

## RESEARCH ARTICLE

# Analytical and clinical performance of different platforms simultaneously detecting 15 antinuclear antibodies

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## Abstract

**Background:** Antinuclear antibodies (ANAs) are invaluable biomarkers for the diagnosis of autoimmune diseases (AIDs). This study aims to compare the performances of line immunoassay (LIA), multiplex bead-based flow fluorescent immunoassay (MBFFI), and magnetic bar code immunofluorescence assay (MBC-IF) to detect ANA-Profile-15S.

**Methods:** In total, 184 samples from AID patients and 50 healthy controls (HCs) were collected. Fifteen ANAs (anti-dsDNA, nucleosome, histone, Sm, PCNA, ribosomal-P, SS-A/Ro52, SS-A/Ro60, SS-B/La, centromere B [CENP-B], Scl-70, U1-snRNP, AMA-M2, Jo-1, and Pm/Scl) were subjected to parallel detection by the LIA, MBFFI, and MBC-IF. The consistency between assays was analyzed. The discrepant results were further examined by chemiluminescent immunoassay (CLIA).

**Results:** Anti-SS-A/Ro52 and SS-A/Ro60 autoantibodies were the most common autoantibodies in ANA positive-profiles, and were detected with equal efficiency by the LIA, MBFFI, and MBC-IF ( $p = 0.101$  and  $p = 0.732$ , respectively). The three assays showed excellent agreement (consistency range: 66.5%–97.5%), and total consistency was 85.8%. The MBFFI and MBC-IF assays were in good agreement in terms of ANA-Profile-15S determination; the kappa coefficient ranged from 0.59 to 0.95, except for the PCNA and PM-Scl. Of the 262 re-assessed divergent results, 124 (47.33%) were positive on CLIA; the various autoantibodies exhibited variable patterns. More importantly, the ANA-Profile-15S results of the MBFFI and MBC-IF accurately identified patients with AID; the area under the curves ranged from 0.642 to 0.919.

**Conclusions:** The novel MBFFI and MBC-IF assay performed well in detecting ANA-Profile-15S. The application of MBFFI and MBC-IF play important roles in laboratory diagnosis of AIDs.

## KEYWORDS

autoantibodies, chemiluminescent immunoassay, line immunoassay, magnetic bar code immunofluorescence assay, multiplex bead-based flow fluorescent immunoassay

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## 1 | INTRODUCTION

Antinuclear antibodies (ANAs) are valuable marks of various autoimmune diseases (AIDs), including systemic lupus erythematosus (SLE), Sjogren's syndrome (SS), systemic sclerosis (SSc), mixed connective tissue disease (MCTD), and idiopathic inflammatory myopathies (IIM, polymyositis and dermatomyositis; and even primary biliary cirrhosis (PBC)).<sup>1,2</sup> Serological detection of ANAs is essential for predicting, screening, and diagnosing AIDs, and also indicates the likelihood of clinical course and complications.<sup>3</sup> ANA screening is internationally recognized as the first step for diagnosing systemic autoimmune diseases.<sup>3</sup> ANA positivity was included as a new entry criterion in the 2019 SLE classification criteria.<sup>4</sup> In addition, positivity for specific ANAs (such as antibodies to SSA/Ro60, U1RNP, centromere protein B [CENPB], RNA polymerase III, and Jo1) are included in classification criteria for other diseases, such as SS, MCTD, SSc, and IIM.<sup>5-7</sup>

Indirect immunofluorescence (IIF) assays using human epithelial type 2 cells (HEp-2 cells), or variants thereof, are the gold standard when screening for ANAs, which were first described in 1950 by Coons and Kaplan.<sup>8,9</sup> However, IIF is laborious and subjective. Moreover, it is affected by intra- and interlaboratory variability, standardization is lacking, and the false-positivity rate is high in healthy individuals.<sup>1</sup> To overcome these limitations, several techniques have been developed to detect the most common ANAs in AID, including enzyme-linked immunoassay (ELISA) and line immunoassay (LIA). Meanwhile, the demand for ANA testing has increased remarkably in recent years, in turn increasing the need for high throughput in clinical laboratories. Therefore, novel automated platforms have been introduced, including chemiluminescent immunoassay (CLIA), multiplex bead-based flow fluorescent immunoassay (MBFFI), magnetic bar code immunofluorescence assay (MBC-IF), and microarray systems.<sup>10-14</sup>

However, inconsistencies among methods are burdensome for those who perform and interpret the tests.<sup>3</sup> Inappropriate interpretation can lead to misdiagnosis, unbefitting therapies, and unnecessary costs. Thus, this study detected antibodies against dsDNA, nucleosome, histone, Sm, PCNA, ribosomal-P, SS-A/Ro52, SS-A/Ro60, SS-B/La, centromere B (CENP-B), Scl-70, U1-snRNP, AMA-M2, Jo-1, and Pm/Scl in a clinical cohort by the LIA, MBFFI, and MBC-IF. We evaluated the performance and consistency of the methods.

## 2 | MATERIALS AND METHODS

### 2.1 | Study population

Serum samples from patients with AIDs ( $n = 184$ ) at the Second Hospital of Shanxi Medical University, and from sex- and age-matched healthy controls (HCs) ( $n = 50$ ), were subjected to ANA-profile-15S detection (15 autoantibodies against dsDNA, nucleosome, histone, Sm, PCNA, Rib-P0, SS-A/Ro 60kDa, SS-A/Ro 52kDa, SS-B/La, CENP-B, Scl-70, U1-snRNP, AMA M2, Jo-1, and PM-Scl) using the

TABLE 1 Characteristics of the study population

	AID ( $n = 184$ )	HC ( $n = 50$ )	<i>p</i> value
Age (years), median (IQR)	51.00 (38.00, 59.00)	51 (35.50, 57.00)	0.635
Female/male, <i>N</i>	169/15	45/5	0.775
ANA by IIF ( <i>N</i> )			
Negative	26	NA	NA
1:80	19	NA	NA
1:160	27	NA	NA
1:320	14	NA	NA
1:640	17	NA	NA
1:1280	69	NA	NA
>1:1280	12	NA	NA
Diagnosis, <i>N</i> (Female/male)			
SLE	70 (64/6)		
SjS	59 (58/1)		
IIM	21 (15/6)		
PBC	11 (10/1)		
SSc	23 (22/1)		

Note: All data were described as numbers except age which was presented as median (interquartile range). Age in both groups were compared using the Mann-Whitney *U* test. Difference in gender was compared by chi-square test.

Abbreviations: AID, autoimmune disease; IIM, idiopathic inflammatory myopathies; IQR, interquartile range; NA, not available; PBC, primary biliary cirrhosis; SLE, systemic lupus erythematosus; SS, Sjogren's syndrome; SSc, systemic sclerosis.

LIA, MBFFI, and MBC-IF. Patients were classified as SLE ( $n = 70$ ), SjS ( $n = 59$ ), IIM ( $n = 21$ ), PBC ( $n = 11$ ), and SSc ( $n = 23$ ) (Table 1). All patients fulfilled the classification criteria for the respective diseases. SLE was diagnosed by clinicians according to the 1997 ACR revised classification criteria,<sup>15</sup> patients with SjS met the diagnostic criteria of the American-European Consensus Group,<sup>16</sup> diagnosis of IIM was based on the 2017 ACR/EULAR classification criteria,<sup>17</sup> patients with PBC met the 2019 AASLD classification criteria,<sup>18</sup> and SSc was diagnosed by reference to the 2013 ACR/EULAR criteria.<sup>19</sup> All blood samples were centrifuged to separate the serum within 3 h of sampling and stored at  $-80^{\circ}\text{C}$ . All tests were performed according to the manufacturers' instructions. This study was approved by the ethics committee of the Second Hospital of Shanxi Medical University (2019YX266) and all individuals gave informed consent.

### 2.2 | LIA assay

A diagnostic ANA kit (Diagnostic Kit for Antinuclear Antibodies, catalog no. 20192400758; Kangrun Biotech®) and automatic LIA analyzer (HELIA BOLT SN201916904; Kangrun Biotech®) were used for LIA assay. The LIA-ANA-Profile is an indirect, membrane-based enzyme immunoassay that provides the qualitative determination of

IgG antibodies against multiple antigens (dsDNA, nucleosome, histone, Sm, PCNA, Rib-P0, SS-A/Ro 60kDa, SS-A/Ro 52kDa, SS-B/La, CENP-B, Scl-70, U1-snRNP, AMA M2, Jo-1, PM-Scl, Mi-2, and Ku). These antigens were placed on a strip that also includes control lines. The antigen band was scanned using a EUROBlotOne analyzer (YG 0153–0101; Euroimmun®) and were interpreted as negative, weakly positive, or positive by comparing the intensity of the of test lines to control lines.

### 2.3 | MBFFI assay

The MBFFI assay used a Super Multiplex Immunoassay System (Tesml F4000; Tellgen®) and an ANAs (IgG) diagnostic kit (catalog no. 20202400548; Tellgen®) for determination of IgG autoantibodies against the ANA-profile antigens (anti-dsDNA, nucleosome, histone, Sm, PCNA, Rib-P0, SS-A/Ro 60kDa, SS-A/Ro 52kDa, SS-B/La, CENP-B, Scl-70, U1-snRNP, AMA M2, Jo-1, PM-Scl, and C1q). All antigens were covalently cross-linked to microspheres with different fluorescence codes, and specific IgG antibodies in samples bound to microspheres with corresponding antigens. Goat anti-human IgG antibody-labeled phycoerythrin (catalog no. 20202400548; Tellgen®) was added, and the microspheres were then re-suspended in sheath fluid. All manufacturer-proposed antibody-positive thresholds (with the exception of that for anti-dsDNA) were >20 arbitrary unit (AU). The cut-off of anti-dsDNA proposed by the manufacturer was >20 international units (IU)/ml.

### 2.4 | MBC-IF assay

The MBC-IF assay (catalog no. 20192401173; LIVZON®) was performed according to the manufacturer's instructions using an automated multi-liquid chip immune analysis system (MCLIA-800; LIVZON®). Magnetically barcoded substrates (with different codes) were coated with the ANA-profile-15S antigens (anti-dsDNA, nucleosome, histone, Sm, PCNA, Rib-P0, SS-A/Ro 60kDa, SS-A/Ro 52kDa, SS-B/La, CENP-B, Scl-70, U1-snRNP, AMA M2, Jo-1, and PM-Scl). The cut-off value for anti-dsDNA positivity was >100IU/ml; all other (manufacturer-suggested) cut-offs were >1 antibody index (AI).

### 2.5 | Statistics

Statistical analysis was performed using the SPSS 22.0 or Graphpad Prism 7. The chi-square test was used to compare the results. Between-assay agreement was determined using the kappa coefficient ( $\kappa < 0.2$ , no agreement;  $0.2 \leq \kappa < 0.4$ , minimal agreement;  $0.4 \leq \kappa < 0.6$ , slight agreement;  $0.6 \leq \kappa < 0.8$ , moderate agreement;  $0.8 \leq \kappa \leq 0.9$ , substantial agreement;  $\kappa > 0.9$ , almost perfect agreement).<sup>20</sup> *p*-values <0.05 were considered statistically significant.

## 3 | RESULTS

### 3.1 | Study population

A total of 234 serum samples were analyzed, of which 184 were from patients with AIDs (median age 51 [38, 59] years; female/male 169/15) including SLE (*n* = 70; median age 39.50 [29.50, 51.25] years; female/male 64/6), SjS (*n* = 59; median age 56.00 [48.00, 63.00] years; female/male 58/1), IIM (*n* = 21; median age 54 [44.50, 59] years; female/male 15/6), PBC (*n* = 11; median age 60 [47, 62] years; female/male 10/1), and SSc (*n* = 23; median age 55 [46, 61] years; female/male 22/1). The basic characteristics of the study population were presented in Table 1. The median ages of patients in the total AID and HC groups were 51 (38, 59) and 51 (35.50, 57) years, respectively (*p* = 0.635).

### 3.2 | Performance of the LIA, MBFFI, and MBC-IF assays

The antigen resources for the ANA-Profile-15S panels of the three manufacturers are shown in Table S1. The performance characteristics of the three systems are summarized in Table S2, and include the conjugation, time to the first result, inspection speed, and cut-off values. The MBFFI conducted the ANA-Profile-15S using a fully automated Super Multiplex Immunoassay System; detection is rapid. For dsDNA, the linear ranges of the MBFFI and MBC-IF assays were 3–300 and 10–600IU/ml, respectively. For other autoantibodies, semi-quantitative detection was conducted by MBFFI and MBC-IF; no clear linear ranges were apparent. No single system was better or more stable in terms of positive rate of all ANAs. However, of the total 3510 results, the MBFFI reported 445 (16.12%) as positive, compared to 377 (13.36%) reported by the LIA and 431 (15.62%) reported by the MBFFI. All three assays reported four (8%) positive results in HCs (Table S3).

### 3.3 | Prevalence of AID-related autoantibodies

The positive ANA-Profile-15S detection rates of the LIA, MBFFI, and MBC-IF for ANA IIF-positive samples are shown in Table 2. Anti-SS-A/Ro52 and SS-A/Ro60 antibodies were the most frequently detected autoantibodies in ANA-positive profiles; the LIA, MBFFI, and MBC-IF assay were equally effective (81 [51.27%] vs. 72 [45.57%] vs. 91 [57.59%], *p* = 0.101; 74 [46.84%] vs. 81 [51.27%] vs. 78 [49.37%], *p* = 0.732, respectively). LIA, MBFFI, and MBC-IF identified very few samples positive for PCNA, Jo-1, or Pm-Scl. For ANA IIF-positive samples, no significant difference in positive rates was observed among the LIA, MBFFI, and MBC-IF assay, except for anti-ds-DNA (35 [22.15%] vs. 56 [35.44%] vs. 57 [36.08%], *p* = 0.011), nucleosome (14 [8.86%] vs. 35 [22.15%] vs. 25 [15.82%], *p* = 0.005), histone (13 [8.23%] vs. 18 [11.39%] vs. 31 [19.62%], *p* = 0.008), and AMA M2 (5 [3.16%] vs. 23 [14.56%] vs. 12 [7.59%], *p* = 0.001). The positive rates of patients with

TABLE 2 Positive detection rates of each item from different test systems according to ANA IIF results

	ANA by IIF (positive, n = 158)				ANA by IIF (negative, n = 26)			
	LIA	MBFFI	MBC-IF	p value	LIA	MBFFI	MBC-IF	p value
ds-DNA (n, %)	35 (22.15)	56 (35.44)	57 (36.08)	<b>0.011</b>	0	0	0	NA
Nucleosome (n, %)	14 (8.86)	35 (22.15)	25 (15.82)	<b>0.005</b>	0	0	0	NA
Histone (n, %)	13 (8.23)	18 (11.39)	31 (19.62)	<b>0.008</b>	0	1 (3.85)	0	NA
Sm (n, %)	27 (17.09)	22 (13.92)	17 (10.76)	0.267	0	0	0	NA
PCNA (n, %)	1 (0.63)	2 (1.26)	1 (0.63)	NA	0	0	0	NA
RIB-P (n, %)	22 (13.92)	23 (14.56)	25 (15.82)	0.889	1 (3.85)	0	0	NA
SS-A/Ro 60 (n, %)	81 (51.27)	72 (45.57)	91 (57.59)	0.101	5 (19.23)	2 (7.69)	5 (19.23)	NA
SS-A/Ro 52 (n, %)	74 (46.84)	81 (51.27)	78 (49.37)	0.732	7 (26.92)	8 (30.77)	6 (23.08)	0.822
SS-B/La (n, %)	25 (15.82)	21 (13.29)	21 (13.29)	0.757	0	1 (3.85)	0	NA
CENP-B (n, %)	11 (6.96)	8 (5.06)	8 (5.06)	0.702	0	0	0	NA
Scl-70 (n, %)	8 (5.06)	10 (6.33)	9 (5.70)	0.889	0	0	0	NA
U1-snRNP (n, %)	45 (28.48)	54 (34.18)	39 (24.68)	0.174	1 (3.85)	1 (3.85)	0	NA
AMA M2 (n, %)	5 (3.16)	23 (14.56)	12 (7.59)	<b>0.001</b>	0	0	0	NA
Jo-1 (n, %)	0	3 (1.90)	1 (0.63)	NA	2 (7.69)	2 (7.69)	3 (11.54)	NA
Pm-Scl (n, %)	0	2 (1.26)	2 (1.26)	NA	0	0	0	NA
Total results (n, %)	361 (15.23)	430 (18.14)	417 (17.59)	<b>0.018</b>	16 (4.10)	15 (3.85)	14 (3.59)	0.933

Note: All data were described as numbers (percentage). *p* values were calculated by chi-square. NA, not available. The *p* values < 0.05 are shown in bold.

different diseases (as determined by the LIA, MBFFI, and MBC-IF) are shown in Table 3. The positive rate did not differ significantly among the three assays for any autoantibody ( $p > 0.05$ ), with the exceptions of the anti-dsDNA and histone antibodies in patients with SLE ( $p = 0.003$  and  $p = 0.031$ , respectively).

### 3.4 | Agreement among the LIA, MBFFI, and MBC-IF assays in terms of ANA-Profile-15S detection

Venn diagram was utilized to assess the overall agreement among the MBFFI, LIA, and MBC-IF (Figure 1). The excellent agreement was shown, range from 66.5% to 97.5%. For each antibody type, agreement among the three methods was assessed (i.e., MBFFI vs. MBC-IF, MBFFI vs. LIA, and MBC-IF vs. LIA). As shown in Table 4, the best agreement in terms of the ANA-Profile-15S was between the MBFFI and MBC-IF (total agreement ranges: 90.17%–99.15%). The total agreement ranges of MBFFI versus LIA and MBCIF versus LIA were 85.47%–99.15% and 84.19%–100%, respectively. The anti-SS-A/Ro 52 yielded almost perfect agreement between the MBFFI and MBC-IF ( $\kappa = 0.95$ ,  $p < 0.001$ ); the anti-nucleosome, RIB-P, SS-A/Ro 60, and CENP-B also displayed strong agreement ( $\kappa = 0.81$ , 0.84, 0.80, and 0.88, respectively; all  $p < 0.001$ ); the dsDNA, histone, Sm, SS-B/La, Scl-70, U1-snRNP, and AMA M2 showed moderate agreement ( $\kappa = 0.74$ , 0.64, 0.69, 0.77, 0.73, 0.70, and 0.60, respectively; all  $p < 0.001$ ). For the MBFFI vs. LIA, the anti-SS-A/Ro

52 and CENP-B displayed strong agreement ( $\kappa = 0.81$  and 0.85, respectively; all  $p < 0.001$ ). For the MBC-IF vs. LIA, the anti-PCNA and Scl-70 were in almost perfect agreement ( $\kappa = 1.00$  and 0.94, respectively; all  $p < 0.001$ ); the RIB-P, SS-A/Ro 60 and SS-A/Ro 52 displayed strong agreement ( $\kappa = 0.82$ , 0.82 and 0.84, respectively; all  $p < 0.001$ ), the nucleosome, Sm, SS-B/La, CENP-B, and U1-snRNP showed moderate agreement ( $\kappa = 0.64$ , 0.60, 0.71, 0.75, and 0.76, respectively; all  $p < 0.001$ ).

### 3.5 | Discrepant data analysis among the MBFFI, LIA, and MBC-IF assays

Although good qualitative agreement was evident among the three assays, there were discrepant data. Therefore, discrepant results were further subjected to CLIA (Table S4). Of the 262 re-assessed results, 124 (47.33%) were positive on CLIA; SLE comprised 84.2% (147/262) of the divergencies. Furthermore, of the 69 results that were MBFFI- and MBC-IF-positive but LIA-negative, 50 (72.46%) were positive on CLIA. Of the six anti-SS-A/Ro 60 and seven AMA M2 that were MBFFI- and MBCIF-positive but LIA-negative, all were positive on CLIA. The 90% (9/10) of anti-SS-A/Ro 52 samples negative on LIA but positive on MBFFI and MBC-IF were positive on CLIA. For anti-dsDNA, 68.42% (13/19) of samples positive on MBFFI and MBCIF, but negative on LIA, were positive on CLIA. Of the 44 results that were MBFFI- and LIA-negative but MBC-IF-positive, 33

**TABLE 3** Positive rates of autoantibodies in different disease cohorts

	LIA	MBFFI	MBC-IF	p value
<b>SLE (70)</b>				
dsDNA (n, %)	24 (34.29)	40 (57.14)	43 (61.43)	<b>0.003</b>
Nucleosome (n, %)	14 (20.00)	27 (38.57)	22 (31.43)	0.059
Histone (n, %)	13 (18.57)	15 (21.43)	26 (37.14)	<b>0.031</b>
Sm (n, %)	18 (25.71)	14 (20.00)	10 (14.29)	0.260
U1-snRNP (n, %)	27 (38.57)	33 (47.14)	21 (30.00)	0.134
SS-A/Ro 60(n, %)	40 (57.14)	30 (42.86)	44 (62.86)	0.052
SS-A/Ro 52 (n, %)	26 (37.14)	27 (38.57)	26 (37.14)	1.000
RIB-P (n, %)	21 (30.00)	21 (30.00)	23 (32.86)	0.947
<b>SjS (59)</b>				
SS-A/Ro 60 (n, %)	38 (64.41)	34 (57.63)	41 (69.49)	0.516
SS-A/Ro 52 (n, %)	40 (67.80)	42 (71.19)	42 (71.19)	0.953
SS-B/La (n, %)	19 (32.20)	18 (30.51)	16 (27.12)	0.884
U1-snRNP (n, %)	9 (15.25)	13 (22.03)	9 (15.25)	0.576
<b>IIM (21)</b>				
Jo-1 (n, %)	2 (9.52)	2 (9.52)	2 (9.52)	NA
SS-A/Ro 60 (n, %)	4 (19.05)	4 (19.05)	4 (19.05)	NA
SS-A/Ro 52 (n, %)	5 (23.81)	7 (33.33)	6 (28.57)	0.952
<b>PBC (11)</b>				
AMA M2 (n, %)	2 (18.18)	6 (54.55)	6 (54.55)	NA
SS-A/Ro 52 (n, %)	4 (36.36)	5 (45.45)	4 (36.36)	1.000
<b>SSc (23)</b>				
Scl-70 (n, %)	8 (34.78)	7 (30.43)	9 (39.13)	0.962
SS-A/Ro 60 (n, %)	3 (13.04)	6 (26.09)	6 (26.09)	0.465
SS-A/Ro 52 (n, %)	6 (26.09)	8 (34.78)	6 (26.09)	0.874
U1-snRNP (n, %)	8 (34.78)	7 (30.43)	7 (30.43)	1.000
CENP-B (n, %)	3 (13.04)	3 (13.04)	3 (13.04)	1.000

Note: p values were calculated by chi-square. The p values < 0.05 are shown in bold.

(75%) were negative on CLIA. The 90% (9/10) of dsDNA samples that were positive on MBC-IF but negative on LIA and MBFFI were also negative results on CLIA. For anti-histone, 92.86% (13/14) of samples negative on LIA and MBFFI but positive on MBC-IF were negative on CLIA. The results of the ANA IIF assays of discrepant samples are shown in Table S5.

### 3.6 | Clinical performance of the MBFFI and MBC-IF for diagnosing specific diseases

The clinical performance of ANA-Profile-15S in terms of AID diagnosis was evaluated for both the MBFFI and MBC-IF, and the results were summarized in Table S6. The areas under the curve (AUCs) of PCNA, SS-A/Ro 60, SS-A/Ro 52, and Jo-1 on MBFFI were all over 0.900, and that for Sm was greater than 0.900 on the MBC-IF, indicating that both the MBFFI and MBC-IF were useful for diagnosing AID. We drew receiver operator characteristic (ROC) curves to compare the significance of most frequently detected antibodies in distinguishing

specific patients and controls (Figure 2). For SLE-specific antibodies, there were no significant differences in the AUCs between MBFFI and MBC-IF for the anti-histone or U1-snRNP ( $Z = 0.106, p = 0.916$ ; and  $Z = 1.547, p = 0.122$ , respectively). The AUCs of MBC-IF for anti-dsDNA, nucleosome, and Sm were significantly larger than those of MBFFI ( $Z = 3.238, p = 0.001$ ;  $Z = 2.338, p = 0.019$ ; and  $Z = 2.438, p = 0.015$ , respectively); however, the AUCs of MBFFI for anti-SS-A/Ro 60, SS-A/Ro 52, and RIB-P were significantly larger than those of MBC-IF ( $Z = 2.571, p = 0.010$ ;  $Z = 4.503, p < 0.001$ ; and  $Z = 3.591, p < 0.001$ , respectively; Figure 2A). Regarding SS-specific antibodies, the MBFFI AUC of anti-SS-A/Ro 52 was significantly larger than that of MBC-IF ( $Z = 4.170, p < 0.001$ ), whereas the MBFFI AUC of U1-snRNP was significantly smaller than that of MBC-IF ( $Z = 2.640, p = 0.008$ ). The AUCs of anti-SS-A/Ro 60 and SS-B/La did not differ significantly between MBFFI and MBC-IF (Figure 2B). In terms of IIM-specific antibodies, the MBFFI AUCs of anti-Jo-1, SS-A/Ro 60, and SS-A/Ro 52 were significantly larger than those of MBC-IF ( $Z = 3.113, p = 0.002$ ;  $Z = 2.889, p = 0.004$ ; and  $Z = 4.557, p < 0.001$ , respectively; Figure 2C). Regarding PBC-specific antibodies, the

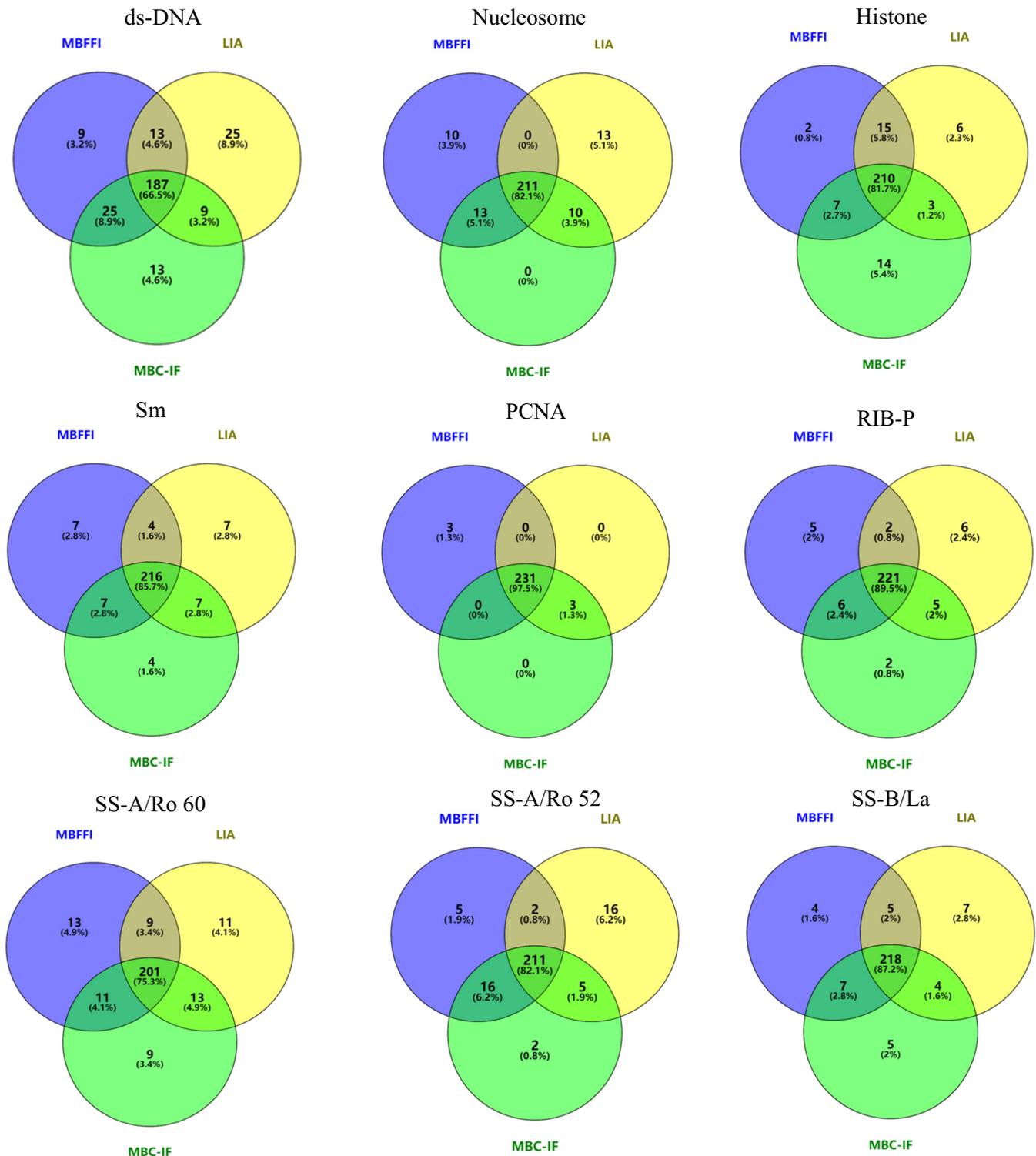


FIGURE 1 Venn diagrams show the agreement between MBFFI, LIA, and MBC-IF for the ANA-Profile-15S ( $n = 234$ )

MBFFI AUCs of the anti-AMA M2 and SS-A/Ro 52 were significantly larger than those of MBC-IF ( $Z = 2.777$ ,  $p = 0.006$ ; and  $Z = 2.516$ ,  $p = 0.012$ , respectively; Figure 2D). For Ssc-specific antibodies, the MBC-IF AUCs of anti-Sci-70, SS-A/Ro 60, and SS-A/Ro 52 were statistically larger than those of MBFFI ( $Z = 1.553$ ,  $p = 0.121$ ;  $Z = 2.313$ ,  $p = 0.021$ ; and  $Z = 2.584$ ,  $p = 0.010$ , respectively); however, the AUC of MBFFI for anti-U1-snRNP was significantly smaller than that of MBC-IF ( $Z = 2.347$ ,  $p = 0.019$ ; Figure 2E).

#### 4 | DISCUSSION

ANA testing is crucial for the diagnosis and management of patients with AID. Internationally, the IIF using HEp-2 cells is recommended as the gold standard for screening ANAs.<sup>21,22</sup> When ANA screening is positive by IIF, it is essential to confirm the autoantibody type, which plays a critical role in stratifying patients with AIDs.<sup>23</sup> In recent years, with the increasing use of ANAs to

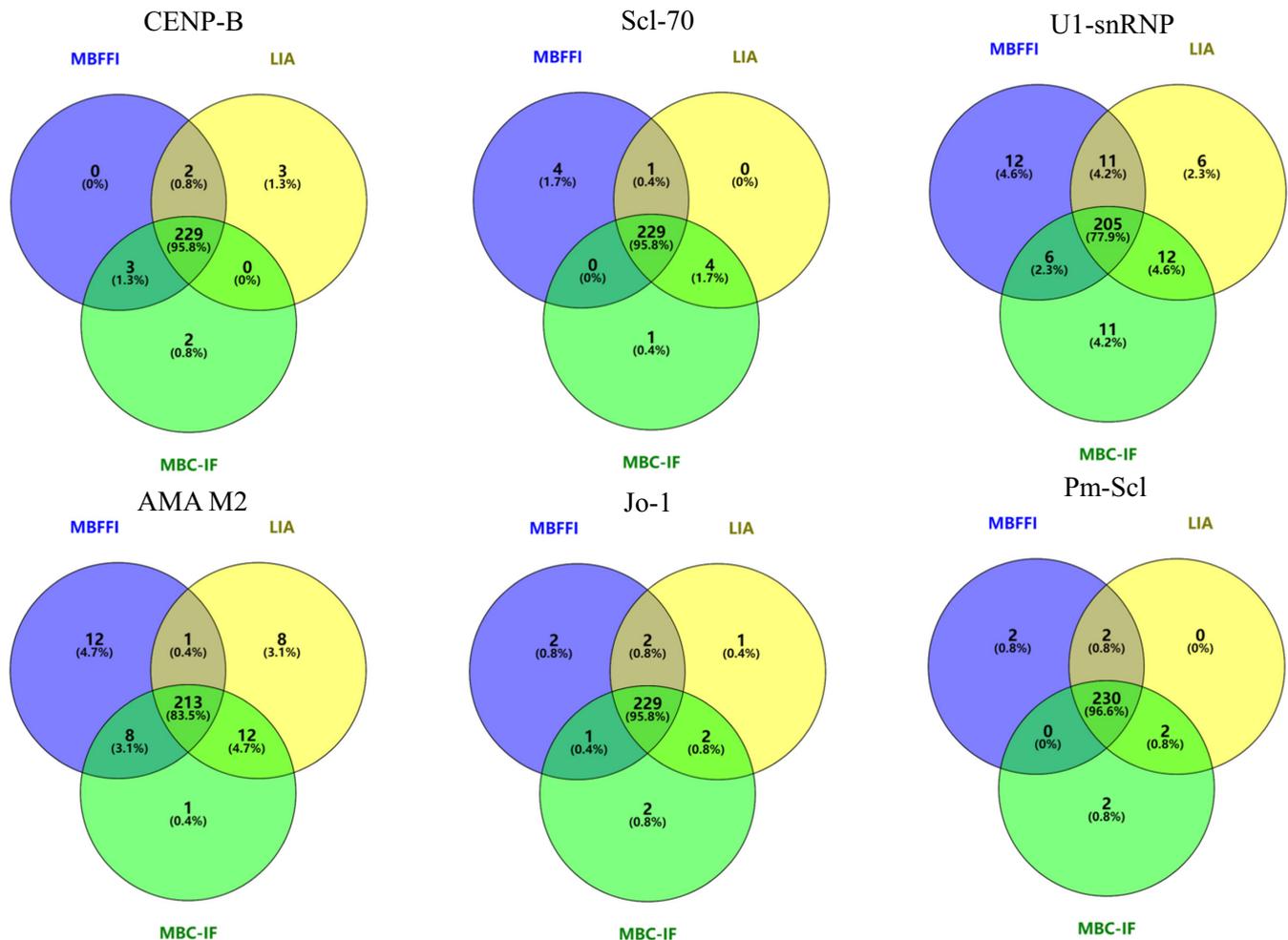


FIGURE 1 (Continued)

diagnose AID, several new ANA detection methods have been developed and commercialized for automated high throughput and simultaneous detection of multiple autoantibodies.<sup>24,25</sup> The MBFFI ANA test system is a fluorescent multiplex method that employs microsphere-based flow cytometric technology and the Tellgen Super Multiplex Immunoassay System, for simultaneous qualitative and semi-quantitative detection of IgG ANAs in human serum.<sup>26</sup> The MBC-IF is an automatic multi-liquid chip immunoassay; the chip was modified by amino, carboxyl, biotin, and other chemical groups, then the antigen was linked to the chip via chemical cross-linking, allowing the detection of autoantibodies. As the absence of performance assessment, we studied 184 patients enrolled in a clinical trial in terms of ANA-Profile-15S using two new platforms (MBFFI and MBC-IF) and conventional LIA; we analyzed the diagnostic and analytical performance, evaluated concordance rates and explored the diagnostic utility.

Our findings suggest that the MBFFI identified the most ANA-Profile-15S-positive outcomes (16.12%) but the positive rate of autoantibody did not differ significantly among the three techniques in ANA IIF-positive samples, with the exception of anti-ds-DNA, nucleosome, histone, and AMA M2. In terms of the positive rates of patients with various diseases, the anti-dsDNA and histone antibody

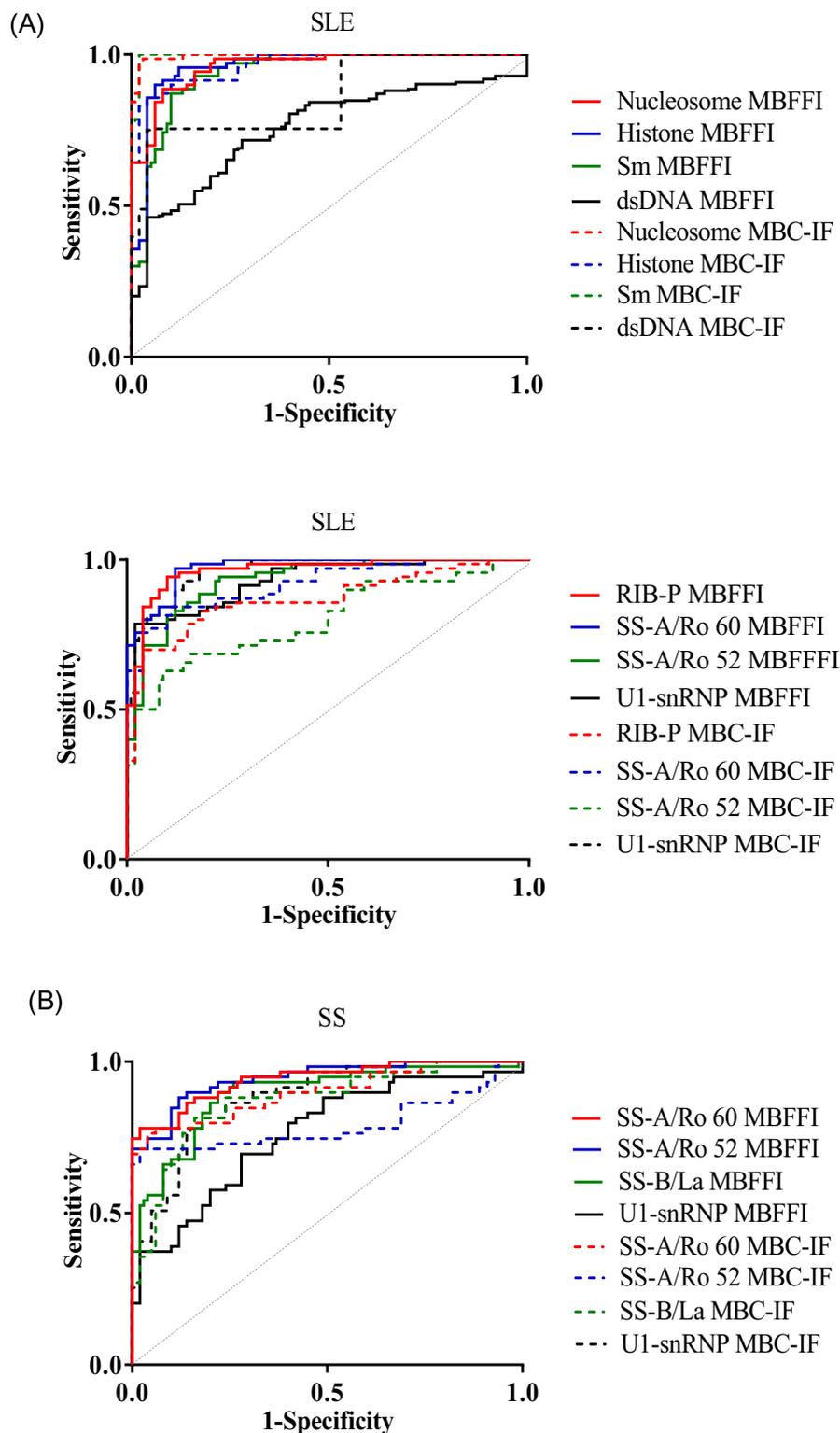
differed among the SLE samples. Notably, samples that were anti-nucleosome-positive on MBFFI and MBC-IF, but negative on LIA, accounted for 62.96% (17/27) of all discrepant results in patients with SLE. Furthermore, 64.71% (11/17) results were positive by CLIA re-analysis. In addition, samples that negative on MBFFI and LIA, but positive on MBC-IF, accounted for 64.71% (11/17) of all anti-histone discrepancies in patients with SLE. However, only one sample was positive on CLIA. Thus, the MBFFI efficiently detects almost all antibodies.

Overall, the kappa coefficients for each autoantibody varied. Frequently detected autoantibodies, including anti-RIB-P, SS-A/Ro 60, SS-A/Ro 52, SS-B/La, CENP-B, and Scl-70 showed moderate, strong, or almost perfect agreement among the three assays (kappa >0.60). Although there were only a few discrepant results, the low number of positive samples and low matched-positive rates yielded low kappa values for anti-PCNA, Jo-1, and Pm-Scl, which may be due to the fact that the kappa value is intrinsically influenced by the distributions of positive and negative samples.<sup>20,27</sup> A similar study compared the LIA-ANA-Profile-17S and EuroLine tests; the autoantibody detection patterns varied and overall agreement was moderate, in accordance with our findings.<sup>28</sup> Another study reported that an LIA and fluorescence enzyme immunoassay showed variability in

TABLE 4 Agreement between different test systems for the ANA-Profile-15S

	MBFFI versus MBC-IF				MBFFI versus LIA				MBC-IF versus LIA						
	PC (%)	NC (%)	TC (%)	Kappa	p value	PC (%)	NC (%)	TC (%)	Kappa	p value	PC (%)	NC (%)	TC (%)	Kappa	p value
ds-DNA	79.31	93.75	90.17	0.74	<0.001	51.72	96.59	85.47	0.55	<0.001	49.12	95.48	84.19	0.51	<0.001
Nucleosome	71.43	100.00	95.73	0.81	<0.001	37.14	99.50	90.17	0.49	<0.001	52.00	99.52	94.44	0.64	<0.001
Histone	89.47	93.49	93.16	0.64	<0.001	57.89	99.07	95.73	0.67	<0.001	35.48	99.01	90.6	0.46	<0.001
Sm	63.64	98.59	95.30	0.69	<0.001	77.27	95.28	93.59	0.66	<0.001	82.35	94.01	93.16	0.60	<0.001
PCNA	0	99.57	98.72	-0.01	0.926	0	99.57	98.72	-0.01	0.926	100.00	100.00	100.00	1.00	<0.001
RIB-P	91.30	97.63	97.01	0.84	<0.001	78.26	97.16	95.30	0.74	<0.001	80.77	98.56	96.58	0.82	<0.001
SS-A/Ro 60	100.00	86.25	90.60	0.80	<0.001	91.89	88.75	89.74	0.77	<0.001	84.38	96.38	91.45	0.82	<0.001
SS-A/Ro 52	94.38	99.31	97.44	0.95	<0.001	84.27	95.17	91.03	0.81	<0.001	88.24	95.30	92.74	0.84	<0.001
SS-B/La	77.27	98.11	96.15	0.77	<0.001	81.82	96.70	95.30	0.74	<0.001	80.95	96.24	94.87	0.71	<0.001
CENP-B	88.89	99.56	99.15	0.88	<0.001	100.00	98.67	98.72	0.85	<0.001	88.89	98.22	97.86	0.75	<0.001
Scl-70	70.00	99.11	97.86	0.73	<0.001	70.00	99.55	98.29	0.77	<0.001	88.89	100.00	99.57	0.94	<0.001
U1-snRNP	64.29	98.31	90.17	0.70	<0.001	75.00	97.75	92.31	0.78	<0.001	87.17	93.85	92.74	0.76	<0.001
AMA M2	47.83	99.53	94.44	0.60	<0.001	17.39	99.53	91.45	0.26	<0.001	33.33	99.55	96.15	0.45	<0.001
Jo-1	60.00	99.13	98.29	0.59	<0.001	40.00	100.00	98.72	0.57	<0.001	40.00	100.00	98.72	0.57	<0.001
Pm-Scl	0	99.14	98.29	-0.01	0.895	0	100.00	99.15	0	1.000	0	100.00	99.57	0	1.000

Abbreviations: PC, positive consistency; NC, negative consistency; TC, total consistency. The p values < 0.05 are shown in bold.



**FIGURE 2** ROC analysis of specific antibodies detected by MBFFI and MBC-IF assays for distinguishing patients in SLE (A), SS (B), IIM (C), PBC (D), and SSc (E) and controls. Full line indicated the AUC of MBFFI assay; dotted line indicated the AUC of MBC-IF assay

terms of their ability to detect different antibodies used to diagnose specific disease. However, variability was reduced, and diagnostic efficiency improved, by combining LIA-ANA-Profile-17S, ANA IIF screening, and EIA ENA.<sup>29</sup> Given the absence of anti-PCNA, Jo-1, and Pm-Scl antibodies in our cohort, further studies on evaluating the agreement of these antibodies are needed.

In this study, the best agreement in terms of the ANA-Profile-15S was that between the MBFFI and MBC-IF; the total agreement ranges was 90.17%–99.15%. The anti-SS-A/Ro 52 showed almost perfect agreement, and the anti-nucleosome, RIB-P, SS-A/Ro60, and CENP-B exhibited strong agreement. Nevertheless, the agreement between MBFFI and LIA, and MBC-IF and LIA, was less

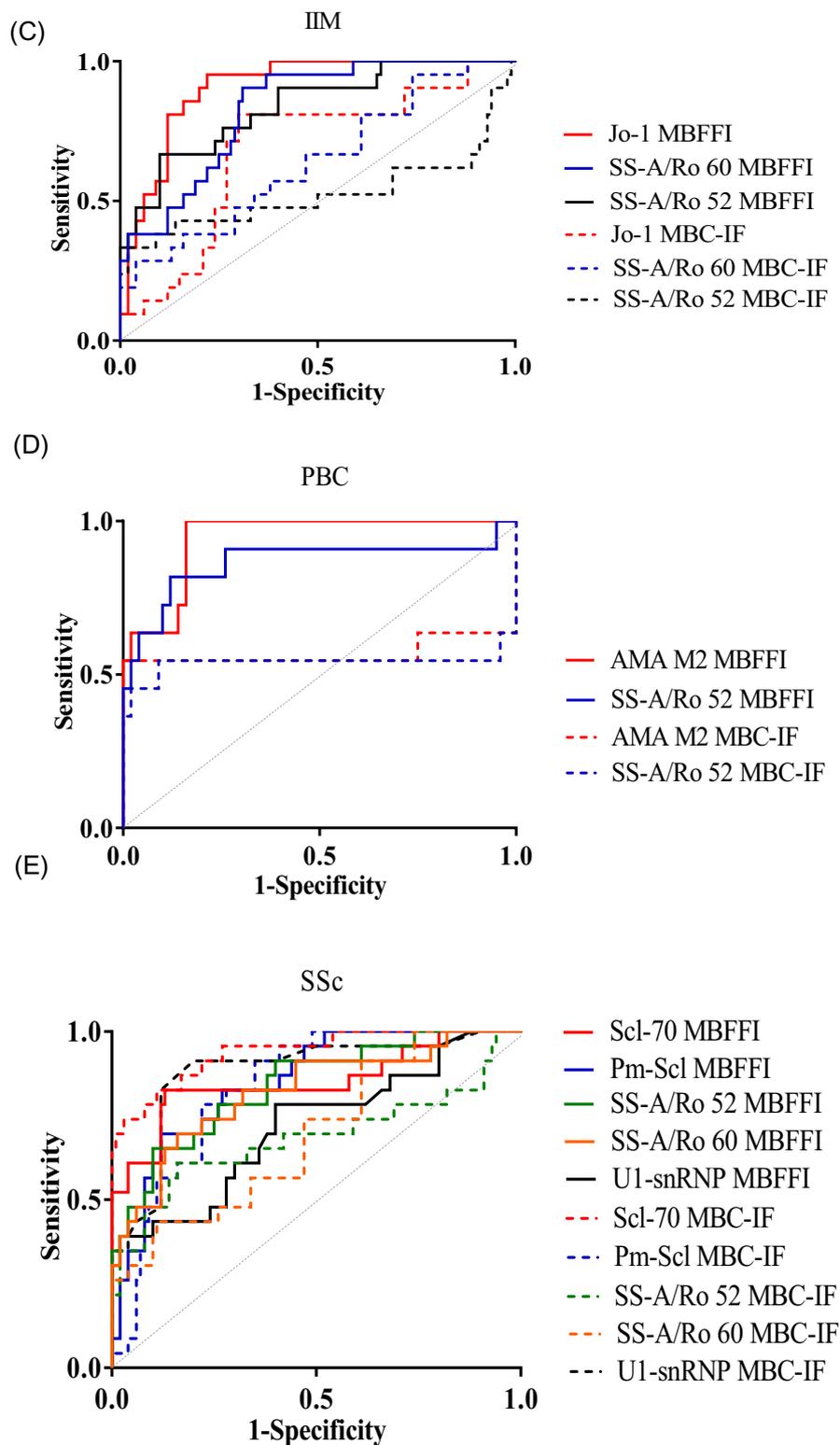


FIGURE 2 (Continued)

strong, probably reflecting technical differences associated with the antigen sources, reagents, and assay conditions. In addition, different conjugates were used for signal detection. On the other hand, LIA does not yield the quantitative values and results rely on manual interpretation, increasing the likelihood of human error.<sup>30</sup> Furthermore, the manufacturer-recommended cut-off values for the three assays differed, as heterogeneous reference sample were

used to derive those cutoffs in the original calculation. The lack of universal internal standards for calibration further increases the risk of discrepancies. Therefore, it is valuable to set up the standardized quantitative ANA-Profile assay to radically resolve the comparison problem in different assays. Based on these findings, we assessed the utility of the ANA-Profile-15S for differentiating AID patients from controls. Our findings demonstrated that ANA-Profile-15S

measured using automated MBFFI and MBC-IF were good biomarkers with larger AUCs in diagnosing AID. However, careful interpretation is required for the following reason: except for anti-dsDNA, the ANA analyses of MBFFI and MBC-IF are not quantitative; intensity units are used in the assays rather than universal internal units, and have not been approved by regulatory agencies. In addition, we found that the AUCs of MBC-IF and MBFFI for the most frequently detected antibodies showed differed significantly in terms of their ability to distinguish specific patients from controls. The AUC differences between MBFFI and MBC-IF further confirmed that consistency evaluations are necessary for clinicians. Considering the small samples in this study, further studies with larger cohorts are essential to carefully evaluate the clinical utility of the ANA-Profile for diagnosing specific diseases.

## 5 | CONCLUSION

This study provided new insight into ANA-Profile expression in AID patients and demonstrated differences among ANA assay kits in terms of the ability to detect ANA reactivity in the serum of patients with established disease. Assay performance depends on the disease being investigated and the associated specific antibodies. Compared to conventional LIA, the MBFFI, and MBC-IF was considered as high-throughput automatic platforms with higher detection rates for ANA-Profile. However, further efforts are needed to develop a standardized, quantitative ANA-Profile assay.

### AUTHOR CONTRIBUTIONS

YQ conducted the experiments, analyzed the data, and wrote the manuscript. CXF contributed to the collection of blood samples and experimental procedures. YLW participated in the collection of clinical data and preprocessing. MF and ZJL participated in the statistical analysis. XCZ and CG participated in revising of the manuscript. JL provided intellectual input and supervision throughout the study and made a substantial contribution to manuscript drafting. All authors contributed to the article and approved the submitted version.

### CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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