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ORIGINAL ARTICLE

Improved accuracy in DFS pattern interpretation using a novel HEp-2 ELITE system

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Abstract

Introduction/objectives Accurate interpretation of DFS70 (dense fine speckled 70) and mixed antinuclear antibodies (ANAs) patterns can be challenging using conventional HEp-2 immunofluorescence (IIF) method. We evaluated a novel HEp-2 IIF substrate (HEp-2 ELITE/DFS70-KO) composed of a mixture of engineered HEp-2 devoid of the DFS70 autoantigen and conventional HEp-2 cells. The study assessed the utility of the new substrate in ANA screening and its advantages.

Method One thousand and five consecutive routine samples sent for ANA screening were tested on both standard HEp-2 and the HEp-2 ELITE DFS70 KO substrates (ImmuGlo ANA HEp-2 and HEp-2 ELITE/DFS70-KO, Trinity Biotech, Buffalo, NY). Anti-DFS70 antibody specificity was additionally determined by immunoblot (IB). Clinical and serological data were included in the analysis of the overall impact of the novel HEp-2 substrate on DFS pattern interpretation.

Results Of the 22 cases suspected as positive for DFS pattern alone or in combination with homogeneous or speckled patterns on conventional HEp-2 cells, 17 were interpreted with a higher accuracy using the new HEp-2 ELITE method as positive for DFS70 (monospecific DFS70 (10), mixed DFS70 (7)), speckled (3), and DFS (2) patterns.

Conclusions The new substrate was not only useful in deciphering unclear mixed ANA patterns but also highly sensitive in detecting DFS70 pattern in comparison to the DFS70 positivity obtained using IB.

Keywords Anti-DFS70 · Antinuclear antibodies · Autoantibodies · Autoimmune disease · HEp2-cells

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Introduction

Presence of circulating antinuclear antibodies (ANAs) in patient serum is widely regarded as the hallmark of systemic autoimmune rheumatic disease [1]. A request for a serological ANA screening test is usually a first step for the laboratory diagnosis.

Although various methods are available for screening of ANAs, indirect immunofluorescence (IIF) method using HEp-2 substrate remains the most widely prevalent method [2–4].

HEp-2 cell substrates are versatile due to their ability to present large proportion of the human cell proteome that contains a majority of the nuclear and cytoplasmic autoantigens [3].

ANA testing on HEp-2 IIF is highly sensitive and presents multiple fluorescent patterns depending on the presence of

various autoantibodies [5]. IIF method provides not only a qualitative result for the presence of ANAs but also a specific pattern directing the selection of an appropriate solid phase confirmatory assay. Despite these advantages, accurate interpretation of IIF pattern can be challenging in certain cases. A recently described dense fine speckled (DFS) pattern in particular can be difficult to interpret due to its similarity to homogeneous-speckled mixed patterns [6–8]. International consensus on ANA patterns committee has described this pattern as AC-2 which is manifested by the presence of heterogeneous fine speckled pattern spread uniformly across the nucleoplasm and the mitotic chromatin [2, 9, 10].

Several studies have reported a high prevalence of this pattern in various disease populations as well as healthy blood donor cohorts [7, 11, 12].

Mariz et al. [13] reported that the likelihood of AARD is low in ANA-positive individuals who present a DFS pattern on HEp-2 test. Therefore, anti-DFS70 antibodies have been proposed as a potential biomarker to discriminate ANA-positive HI from ANA-positive patients with AARD potentially resulting in considerable cost-savings [14–21].

Based on these reports, anti-DFS70 antibodies represent a novel prognostic biomarker that can aid in the interpretation of unexplained positive IIF ANA test results, especially considering the shift in patterns of referrals for ANA testing [21]. Recently, Infantino et al. [11, 22] reported a high prevalence of the anti-DFS70 antibodies in the UCTD patients and further proposed them as novel marker in the evolution of UCTD to CTD. DFS pattern predominantly is due to an autoimmune response towards an autoantigen referred to as LEDGF (lens epithelium–derived growth factor) or PSIP1 gene product which migrates as a 70 kDa band on western blots [10, 23]. Due to this reason, DFS pattern resulting from anti-LEDGF/PSIP1 autoantibodies is specifically referred to as DFS70 pattern.

Multiple studies have highlighted the importance of accurately identifying the DFS70/DFS pattern and differentiating it from homogeneous or speckled or mixed homogeneous-speckled patterns which are associated with systemic autoimmune diseases [7, 24–27].

Variations in the presentation of DFS pattern on HEp-2 cells by various manufacturers [19, 28] can further increase the challenges associated with its accurate interpretation [25, 29, 30].

DFS70 autoantibodies have been found alone (monopositive) as well as co-occurring with other disease-associated ANAs [7, 24].

This phenomenon further affects the accuracy of the ANA screening step and significantly impacts the subsequent reflex testing algorithms [7].

In this study, we evaluated a novel IIF HEp-2 ELITE/DFS70-KO substrate composed of a mixture of natural unmodified HEp-2 cells and engineered HEp-2 cells devoid of a functional PSIP1/LEDGF/DFS70 gene [6, 7].

Material and methods

Patients

One thousand and five consecutive samples received by the Laboratory of Immunology and Allergy of San Giovanni di Dio Hospital for routine screening of ANAs were evaluated IIF at 1:80 dilution using a conventional HEp-2 substrate (ImmuGlo™ ANA HEp-2 Substrate, Trinity Biotech, Buffalo, NY) and a novel engineered HEp-2 substrate (HEp-2 ELITE/DFS70-KO, Trinity Biotech, Buffalo, NY).

All samples were tested for anti-dsDNA and anti-ENA (extractable nuclear antigens) by BioPlex 2200 multiplex flow immunoassay (MFI). Anti-DFS pattern on HEp-2 cells was tested by DFS70 line immunoassay (LIA) (Euroimmun AG, Luebeck, Germany). Clinical and serological information were included in analyzing the overall impact of the novel HEp-2 DFS70-KO substrate on DFS pattern interpretation. All patients gave their informed consent to this retrospective study according to the Declaration of Helsinki and to Italian legislation (Authorization of the Privacy Guarantor No. 9, 12 December 2013).

Methods

Immunofluorescence assay on HEp-2 cells

Conventional HEp-2 substrate Diluted serum samples are incubated on fixed monolayer cultures of HEp-2 cells on glass slide wells. Bound autoantibodies are retained and unbound serum components are washed off. Subsequent incubation with anti-human IgG antibodies coupled to FITC (fluorescein isothiocyanate) and washing step retains and labels the bound autoantibodies of type IgG which can be visualized under a fluorescent microscope. Presence of fluorescent signal above background levels and a distinct pattern indicates a positive result.

HEp-2 ELITE/DFS70-KO substrate This substrate uses an identical principle and procedure to those of the conventional HEp-2 IIF method. It is composed of a mixture of approximately 10% conventional HEp-2 that preserves the entire proteome and 90% engineered HEp-2 cells that are devoid of the autoantigen LEDGF/PSIP1. The engineered cells are not able to present any binding sites to the anti-DFS70 antibodies specifically targeted to the epitopes on LEDGF/PSIP1 antigen. A

distinct difference in resulting pattern is only observed in case of patients/samples positive for anti-LEDGF/PSIP1 antibodies which produce a DFS (AC-2) reaction more specifically referred to as DFS70. In case of a reaction with anti-DFS70 antibody positive serum, 10% of the cells on slide well are labeled with a distinct DFS70/DFS/AC-2 pattern and the rest of the 90% cells (engineered HEp-2) are negative in monospecific cases. If it were a DFS pattern resulting from binding of unknown autoantibody specificity, all 100% cells (both unmodified/wild type and PSIP1−/− cells) will show the AC-2 pattern. Due to this design, Elite method is able to not only distinguish the dense fine speckled 70 pattern from the DFS pattern of unknown autoantibody specificity with greater ease but also is able to distinguish monospecific DFS70 patterns with greater accuracy while simultaneously revealing any underlying co-occurring ANA patterns that may be concealed on a conventional HEp-2 [6, 7]. Reader used "/" when two or more patterns were not clearly distinguished for a sample, and "+" was used when two or more co-occurring patterns were recognized with confidence.

Anti-DFS70 antibodies determined by line immunoassay

All the 22 DFS-positive samples were tested by the EUROLINE ANA Profile 3 lineblot, Euroimmun (Ro60, Ro52, La, RNP, Sm, Scl70, CENP-B, PM-Scl, Jo-1, PCNA, dsDNA, nucleosomes, histones, ribosomal P-proteins, AMA M2, DFS70) using a full-length DFS70 antigen [31]. Sera were incubated in accordance with the standard protocol provided by the manufacturer (30 min in serum, 30 min in anti-human immunoglobulin G/alkaline phosphatase, and 10 min in 5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium substrate). Reaction intensities expressed in grayscale units were automatically evaluated using commercially available EUROLineScan software (Euroimmun AG, Luebeck, Germany).

Anti-dsDNA and anti-ENA analyzed with BioPlex2200 ANA screen

BioPlex® 2200 (Bio-Rad, Hercules, CA) system is an automated analyzer that uses multiplex bead technology (Luminex, Austin, TX, USA) to simultaneously detect antibodies to several antigens in a single tube. The BioPlex® 2200 ANA Screen kit is intended for the qualitative screening of anti-nuclear antibodies (ANAs), the quantitative detection of antibodies to dsDNA, and the semi-quantitative detection of ten separate antibodies (chromatin, ribosomal P, SS-A, SS-B, Sm, SmRNP, RNP, Scl-70, Jo-1, and centromere B) in human serum and/or EDTA or heparinized plasma. Results are expressed in IU/mL for anti-dsDNA and antibody index (AI) for ANA antibodies, and the results' interpretations established by the manufacturer are 5–9 IU/mL (indeterminate) and ≥ 10 IU/mL (positive) for dsDNA and ≥ 1.0 AI (positive) for ANA antibodies [32].

Statistical analysis

Kappa statistic was used to measure the degree of agreement between raters for HEp-2 Elite method. A kappa statistic of <0.20, <0.40, <0.60, and <0.80 indicates poor, fair, moderate, and good agreement levels between raters, respectively. Kappa statistic between 0.80 and 1 indicates very good agreement. Inter-rater agreement was calculated using Analyze-it for Microsoft Excel 4.95 (Analyze-it Software, Ltd. http://www.analyse-it.com).

Results

Out of the 1005 consecutively tested routine ANA screening samples, 22 (2.2%) cases were suspected to have a DFS pattern using conventional HEp-2 IIF method (Table 1). The majority (16 out of 22) of these samples were also suspected to present with a homogeneous or speckled or mixed homogeneous-speckled patterns. All of these samples suspected to present monospecific or mixed DFS or mixed non-DFS patterns were evaluated using a novel HEp-2 ELITE DFS70 KO substrates by three expert readers. When 2 out of the 3 readers matched the result, a consensus result was assigned. Six out of the 22 samples with monospecific or mixed DFS pattern suspicion had an AARD (antinuclear antibody–associated rheumatic diseases) association, while the other 16 samples had a clinical diagnosis spanning many autoimmune diseases and non-autoimmune states such as arthritis and pregnancy.

Using the HEp-2 ELITE system, 17 out of the 22 cases were determined as positive for DFS70 antibodies (monospecific DFS70 (10), mixed DFS70 (7)); of the remaining 5 cases, 3 had a speckled pattern and 2 had a DFS pattern. A total of 6 samples were diagnosed to have an AARD association. Four out of these 6 samples were determined to have a DFS70 pattern co-occurring with a speckled pattern (sample # 2, 9, 16, and 17; Table 1).

Four out of the 6 AARD-associated cases were confirmed as positive for antibodies to one or more of the anti-ENAs (anti-SSA52, anti-SSA60, and/or anti-SSB) or anti-dsDNA by LIA, and two others (sample # 16 and 17) were negative for anti-ENA and anti-dsDNA but presented with a DFS or DFS/H/S IIF pattern by conventional HEp-2 cells, and a mix of DFS70 and speckled patterns using HEp-2 ELITE substrate.

In the absence of a gold standard result for the DFS/DFS70 suspicion, a consensus IIF pattern was determined based on

Table 1 Clinical and serological analysis of 22 ANA-positive cases suspected to have a DFS pattern

Consensus ANA pattern			Anti-ENA/dsDNA profile		Diagnosis/suspicion	
Sample #	Conventional HEp-2	HEp-2 Elite	Bio-Plex 2202	IB/LIA	AARD diagnosis	Disease state suspicion
1	DFS	DFS70	Neg	DFS70, Histone	Yes	UCTD
2	DFS/S	S + DFS70	SSA60	SSA60, dsDNA (+/−)	Yes	SLE
3	DFS/H/S	S + DFS70	SSA60	DFS70	No	Celiac disease
4	DFS/H/S	DFS70	Neg	Neg	No	AARD suspect
5	DFS/H	DFS70	Neg	Neg	No	AARD suspect
6	DFS/S	S	SSA60, SSA52	SSA60, SSA52	Yes	SLE
7	DFS/S	DFS70	Neg	Neg	No	Psoriatic Arthritis
8	DFS	DFS	Neg	Neg	No	AARD suspect
9	DFS/H/S	S + DFS70	SSA60, SSB	SSA60, SSA52, SSB, DFS70	Yes	Sjogren's syndrome
10	DFS/S	S	Neg	Neg	No	AARD suspect
11	DFS/S	DFS70	Neg	Neg	No	Diabetes
12	DFS/S	S + DFS70	Neg	Neg	No	Nephropathia
13	DFS	DFS70	Neg	DFS70	No	Celiac disease
14	DFS	DFS70	Neg	DFS70	No	Pregnancy
15	DFS/H/S	DFS70	Neg	DFS70	No	F. Raynaud
16	DFS	S + DFS70	Neg	DFS70	Yes	UCTD
17	DFS/H/S	S + DFS70	Neg	DFS70	Yes	UCTD
18	DFS/H/S	DFS70	Neg	Neg	No	RA
19	DFS	DFS70	Neg	DFS70	No	Hypothyroidism
20	DFS/H/S	S	Neg	Neg	No	Arthritis wrist
21	DFS/H/S	S + DFS70	Neg	Neg	No	Hypothyroidism, arthralgia
22	DFS/H/S	DFS	Neg	Neg	No	RA

IB, immunoblot; *LIA*, line immunoassay; *ANA*, antinuclear antibodies; *AARD*, ANA-associated rheumatic disease; *DFS*, dense fine speckled; *DFS70*, dense fine speckled 70; *S*, speckled; *H*, homogeneous pattern; "/" indicates either-or unclear pattern, "+" indicates co-occurring patterns

the majority result of 3 interpretations (Table 2). As shown in Table 2, reader 1 determined all 22 samples as DFS70 (with or without co-occurring patterns), but readers 2 and 3 distinguished DFS from DFS70 patterns.

Agreement analysis between the readers for patterns reported as DFS/DFS70, monospecific DFS/DFS70, or mixed DFS70 cases was performed using Cohen's Kappa coefficient (Table 3). For monospecific DFS70 detection using the ELITE method, the Kappa values ranged from 0.51 to 0.58. Kappa agreement was lowest for mixed DFS70 cases (0.05–0.21).

Of the 22 evaluated cases, two were determined as DFS pattern on the ELITE method (Table 4). Both of these were described as DFS or DFS/H/S patterns for the consensus result on the conventional substrate (Table 4). Out of the 17 samples that were identified as positive for DFS70 pattern using the ELITE method, ten were determined as monospecific for anti-DFS70 antibodies. Five (50%) out of these ten cases were confirmed as positive for anti-DFS70 antibodies using LIA. Out of the 7 cases that were mixed DFS70 pattern by the ELITE method, 4 (57.1%) were confirmed as anti-DFS70 positive by LIA. Three out of the seven (42.9%) mixed anti-DFS70 cases with co-occurring speckled pattern as determined by the ELITE method were confirmed as anti-ENA/-dsDNA positive by LIA and/or BioPlex2200. Out of the 17 cases identified as positive for anti-DFS70 antibodies (monospecific and mixed), 9 (52.9%) cases in total were confirmed by LIA and 8 (47.1%) cases could not be confirmed by LIA. All 3 unclear samples by conventional HEp-2 that were determined in consensus as DFS/speckled pattern or DFS/homogeneous/speckled pattern were reported as positive for speckled pattern using the ELITE substrate. All three cases identified as speckled pattern using the ELITE method were negative by LIA for anti-DFS70 antibodies. One of the speckled samples (sample # 6; Table 1) previously diagnosed with SLE and suspected as DFS/speckled pattern by conventional HEp-2 and positive for anti-SSA60 and anti-SSA52 by LIA/BiopPlex2200 was determined as speckled pattern by 2 out of the three readers using the ELITE method.

Despite the variability in reporting the anti-DFS/DFS70 antibody positivity across the readers using the ELITE method, readers 1, 2, and 3 determined 9, 14, and 8 cases,

Table 2 IIF results by three readers using the HEp-2 ELITE DFS70 KO ELITE method and derived consensus

Sample No.	Reader 1	Reader 2	Reader 3	Consensus
1	DFS70	DFS70	DFS70	DFS70
2	S + DFS70	S + DFS70	S	S + DFS70
3	S + DFS70	S + DFS70	S + DFS70	S + DFS70
4	DFS70	DFS70	DFS	DFS70
5	DFS70	DFS70	DFS/H	DFS70
6	S + DFS70	S	S	S
7	DFS70	DFS	S	DFS70
8	S + DFS70	DFS	DFS	DFS
9	S + DFS70	DFS	S + DFS70	S + DFS70
10	S + DFS70	S	S	S
11	DF570	DFS	S	DFS70
12	S + DFS70	S + DFS70	S	S + DFS70
13	DFS70	DFS70	DFS70	DFS70
14	DFS70	DFS70	DFS70	DFS70
15	DFS70	DFS70	DFS70	DFS70
16	S + DFS70	S + DFS70	S + DFS70	S + DFS70
17	S + DFS70	S + DFS70	S + DFS70	S + DFS70
18	S + DFS70	DFS	H/S	DFS70
19	DFS70	DFS70	DFS70	DFS70
20	S + DFS70	S	S	S
21	S + DFS70	DFS	S	S + DFS70
22	S + DF570	DFS	DFS	DFS

DES, dense fine speckled; *DFS70*, dense fine speckled 70; *S*, speckled; *H*, homogeneous pattern; "1" indicates either of the patterns, "+" indicates co-occurring patterns. Consensus determination is challenging in cases where reader response was varied (examples: sample nos. 7, 11, 18, and 21)

respectively, as monospecific for anti-DFS70 antibodies (Tables 2 and 5). Reader 1 determined all 22 (100%) cases as positive for DFS70 or DFS pattern using the ELITE method, but only 9 (40.9%) of these were positive by LIA. On the other extreme, reader 3 determined only 13 cases (59%) as positive for DFS/DFS70 pattern (8 monospecific DFS70, 5 mixed DFS70), out of which 9 (81.8%) were confirmed by LIA (Table 5).

Table 4 Cross tabulation of IIF patterns for consensus pattern using ELITE vs. conventional HEp-2 methods. $N = 22$. Number of cases positive for anti-DFS70 by IB is indicated in parenthesis

		Pattern on conventional HEp-2			
Consensus pattern on HEp-2 Elite		DFS	DFS/H	DFS/S	DFS/H/S
	DFS	1 (0)	0(0)	0(0)	1(0)
	DFS70	4 (4)	1(0)	2(0)	(1)
	DFS7O + S	1 (1)	0(0)	0(0)	4 (3)
	S	0 (0)	0 (0)	2 (0)	1 (0)

Discussion

Despite the advances in multiplex solid phase testing approaches for autoantibodies detection, IIF remains as one of the dominant methods for their screening. Interpretation of ANA patterns requires experience and in certain cases such as DFS/DFS70 pattern, it can pose a challenge even for the more experienced readers. In this study, 2.2% ($n = 22$) of the 1005 ANA screening samples were suspected as having a DFS/DFS70 pattern using a conventional HEp-2 IIF method. Out of these, only 6 cases had a monospecific DFS pattern by consensus. All the three readers participating in this study had difficulty in the interpretation of a specific ANA pattern for 16 out of the 22 cases (Table 1). In contrast, by using the novel HEp-2 ELITE substrate, all three readers correctly distinguished between monospecific DFS70, mixed DFS70, and disease-associated ANA (speckled and homogeneous) patterns (Table 2) with the exception of two misinterpretations by reader 3. Seventeen (77.3%) out of the 22 samples were presented with a DFS70 pattern with or without a co-occurring pattern as per consensus result using HEp-2 ELITE. Two samples (#8 and #22) were interpreted as having DFS pattern that was not associated with PSIP1/LEDGF. Both of these samples were not only negative for antibodies to DFS70 but also for anti-ENAs and anti-dsDNA antibodies by solid phase assays.

Absence of the consensus by using the conventional HEp-2 substrate, in picking a specific pattern with confidence by

Table 3 Agreement analysis across three readers for DFS and related patterns is presented. Average (%) agreement for DFS/DFS70, monospecific DFS/DFS70, and mixed DFS7 patterns across 3 readers were 72.7%, 75.8%, and 66.6%, respectively

	DFS/DFS70		Monospecific DFS/DFS70		Mixed DFS70	
	(*n*), % agreement, (95% CI)	Kappa, (95% CI)	(*n*), % agreement, (95% CI)	Kappa, (95% CI)	(*n*), % agreement, (95% CI)	Kappa, (95% CI)
Reader 1 vs reader 2	(19), 86.4, (66.7–95.3)	0.36, (− 0.16–0.88)	(17), 77.3, (56.6–89.9)	0.58, (0.29–0.88)	(14), 63.6, (43.0–80.3)	0.21, (0.08–0.63)
Reader 2 vs reader 3	(16), 72.7, (51.8–86.8)	0.43, (0.11–0.75)	(16), 72.7, (51.8–86.8)	0.51, (0.22–0.80)	(18), 81.8, (61.5–92.7)	0.2, (0.06–0.92)
Reader 3 vs reader 1	(13), 59.1, (38.7–76.7)	0.11, (− 0.10–0.32)	(17), 77.3, (56.6–89.9)	0.53, (0.18–0.89)	(12), 54.5, (34.7–73.1)	0.05, (− 0.10–0.49)

Table 5 Cross tabulation of IIF patterns by the HEp-2 ELITE method along with DFS70 positivity by LIA (in parenthesis) for each reader and consensus is summarized.

HEp-2 Elite DFS70 KO substrate					
# of cases with below criterion	Conventional HEp-2	Reader 1	Reader 2	Reader 3	Consensus by Elite
DFS70/DFS suspicion	22 (9)	22 (9)	19 (9)	13 (9)	19 (9)
Monospecific DFS70/DFS suspicion	Unclear	9 (5)	14 (6)	8 (5)	13 (5)
Mixed DFS70/DFS suspicion	Unclear	13 (4)	5 (3)	5 (4)	7 (4)
Non DFS70/DFS suspicion	Unclear	0 (0)	3 (0)	8 (0)	3 (0)

three readers, prevented performing an inter-rater agreement analysis for this method. This difficulty in interpretation was ameliorated for all three readers when the HEp-2 ELITE method was used, which enabled inter-rater agreement analysis (Table 3). The kappa agreement was highest for monospecific DFS70 pattern and lowest for mixed DFS70 cases. Visual interpretation of IIF results is highly subjective, especially when determining if a weak positive reaction is above or below the cut-off intensity. Only ten cases (45.5%) were determined as monopositive for DFS70 pattern by consensus, out of which 5 were confirmed as positive by LIA. Three (one confirmed by LIA) out of these 10 monopositive cases were suspected or diagnosed with an AARD. Seven out of the 22 cases were determined as positive for both DFS70 and speckled pattern using the HEp-2 ELITE method. Five out of these 7 cases were confirmed for DFS70 positivity by LIA, and 4 out of these 7 cases were associated with an AARD suspicion or diagnosis. HEp-2 ELITE method provided additional value during the ANA screening step by allowing the determination of mixed patterns with improved confidence. As described in Table 4, for all of the cases determined as DFS/H/S or DFS/S or DFS/H patterns using conventional HEp-2 cells, a specific consensus pattern was derived using the HEp-2 ELITE method. All of the samples determined as speckled pattern by HEp-2 ELITE were also negative for anti-DFS70 antibodies by LIA. Using the conventional HEp-2, it was difficult to distinguish between monospecific or mixed DFS70/DFS pattern (Table 5). However, this was possible for all three readers using the HEp-2 ELITE method.

Results from this study indicate that this new generation of HEp-2 cells may aid in easier interpretation of DFS70 patterns and further improve accuracy of ANA screening method. Results from this cohort also indicate that monospecific anti-DFS70 positivity cannot completely rule out an AARD.

In conclusion, factors such as mixed patterns (DFS/DFS70 co-occurring with another pattern), user subjectivity, and quality of IIF substrates may negatively impact the accuracy of IIF result. The new HEp-2 ELITE IIF method shows promise in unraveling the mixed ANA patterns, improving the accuracy of DFS70 pattern recognition and decreasing the gap between results by IIF and solid phase assays, especially for DFS70 pattern.

Compliance with ethical standards

Disclosures None.

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