Evaluation of diagnostic performance of a new multiparametric microblot array-based immunoassay for anti-DFS70 detection.

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Background and aims

The ICAP classified the nuclear speckled pattern with stained metaphase plate as dense fine speckled (AC-2) or fine speckled (AC-30). The aim of this study was to evaluate the ability of a new microblot array (MBA) to correctly identify samples as anti-DFS70 positive (AC-2) or negative (AC-30) and compare it to a fluorescence enzyme immunoassay (FEIA) and immunoblotting (IB).

Methods:

The study included 96 consecutive samples showing a nuclear speckled with mitotic plate pattern on HEp-2 indirect immunofluorescence assay, that were analyzed using three different methods to detect anti-DFS70 and anti-ENA antibodies: **dot blot ANA+DFS70** (Sm, RNP, Sm/RNP, SSa-60kD, SSB, Scl 70, PMScl-100, Ku, CENPA/B, PCNA, Mi2, DFS70) (Alphadia), **EliA DFS70 plus EliA CTD Screen** (U1RNP (RNP70, A, C), SS-A/Ro (60 kDa, 52 kDa), SS-B/La, centromere B, Scl-70, Jo-1, fibrillarin, RNA Pol III, Rib-P, PM-Scl, PCNA, Mi-2, Sm, dsDNA) (Thermofisher) and **MicroBlot-array** (Jo-1, PL-7, PL-12, EJ, OJ, KS, Ha, Zo, SAE-1, SAE-2, SRP, Mi-2, TIF1γ, MDA5, NXP2, PMScl-100, PMScl-75, Scl70, CENPA, CENPB, RNA polymerase III, NOR90, Th/To, PDGFR-β, fibrillarin, Ro52, Ro60, La, RNP-A, RNP 68/70, RNP C, SmB, SmD, PCNA, Rib P0, Ku 38 nucleolin, histone, nucleosome, dsDNA, M2, DFS70) (TestLine Clinical Diagnostics s.r.o.).

Results:

In a setting of anti-nuclear antibodies positive samples, showing a nuclear speckled with mitotic plate, the AC-2 pattern resulted more prevalent than AC-30 with all methods (MBA:63.5%; FEIA:68.8%; IB:66.6%). The frequency of anti-DFS70 positive samples confirmed with MBA (61/96) was no statistically different when compared with FEIA (66/96) or IB (64/96) (Table 1). A substantial concordance rate (k:0.60-0.88) was obtained when MBA was compared with FEIA and IB in the detection of monospecific anti-DFS70 positive or negative samples, while a moderate degree of concordance (k:0.43-0.56) was found among methods in the cases of additional anti-ENA positivity (Table 2). MBA method was able to detect the highest number of additional autoantibody positivity (Figure 1).

Table 1. Frequency of confirmed anti-DFS70 positive samples in routine ANA cohort

	Positive samples (n)	Anti-DFS70 positive samples (%)
MBA	61	63.5
FEIA	66	68.8
IB	64	66.6

Conclusions:

- MBA demonstrated very good performance in detecting anti-DFS70 positive (AC-2) and negative (AC-30) samples ,
- The performance of MBA in detecting anti-DFS70 positive (AC-2) and negative (AC-30) samples is comparable with FEIA and IB methods.
- Given the larger panel of antibody specificities, MBA was able to identify an higher number of concomitant anti-ENA positivity and therefore may represent a valid alternative to the current commercially available tests.

Figure 1. Percentage of anti-DFS70 positive and negative samples with or without concomitant ENA positivity, compared with three different methods.

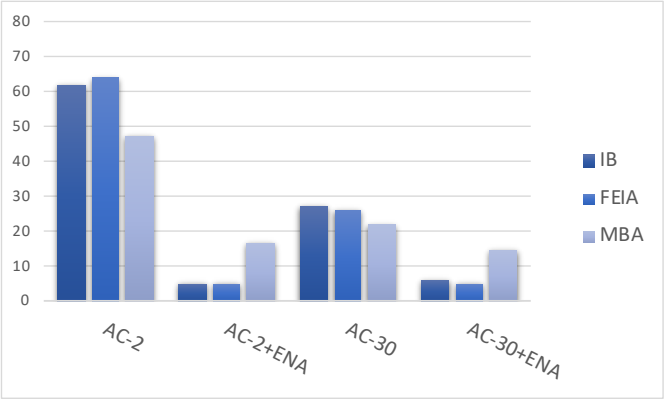

Table 2. Concordance rate among MBA, FEIA and IB methods in the detection of AC-2 or AC-30 samples as monospecific or associated with additional anti-ENA autoantibodies.

	MBA (n)	IB (n)	Agreement (%)	Cohen's K	FEIA (n)	Agreement (%)	Cohen's K
Anti-DFS70 positive (AC-2)	45	59	84.5	0.71	61	80.0	0.60
Anti-DFS70 positive+ENA	16	5	88.5	0.43	5	88.5	0.43
Anti- DFS70 negative (AC-30)	21	26	94.6	0.86	25	95.8	0.88
Anti- DFS70 negative+ENA	14	6	91.7	0.56	5	90.6	0.48