Diagnostic value of a novel ANA Screen ELISA

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Background: Systemic autoimmune rheumatic diseases (SARD) are characterized by circulating anti-nuclear antibodies (ANA). Historically, ANA are commonly detected by an indirect immunofluorescence (IIF) assay using HEp-2 cells and confirmed by ELISA, line immunoassay (LIA) or addressable laser bead immunoassays (ALBIA). It is widely appreciated that the detection of ANA by IIF has several economic and diagnostic drawbacks. Most importantly, IIF is difficult to automate and some clinical relevant autoantibodies (aab) such as anti-ribosomal P, anti-Jo-1 and anti-Ro antibodies are difficult to detect. Therefore, alternatives to ANA screening methods may represent the future of ANA testing. The objective of our study was to evaluate the clinical usefulness of a novel ANA Screening ELISA.

Methods: Sera collected from children with systemic lupus erythematosus (SLE, n=131) and systemic sclerosis patients (SSc, n=100) were tested by IIF (HEp-2 cells), ANA Screen ELISA (Dr. Fooke Laboratorien) and *recom*Line ANA/ENA (LIA, Mikrogen). Discrepant samples were further analysed by ALBIA (QuantaPlex ENA8, INOVA).

	ANA Screen	LIA	ALBIA	HEp2
RNP68	√	√	√	√
RNP-A	√	√	√	√
RNP-C	√	√	√	√
SmBB'	√	√	-	√
SmD	√	√	√	√
Ro52	√	√	√	√
Ro60 (SS-A)	√	√	√	√
La	√	√	√	√
Jo-1	√	√	√	√
CENP-B	√	√	-	√
PCNA	-	√	-	√
Scl-70	√	√	√	√
Ribosomal P	-	√	√	√
Chromatin	-	-	√	√
Histone	-	√	-	√
dsDNA	√	√	-	√
PM1-Alpha	√	-	-	√
No.(%) pos SLE (n=131)	111 (85%)	104 (79%)	n.d.	130 (99%
No.(%) pos SSc (n=100)	75 (75%)	77 (77%)	n.d.	90 (90%)
No.(%) pos all (n=231)	186 (81%)	181 (78%)	n.d.	220 (95%)

Figure 1 Antigen composition and number (%) of positive results of the assays used. Cut-off ANA Screen 1 RU.

Results and findings: Number and percentage of positive results are shown in Figure 1. In SLE sera, the highest prevalence of aab by LIA was found for anti-Ro52 (47%) and in SSc sera for anti-CENP (34%). The agreement between ANA ELISA, IIF and LIA is shown in Figure 2. The ANA ELISA failed to identify 10/181 (5.5%) samples (4 SLE / 6 SSc) as ANA positive when compared to the LIA confirmation test. 3 of those 10 samples (33.3%) showed reactivity to antigens not present in the ANA ELISA (ribosomal P, PCNA or histone). 19/186 (10.2%) samples (15 SLE / 4 SSc) were positive by ANA ELISA but negative by LIA when antigens contained in the ELISA were considered. In 4 of those 19 samples aab to other antigens were detectable including Rib-P and histone. In three ANA ELISA positive / LIA negative samples aab to RNP, Ro60 or chromatin could be detected by ALBIA (data not shown). 5/11 of ANA IIF negative samples had detectable levels of aab by LIA (including RNP-C, RNP-A, SmB, Ro52, La and Scl-70) and 3/11 by ANA ELISA. It is noteworthy that the anti-Scl-70 positive ANA IIF negative sample was positive by ANA ELISA (Table 1).

ANA ELISA vs. ENA LIA, Agreement: 89.2%; *kappa=0.67; p<0.0001*

ENA LIA	ANA ELISA pos	neg	Total
pos	171	10	181
neg	15	35	50
Total	186	45	231

ENA LIA vs. ANA IIF, Agreement: 78.8%; *kappa=0.13; p=0.0066*

ANA IIF	ENA LIA pos	neg	Total
pos	176	44	220
neg	5	6	11
Total	181	50	231

ANA ELISA vs. ANA IIF, Agreement: 82.7%; *kappa=0.23; p<0.0001*

ANA IIF	ANA ELISA pos	neg	Total
pos	183	37	220
neg	3	8	11
Total	186	45	231

Figure 2 Agreement between IIF, ANA Screen ELISA and line immunoassay (LIA).

Table 1 Autoantibody profile of ANA IIF negative samples (LIA and ANA Screen ELISA).

Serum	RNP68	RNPA	RNPC	SmB	SmD	Ro60	Ro52	LaSSB	RibP	PCNA	CENPB	Scl70	Jo1	Histon	dsDNA	ANA
SLE 36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.60
SSc 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.73
SSc 15	0	3	1	0.5	0	0	0	0	0.5	0	0	0	0	0	0	0.71
SSc 19	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	1.60
SSc 33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.46
SSc 42	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0.70
SSc 48	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0.66
SSc 50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.67
SSc 53	0	0	0	0	0	0	3	2	0	0	0	0	0	0	0	2.44
SSc 61	0	0	0	1	0.5	0.5	0	0	0	0	0	0	0	0.5	0	0.89
SSc 97	0	0	0	1	0.5	0	0.5	0	0	0	0	2	0	0.5	0.5	1.80
no pos.	0	1	1	3	0	0	1	1	0	0	0	1	0	0	0	3

LIA positive results are shown in blue; ELISA positives are indicated in red

Conclusion: Although the sensitivity of the ANA ELISA was lower than the IIF test, the ANA ELISA may represent a promising alternative to IIF on HEp-2 cells because of its high specificity and good agreement with the IIF test (82.7%) and confirmation assays such as LIA (89.2%). The minor discrepancies between ANA ELISA and LIA might be explained by the different antigen composition and by the different methodology. The ENA positive IIF negative sample confirms the sensitivity limitation of IIF for certain aab.