

Agreement of three commercial anti-extractable nuclear antigen tests: EUROASSAY Anti-ENA Profile, Polycheck Autoimmune Test and FIDIS^{IS} Connective Profile

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Background: Detection of antibodies to extractable nuclear antigens (ENAs) is needed for the diagnosis in systemic autoimmune diseases. In this study, we compared three reagents using line immunoblot assay (LIA) or multiplex bead immunoassay for detecting the anti-ENAs.

Methods: A total of 89 sera were tested by 3 different assays: EUROASSAY Anti-ENA Profile (Euroimmune, Germany), Polycheck Autoimmune Test (Biocheck GmbH, Germany), and FIDISTM Connective Profile (Biomedical Diagnostics, France). The following individual ENAs were investigated: Sm, SS-A (Ro), SS-B (La), Scl-70, Jo-1 and RNP. We reviewed medical records to investigate the discrepant results among three methods.

Results: Overall percent agreements were 96.1% between EUROASSAY Anti-ENA Profile and FIDISTM Connective profile; 90.4% between EUROASSAY Anti-ENA Profile and Polycheck Autoimmune Test using the manufacturers' cutoff; 96.4% between EUROASSAY Anti-ENA Profile and Polycheck Autoimmune Test using a upward cutoff; 90.4% between FIDISTM Connective profile and Polycheck Autoimmune Test the manufacturers' cutoff; and 96.4% between FIDISTM Connective profile and Polycheck Autoimmune Test a upward cutoff.

Conclusions: The three assays showed excellent agreement with each other. With appropriate cutoff, the all three assays for six of the anti-ENA tests investigated in this study can be used in clinical laboratories for detecting the anti-ENAs.

Key Words: Anti-extractable nuclear antigen antibodies, Antinuclear antibodies, Autoimmunity

Systemic autoimmune disorder is characterized by the presence of auto-antibodies for specific antigens of the nucleus, the cytoplasm or the nuclear membrane. Anti-nuclear antibody (ANA) tests are used to check for the presence of auto-antibodies in a systemic autoimmune disorder.^{1,2} A

systemic autoimmune disorder is distinctively related to more than one extractable nuclear antigen (ENA). Therefore, when ANA tests are positive in patients with suspected autoimmune disease, anti-ENA tests are performed to screen specific diseases. However, since it has been reported that

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anti-ENA antibodies were detected even in negative specimens from ANA tests, there is a compelling argument that the application of anti-ENA tests should be expanded.^{3,4}

Since the double immunodiffusion (DID) test was first used as an anti-ENA test in 1975, it has been considered the standard test method until now. However, with this method each antibody needs to be tested separately and since the response time is long, the test takes a long time to complete. In addition, it has disadvantages of low reproducibility, low sensitivity, and difficulty in determining, if the precipitation line is not formed clearly. Due to these drawbacks, many laboratories started using the enzyme linked immunosorbent assay, (ELISA) instead of the double immunodiffusion.¹ Recently, the line immunoblot assay (LIA) and multiplex bead immunoassay were introduced. These test methods are characterized by the ability to detect multiple antibodies simultaneously. Additionally, the required time for testing is short, which is useful in clinical laboratories, which routinely handle many specimens. Furthermore, their sensitivity and specificity are high and therefore have the advantage of deriving objective results, so they are being used instead of double immunodiffusion.⁵⁻⁷ Among them, the EUROASSAY Anti-ENA Profile (Euroimmun AG, Luebeck, Germany) Kit, which is one of the LIA methods, has been used in many clinical laboratories since it was introduced in 2008.⁸ The Polycheck Autoimmune Test (Biocheck GmbH,

Münster, Germany) is another LIA method, similar to the EUROASSAY Anti-ENA Profile, to analyze the strength of color reaction semiquantitatively through antibody-antigen binding using a designated scanner and program nitrocellulose with 20 antigens and 5 calibrators on a nitrocellulose membrane. The FIDIS™ Connective Profile (Biomedical Diagnostics, Marne la Vallée, France) Kit measures the fluorescence of antibodies attached to each bead by reacting antigens attached to beads with anti-ENA antibodies in serum. Types and amount of measured fluorescence are analyzed using the Luminex 100 System (Luminex, Austin, TX, USA) and its dedicated program to identify anti-ENA antibodies. This method has excellent sensitivity compared to other test methods.⁹ In addition, it can quantify 9 types of antibodies at the same time using a small amount of specimen and it can also process a large number of specimens since it uses 96-well microplates.

There are no studies in Korea evaluating the usefulness of anti-ENA antibody tests using the newly introduced Polycheck Autoimmune Test and FIDIS™ connective profile kit. Therefore, this study is to compare and evaluate the concordance rate after conducting the tests with three kinds of commercialized kits, the EUROASSAY Anti-ENA Profile, the Polycheck Autoimmune Test, and the FIDIS™ Connective profile kits using the same specimen.

MATERIALS AND METHODS

1. Specimens

The study was performed with specimens from 89 patients who were ordered to have anti-ENA antibody tests from January 2012 to December 2012. In subject groups, there were 22 males and 67 females and their average age was 45.2 years old. The clinical diagnosis of the subject groups was based on the medical records at the time the tests were requested. There were 33 patients with systemic rheumatic diseases and 56 patients with other diseases. The patients with systemic rheumatic diseases include 9 cases of systemic lupus erythematosus (SLE), 10 cases of Sjögren syndrome, 3 cases of Raynaud's syndrome, 4 cases of rheumatoid arthritis, 1 case of scleroderma, 2 cases of systemic sclerosis, 1 case of ankylosing spondylitis, 1 case of Behcet's syndrome and 2 cases of undifferentiated connective tissue disease. All of them were diagnosed based on the diagnostic criteria suggested by the American College of Rheumatology.^{10,11}

2. Detection of ENA Auto-antibodies

With the same specimens, tests were performed using EUROASSAY Anti-ENA Profile and Polycheck Autoimmune Test Kits, which both use the principle of LIA, and a third test the FIDIS™ Connective profile kit, which uses a multiplex bead immunoassay.

1) Detection of anti-ENA auto-antibodies using EUROASSAY Anti-ENA Profile Kit

The EUROASSAY Anti-ENA Profile test was performed according to the manufacturer's instructions. After placing the test strip inside each channel on the incubation tray, 1.5 mL of buffer was dispensed into each channel and pre-treated for 5 minutes by rocking on a shaker. After pretreatment, the serum of the patient was dispensed into each channel and left to react for 30 minutes at room temperature, maintaining it on the shaker. After washing three times for 5 minutes after the reaction, the alkaline phosphatase labeled anti-human IgG was dispensed into each channel and left to react for 30 minutes at room temperature. After washing it three times for 5 minutes again, the serum was left it to react for 10 minutes at room temperature in order for the brown control line to appear by adding the substrate. After 10 minutes, distilled water was added to stop the reaction and it was washed three times. Then the reaction strength was indicated from 0 to 4+ according to the positive acceptance criteria from the manufacturer using a dedicated scanner and analysis program. A strength of 1+ or over was rated as a positive.

2) Detection of anti-ENA auto-antibodies using Polycheck Autoimmune Test Kit

The test was performed using the Polycheck Autoimmune Test kit according to the manufacturer's instructions. 250 µL of stat solution was dispensed into the specimen application part of

the cassette and removed after 1 minute using the absorbent paper. According to the manufacturer's instructions, all reactions were carried out with constant shaking at 30 rpm at room temperature. After diluting the specimen 1:100 using the diluent, 250 μ L of diluted specimen was dispensed into the cassette and left to react for 45 minutes at room temperature. 250 μ L of wash buffer was added into the cassette and washed for 5 minutes and the wash process was repeated 2 times. After dispensing 250 μ L of antibodies into the cassette and leaving it to react for 30 minutes, it was washed using 1 mL of wash buffer. 250 μ L of substrate solution was added and left to react for 20 minutes in the dark. After washing using 1 mL of wash buffer and drying, the cassette was analyzed using a dedicated scanner and Biocheck Image Software. The positive acceptance criteria was determined when the criteria was set for 1+ or over and 2+ or over, respectively, according to the manufacturer's instructions.

3) Detection of anti-ENA auto-antibodies using FIDIS™ connective profile kit

The test was performed using a FIDIS™ connective profile kit according to the manufacturer's instructions. Briefly, 50 μ L of microspheres reagent and the patient's specimen diluted with 100 μ L diluent were added into each well of a 96 well plate and then incubated for 30 minutes at room temperature in the dark. It was washed 2 times using 300 μ L of wash buffer and then the surface was dried. 100 μ L of anti-human IgG con-

jugate was then added to each well and incubate for 30 minutes in the dark. After washing each well using 100 μ L of diluent, the results were obtained using MLX-BOOSTER software (Biomedical Diagnostics, Marne La Vallee, France) in the Luminex 100 system. According to the manufacturer's instructions, < 30 IU/mL was considered a negative, $30 - 40$ IU/mL was borderline, and > 40 IU/mL was considered to be a positive.

4) Comparison of the concordance rate between test methods

The concordance rate between test results was evaluated with 6 items for analysis including: anti-Sm, anti-RNP, anti-SS-A (Ro), anti-SS-B (La), anti-Scl-70, and anti-Jo-1, which were all included commonly in each kit. For the Polycheck Autoimmune Test kit that applies two positive criteria, the concordance rate based on each borderline value was evaluated.

5) Statistical Analysis

The concordance rate was calculated as the % of patient numbers that showed the same results from two methods. In order to evaluate the concordance rate between test methods, Kappa statistics was used.¹² For interpretation of Kappa values it was determined that 0.20 or less was poor, 0.21-0.60 was fair to moderate, 0.61 or over was in good agreement. $P < 0.05$ was determined as statistically significant. The SPSS 12.0 (SPSS Inc., Chicago, IL, USA) program was used for statistical analysis.

RESULTS

1. Concordance rate of the EUROASSAY Anti-ENA Profile kit and the Polycheck Autoimmune Test kit

The concordance rate of detecting ENA auto-antibodies using the EUROASSAY Anti-ENA Profile kit and the Polycheck Autoimmune Test kit was as follows when the positive reading criteria of the Polycheck Autoimmune Test kit was 1+ or over in accordance with the manufacturer's instructions: anti-Sm antibody 84.3% ($\kappa = 0.11$, $P = 0.027$), anti-SS-A antibody 88.8% ($\kappa = 0.74$, $P = 0.000$), anti-SS-B antibody 80.9% ($\kappa = 0.31$,

$P = 0.000$), anti-Scl-70 antibody 94.4% ($\kappa = 0.59$, $P = 0.000$), anti-Jo-1 antibody 96.6%, and anti-RNP antibody 95.5% ($\kappa = 0.59$, $P = 0.000$) (Table 1). If the positive reading criteria of the Polycheck Autoimmune Test kit was adjusted upward to 2+ or over, the concordance rate increased as follows: anti-Sm antibody 96.6% ($\kappa = 0.39$, $P = 0.000$), anti-SS-A antibody 95.5% ($\kappa = 0.88$, $P = 0.000$), anti-SS-B antibody 91.0% ($\kappa = 0.46$, $P = 0.000$), anti-Scl-70 antibody 98.9% ($\kappa = 0.88$, $P = 0.000$), anti-Jo-1 antibody 98.9%, and anti-RNP antibody 95.5% ($\kappa = 0.59$, $P = 0.000$) (Table 1).

Table 1. Comparison of the results for antibodies to extractable nuclear antigens by EUROASSAY Anti-ENA Profile and Polycheck Autoimmune Test (N = 89)

Cutoff of Polycheck Autoimmune Test = grade 1							
Antigen	Concordant results		Discrepant results		Concordance rate (%)	Kappa coefficient (95% CI)	P-value
	EUROASSAY-/ Polycheck-	EUROASSAY+/ Polycheck+	EUROASSAY-/ Polycheck+	EUROASSAY+/ Polycheck-			
Sm	74	1	14	0	84.3	0.11 (-0.32-0.53)	0.027
SSA	57	22	8	2	88.8	0.74 (0.58-0.89)	0.000
SSB	67	5	17	0	80.9	0.31 (0.01-0.60)	0.000
scl-70	80	4	5	0	94.4	0.59 (0.24-0.94)	0.000
Jo-1	86	0	2	1	96.6	N/A	N/A
nRNP	83	2	0	4	95.5	0.48 (-0.01-0.98)	0.000

Cutoff of Polycheck Autoimmune Test = grade 2							
Antigen	Concordant results		Discrepant results		Concordance rate (%)	Kappa coefficient (95% CI)	P-value
	EUROASSAY-/ Polycheck-	EUROASSAY+/ Polycheck+	EUROASSAY-/ Polycheck+	EUROASSAY+/ Polycheck-			
Sm	85	1	3	0	96.6	0.39 (-0.29-1.07)	0.000
SSA	64	21	1	3	95.5	0.88 (0.77-0.99)	0.000
SSB	77	4	7	1	91.0	0.46 (0.10-0.82)	0.000
scl-70	84	4	1	0	98.9	0.88 (0.66-1.11)	0.000
Jo-1	88	0	0	1	98.9	N/A	N/A
nRNP	83	2	0	4	95.5	0.48 (-0.01-0.98)	0.000

Abbreviations: CI, confidence interval; N/A, did not calculated; RNP, ribonucleic protein; Scl-70, scleroderma 70.

2. Concordance rate of EUROASSAY Anti-ENA Profile kit and FIDIS™ connective profile kit

The concordance rate of detecting ENA auto-antibodies using the EUROASSAY Anti-ENA Profile kit and the FIDIS™ connective profile kit was high as follows: anti-Sm antibody 96.6% ($\kappa = 0.39, P = 0.000$), anti-SS-A antibody 89.9% ($\kappa = 0.74, P = 0.000$), anti-SS-B antibody 95.5% ($\kappa = 0.69, P = 0.000$), anti-Scl-70 antibody 98.9% ($\kappa = 0.88, P = 0.000$), anti-Jo-1 antibody 98.9%, and anti-RNP antibody 96.6% ($\kappa = 0.65, P = 0.000$) (Table 2).

3. Concordance rate of Polycheck Autoimmune Test kit and FIDIS™ connective profile kit

The concordance rate of detecting ENA auto-antibodies using the Polycheck Autoimmune Test kit and the FIDIS™ connective profile kit was as follows when the positive reading criteria of Polycheck Autoimmune Test kit was set as 1+ or over in accordance with the manufacturer's instructions: anti-Sm antibody 84.3% ($\kappa = 0.11, P$

$= 0.026$), anti-SS-A antibody 85.4% ($\kappa = 0.65, P = 0.000$), anti-SS-B antibody 83.1% ($\kappa = 0.44, P = 0.000$), anti-Scl-70 antibody 93.3% ($\kappa = 0.54, P = 0.000$), anti-Jo-1 antibody 97.8%, and anti-RNP antibody 98.9% ($\kappa = 0.79, P = 0.000$) (Table 3). If the positive reading criteria of the Polycheck Autoimmune Test kit was adjusted upward to 2+ or over, the concordance rate increased as follows: anti-Sm antibody 96.6% ($\kappa = 0.39, P = 0.000$), anti-SS-A antibody 92.1% ($\kappa = 0.79, P = 0.000$), anti-SS-B antibody 93.3% ($\kappa = 0.66, P = 0.000$), anti-Scl-70 antibody 97.8% ($\kappa = 0.79, P = 0.000$), anti-Jo-1 antibody 100.0%, and anti-RNP antibody 98.9% ($\kappa = 0.79, P = 0.000$) (Table 3).

4. Comparison of Test Concordance Rate in Patient Group with Systemic Rheumatic Disease

The positive rates for all six of the anti-ENA antibodies using the EUROASSAY Anti-ENA Profile kit and the FIDIS™ connective profile kit in a patient group ($n = 33$) with systemic rheumatic disease were 87.9% (29/33) and 81.8% (27/33),

Table 2. Comparison of the results for antibodies to extractable nuclear antigens by EUROASSAY Anti-ENA Profile and FIDIS Connective Profile (N = 89)

Antigen	Concordant results		Discrepant results		Concordance rate (%)	Kappa coefficient (95% CI)	P-value
	EUROASSAY-/FIDIS	EUROASSAY+/FIDIS+	EUROASSAY-/FIDIS+	EUROASSAY+/FIDIS-			
Sm	85	1	0	3	96.6	0.39 (-0.29-1.07)	0.000
SSA	61	19	6	3	89.9	0.74 (0.58-0.90)	0.000
SSB	80	5	4	0	95.5	0.69 (0.40-0.99)	0.000
scl-70	84	4	1	0	98.9	0.88 (0.66-1.11)	0.000
Jo-1	88	0	0	1	98.9	N/A	N/A
RNP	83	3	0	3	96.6	0.65 (0.26-1.04)	0.000

Abbreviations: CI, confidence interval; N/A, did not calculated; RNP, ribonucleic protein; Scl-70, scleroderma 70.

respectively. The concordance rates for the two tests showed an excellent concordance rate as anti-Sm antibody 100.0% ($\kappa = 1.00$, $P = 0.000$), anti-SS-A antibody 93.9% ($\kappa = 0.88$, $P = 0.000$), anti-SS-B antibody 93.9% ($\kappa = 0.77$, $P = 0.000$), anti-Scl-70 antibody 100.0% ($\kappa = 1.00$, $P = 0.000$), anti-Jo-1 antibody 100.0%, and anti-RNP antibody 93.9% ($\kappa = 0.64$, $P = 0.000$) (Table 4).

When the borderline value of the Polycheck Autoimmune Test kit in the patient group with systemic rheumatic disease (n=33) was set as 1+ or over in accordance with the manufacturer's instructions, the concordance rate with the EUROASSAY Anti-ENA Profile reagents was: an-

ti-Sm antibody 72.7% ($\kappa = 0.13$, $P = 0.124$), anti-SS-A antibody 84.8% ($\kappa = 0.70$, $P = 0.000$), anti-SS-B antibody 66.7% ($\kappa = 0.28$, $P = 0.019$), anti-Scl-70 antibody 90.9% ($\kappa = 0.62$, $P = 0.000$), anti-Jo-1 antibody 100.0%, and anti-RNP antibody 90.9% ($\kappa = 0.37$, $P = 0.000$). The concordance rate with the FIDIS™ connective profile kit was found to be: anti-Sm antibody 72.7% ($\kappa = 0.13$, $P = 0.124$), anti-SS-A antibody 81.8% ($\kappa = 0.64$, $P = 0.000$), anti-SS-B antibody 72.7% ($\kappa = 0.39$, $P = 0.005$), anti-Scl-70 antibody 90.9% ($\kappa = 0.62$, $P = 0.000$), anti-Jo-1 antibody 100.0%, and anti-RNP antibody 97.0% ($\kappa = 0.65$, $P = 0.000$). When the borderline values for the Polycheck Autoimmune Test

Table 3. Comparison of the results for antibodies to extractable nuclear antigens by Polycheck Autoimmune Test FIDIS Connective Profile (N = 89)

Cutoff of Polycheck Autoimmune Test = grade 1							
Antigen	Concordant results		Discrepant results		Concordance rate (%)	Kappa coefficient (95% CI)	P-value
	Polycheck-/FIDIS-	Polycheck+/FIDIS+	Polycheck-/FIDIS-	Polycheck+/FIDIS+			
Sm	74	1	0	14	84.3	0.11 (-0.32-0.54)	0.026
SSA	57	19	2	11	85.4	0.65 (0.47-0.82)	0.000
SSB	66	8	1	14	83.1	0.44 (0.17-0.70)	0.000
scl-70	79	4	1	5	93.3	0.54 (0.18-0.90)	0.000
Jo-1	87	0	0	2	97.8	N/A	N/A
nRNP	86	2	1	0	98.9	0.79 (0.40-1.20)	0.000

Cutoff of Polycheck Autoimmune Test = grade 2							
Antigen	Concordant results		Discrepant results		Concordance rate (%)	Kappa coefficient (95% CI)	P-value
	Polycheck-/FIDIS-	Polycheck+/FIDIS+	Polycheck-/FIDIS-	Polycheck+/FIDIS+			
Sm	85	1	3	0	96.6	0.39 (-0.29-1.07)	0.000
SSA	64	21	1	3	92.1	0.79 (0.63-0.94)	0.000
SSB	77	4	7	1	93.3	0.66 (0.40-0.92)	0.000
scl-70	84	4	1	0	97.8	0.79 (0.50-1.08)	0.000
Jo-1	88	0	0	1	100.0	N/A	N/A
nRNP	83	2	0	4	98.9	0.79 (0.39-1.20)	0.000

Abbreviations: CI, confidence interval; N/A, did not calculated; RNP, ribonucleic protein; Scl-70, scleroderma 70.

Table 4. Comparison of the results for antibodies to extractable nuclear antigens by EUROASSAY Anti-ENA Profile and FIDIS Connective profile in the systemic autoimmune disorder (n = 33).

Antigen	Concordant results		Discrepant results		Concordance rate (%)	Kappa coefficient (95% CI)	P-value
	EUROASSAY-/ FIDIS	EUROASSAY+/ FIDIS+	EUROASSAY-/ FIDIS+	EUROASSAY+/ FIDIS-			
Sm	32	1	0	0	100.0	1.00 (1.00–1.00)	0.000
SSA	16	15	0	2	93.9	0.88 (0.72–1.04)	0.000
SSB	27	4	2	0	93.9	0.77 (0.45–1.08)	0.000
scl-70	30	3	0	0	100.0	1.00 (1.00–1.00)	0.000
Jo-1	33	0	0	0	100.0	N/A	N/A
RNP	29	2	0	2	93.9	0.64 (0.15–1.12)	0.000

Abbreviations: SLE, systemic lupus erythematosus; CI, confidence interval; N/A, did not calculated; RNP, ribonucleic protein; Scl-70, scleroderma 70.

kit were adjusted upward to 2+ or over, the concordance rate with EUROASSAY Anti-ENA Profile kit was: anti-Sm antibody 93.9% ($\kappa = 0.48$, $P = 0.001$), anti-SS-A antibody 93.9% ($\kappa = 0.88$, $P = 0.000$), anti-SS-B antibody 90.9% ($\kappa = 0.68$, $P = 0.000$), anti-Scl-70 antibody 100.0% ($\kappa = 1.00$, $P = 0.000$), anti-Jo-1 antibody 100.0%, and anti-RNP antibody 93.9% ($\kappa = 0.64$, $P = 0.000$). The concordance rates with FIDIS™ connective profile kit were found to be increased: anti-Sm antibody 93.9% ($\kappa = 0.48$, $P = 0.001$), anti-SS-A antibody 90.9% ($\kappa = 0.82$, $P = 0.000$), anti-SS-B antibody 90.9% ($\kappa = 0.67$, $P = 0.000$), anti-Scl-70 antibody 97.0% ($\kappa = 0.84$, $P = 0.000$), anti-Jo-1 antibody 100.0%, and anti-RNP antibody 97.0% ($\kappa = 0.65$, $P = 0.000$) (data not shown).

DISCUSSION

Detecting anti-ENA antibodies helps in screening diagnosis and prognosis determination for

various systemic autoimmune disorders.¹ It is also helpful to overcome some of the testing limitations such as nonspecific positive reactions and false negative rate of ANA. Due to the diagnostic values of these anti-ENA tests, the clinical demands for this level of accurate results are greatly increased.

Initial tests for anti-ENA antibodies were performed using double immunodiffusion at the early test development stage, but it had the disadvantages of low sensitivity and difficulty in reading.¹³ In the early 1980s, the ELISA method was developed, and while its sensitivity was high, it had the disadvantage of being able to test only one type of antibody at a time.⁶ Systemic autoimmune disorders have nonspecific symptoms but similar characteristics to each other, so for clinical doctors who choose the test based on clinical symptoms there was difficulty in choosing the appropriate tests. Therefore, it is essential to test various anti-ENA antibodies for diagnosis, leading to increases in test costs. In order to overcome

these problems, a method to test several antibodies simultaneously was developed.⁵⁻⁹

Among the next generation of tests, the LIA method was simple and had excellent sensitivity and specificity so it has been used in many clinical laboratories and its usefulness has been reviewed by many researchers.^{5,14} Lo ´pez-Longo et al. compared the LIA method with counter-immunoelectrophoresis (CIE), ELISA and immunoblotting for detecting antinuclear antibodies from the specimens of patients with rheumatic disease. They reported that when compared with ELISA, the greatest advantage of the LIA method was its ability to detect several antibodies simultaneously and inexpensive test costs.⁵

Multiplex bead immunoassay measures the fluorescence of antibody attached to each bead by reacting antigens attached beads with anti-ENA antibodies in serum. Types and amount of measured fluorescence are analyzed using Luminex 100 system (Luminex, Austin, TX, USA) and a dedicated program and it is able to identify anti-ENA antibodies, which are known to have very excellent specificity. Vercammen et al. reported that in the study of evaluating the anti-ENA detection abilities in various test methods with patients with connective tissues disease, the concordance rate of multiplex bead immunoassay and double immunodiffusion, which is a standard method of anti-ENA detection, was as high as 95-100% and its diagnostic specificity was excellent at 88-100%.¹⁵

The concordance rate analysis of test results used in this study, was highest between

EUROASSAY Anti-ENA Profile reagents and FIDIS™ connective profile reagents as 89.9-98.9% (Table 2). The Polycheck Autoimmune Test kit showed relatively high sensitivity and there were many cases showing only positive results in the Polycheck Autoimmune Test kit. Therefore, when adjusted to higher than the borderline value of the Polycheck Autoimmune Test kit recommended by the manufacturer, the concordance rate with EUROASSAY Anti-ENA Profile reagents increased from an average of 90.1% to 96.1%, and the concordance rate with the FIDIS™ connective profile kit increased from an average of 90.4% to 96.4%.

The many existing studies have reported various sensitivities and specificities for each test kit. The causes of discrepancy in results between kits were the degree of antigen's specificity being used, the binding strength of different antibodies, differences in source of antigens and the use of different borderline values for each test kit.^{9,10,15}

In this study, there were many cases that the Polycheck Autoimmune Test kit alone showed positive. In previous studies, the enzyme immunoassay also had been pointed out as drawbacks in anti-ENA tests since it leads to confusion in diagnosis due to high sensitivity. In this study, when the manufacturer's reading criteria was followed, it was confirmed that a number of false positive results were obtained. To clinically use the anti-ENA antibody tests for the diagnosis of autoimmune diseases, high specificity is required rather than high sensitivity. Therefore, in labo-

ratories using the Polycheck Autoimmune Test kit, it may be necessary to adjust the borderline value upward after consultation with the clinician. In addition, anti-Sm antibodies showed that the concordance rate was high but the Kappa value was 0.02 or less, therefore care must be taken to interpret the results.

The biggest limitation of this study was that it was not compared with the double immunodiffusion test, which is a standard test. The double immunodiffusion test is recognized as a standard test due to high specificity despite several disadvantages. In addition, there was a limit in determining the clinical sensitivity and specificity because sufficient number of positive specimens could not be obtained for each item due to the lack of specimens.

In conclusion, if the EUROASSAY Anti-ENA Profile, the FIDIS™ connective profile, and the Polycheck Autoimmune Test kit are used when the appropriate borderline value is set, it is thought that it can be applied to patient's specimen since the concordance rate between each reagent is excellent. In the future, the results of this study can be continued by obtaining positive specimens from patients with various systemic autoimmune disorders.

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