showed poor agreement for three antibody specificities (anti-dsDNA, anti-Sm, and anti-RNP). Nevertheless, overall detection results using LIA-ANA-Profile-17S was complementary with the results of EliA ENA and ANA IIF.

The sensitivity of LIA-ANA-Profile-17S for SLE diagnosis was lower than that of EliA ENA, but the specificity for SjS diagnosis was similar between the two assays. LIA-ANA-Profile-17S and EliA ENA showed similar diagnostic performance for diagnosis of AARDs. If LIA-ANA-Profile-17S is included in the current diagnostic process where ANA IIF screening and EliA ENA identification are performed sequentially, ANA profiles can be identified simultaneously and antibody specificities can be selected to optimize diagnostic efficiency.

## Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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