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Multiparametric antibody assays compared to indirect immunofluorescence in screening for anti-nuclear antibodies in patients with autoimmune rheumatic diseases

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ANA

International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies

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The European Autoimmunity Standardization Initiative representing 15 European countries and the International Union of Immunologic Societies/World Health Organization/Arthritis Foundation/Centers for Disease Control and Prevention autoantibody standardising committee.

- ✓ **The detection of ANA is the first level test for lab diagnosis of SARD.**
- ✓ **IIF on HEp-2 cells is the reference method for ANA screening for its high sensitivity.**
- ✓ **IIF limitations are acknowledged.**

Shortcomings of the manual IIF assay

- Low specificity
- High intra- and inter-laboratory variability
- Operator-dependent
- Low sensitivity for anti-Ro and anti-synthetase Abs
- Semiquantitative results
- Long turn-around-time

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American College of Rheumatology
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SPECIAL ARTICLE

2019 European League Against Rheumatism/American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus

Use ANA of ≥1:80 as an entry criterion.

In view of ongoing work on the standardization of serology and potential future advances in the field, the steering committee recommended the provision "**Testing by immunofluorescence on HEp-2 cells or a solid-phase ANA screening immunoassay with at least equivalent performance is highly recommended**."

Diagnostic accuracy of immunological methods in detecting ANA

	Sens	Spec
Indirect immunofluorescence (IIF) on HEp-2 cells (±100 Ag)	High	Low
Immunometric methods (ELISA, FEIA, CLIA, ALBIA) (8-16 Ag)	Less than IIF	High

The lower sensitivity of the solid-phase assays *vs*. HEp-2 IIF can be improved using more antigens in the test panel?

Bizzaro N, et al. Arthritis Res Ther 2022;24:278.

Particle-based multi-analyte technology (PMAT)

New automated multiparametric analytical system for the simultaneous detection of antibodies in autoimmune diseases

1257 samples studied (791 CTD and 446 controls)

The samples were tested for the following **29** autoantibodies:

dsDNA, DFS70, U_1RNP, Sm, Ro60, Ro52/TRIM21, La, Scl70, Jo1, CENP-B, Ribo-P, RNA Pol III, fibrillarin, Th/To (Rpp25, Rpp38), Ku, BICD2, PM/Scl, Mi-2, HMGCR, NXP2, MDA5, PL-7, PL-12, EJ, SRP, TIF1g, SAE, OJ

PMAT-Aptiva

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Autoantibody prevalence in pathology groups (%)

	BICD2	Fibrillarin	Ku	PM1-Alpha	RNA Pol III	Th/To Rpp25	Th/To Rpp38	dsDNA	U1RNP	Sm	Ro52	Ro60	La	Scl70	Jo-1	Centromere	DFS70	Ribo-P
SLE (166)	0.7	2.7	7.4	1.9	0	0	0	58.4	41.6	14.5	30.3	54.5	23.6	1.8	0	0	4.8	12
SSc (133)	0	0.8	0	0.8	2.4	0	0	5.3	7.6	0	13.6	17.4	2.3	34.1	0	50.8	7.6	0
pSS (279)	0	1.1	1.5	0	0.4	0	0.7	8.0	9.1	0.4	66.9	69.5	49.5	2.9	0	2.5	2.9	1.8
IIM (103)	0	0.9	2.8	1.9	1.9	0	0.9	2.8	6.6	0	24.5	13.2	4.7	1.9	24.5	5.7	2.7	0
UCTD (106)	0	0	4.8	1.9	1.9	0	1.0	18.4	18.4	1.0	24.3	39.8	13.6	2.9	1.9	3.9	1.9	4.8

	EJ	HMGCR	MDA5	Mi-2	NXP2	OJ	PL-12	PL-7	SAE	SRP	TIF1γ
SLE (166)	0	0	1.3	1.3	0.6	0	0	0	0	0	0
SSc (133)	0	0	0	0	0	0	0.8	0	0	0	0
pSS (279)	0	0	0	0	0	0	0	0	0	0.4	0
IIM (103)	2.9	9.7	5.8	12.6	2.8	10.7	1.9	1.0	3.9	1.0	2.8
UCTD (106)	0	0	1.0	0	0	0	0	0	0	0	0

Overall diagnostic sensitivity of Aptiva PMAT 82.6%

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Diagnostic specificity of Aptiva PMAT

Comprehensive

	Cutoff (RUO)	SP %
BICD2	1	99.8
Fibrillarin	1	98.7
Ku	1	99.1
PM1-Alpha	1	99.6
RNA_Pol_III	1	99.8
Th/To_Rpp25	1	100
Th/To_Rpp38	1	98.5

Essential

	Cutoff (FDA appr)	SP %
dsDNA	5	93.7
RNP	5	98.0
Sm	5	99.8
Ro52	5	98.5
Ro60	5	94.8
La	5	100
Scl-70	5	97.6
Jo-1	5	99.8
Centromere	5	97.4
DFS70	5	96.7
Ribo-P	5	98.9

Autoimmune myopathy

	Cutoff (RUO)	SP %
EJ	1	99.1
HMGCR	1	99.6
Jo-1	1	100
MDA5	1	99.8
Mi-2	1	99.3
NXP2	1	99.2
OJ	1	99.6
PL-12	1	100
PL-7	1	100
SAE	1	99.8
SRP	1	99.3
TIF1y	1	99.8

Mean diagnostic specificity 98.8%

Overall diagnostic specificity of Aptiva PMAT 77.8%

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The overall specificity of multiparametric methods

Even if the cutoffs are positioned to have a 99% specificity for each one of the antibodies being tested, the 1% false positive rate for each antibody is added to that of all the other antibodies targeting the antigens included in the panel.

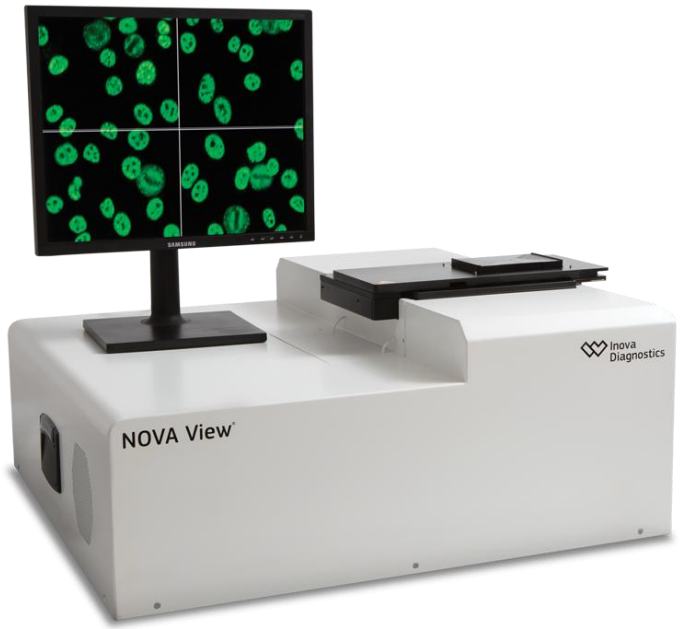
Therefore, if 30 antibodies are studied simultaneously, each one with a specificity of 99%, the overall specificity of the system will be around 70%.

Diagnostic performance of particle-based multi-analyte technology compared to indirect immunofluorescence in screening for anti-nuclear antibodies in patients with autoimmune rheumatic diseases

F. Buzzulini[1], D. Villalta[1], G. Previtali[2], M.G. Alessio[2], G. Cafaro[3], E. Bartoloni[3], R. Gerli[3], N. Bizzaro[4], on behalf of the Forum Interdisciplinare per la Ricerca nelle Malattie Autoimmuni (FIRMA Group)

Clinical and Experimental Rheumatology 2025; 43: 1622-1628.

THE PMAT WAS COMPARED WITH THE IMMUNOFLUORESCENCE TEST



ANAs were detected using an automated HEp-2 IIF method (QUANTA LITE, Inova Diagnostics).

Sera were diluted to 1:80 with phosphate-buffered saline (PBS), and positive samples were titrated to a maximum dilution of 1:1280.

Results

SARD patients (782)

		HEp-2 IIF 1:80 +	HEp-2 IIF 1:80 -
Aptiva PMAT	+	612 (78.3%)	34 (4.3%)
	-	114 (14.6%)	22 (2.8%)

Concordance 81.1%

Controls (459)

		HEp-2 IIF 1:80 +	HEp-2 IIF 1:80 -
Aptiva PMAT	+	57 (12.4%)	45 (9.8%)
	-	154 (33.5%)	203 (44.3%)

Concordance 56.7%

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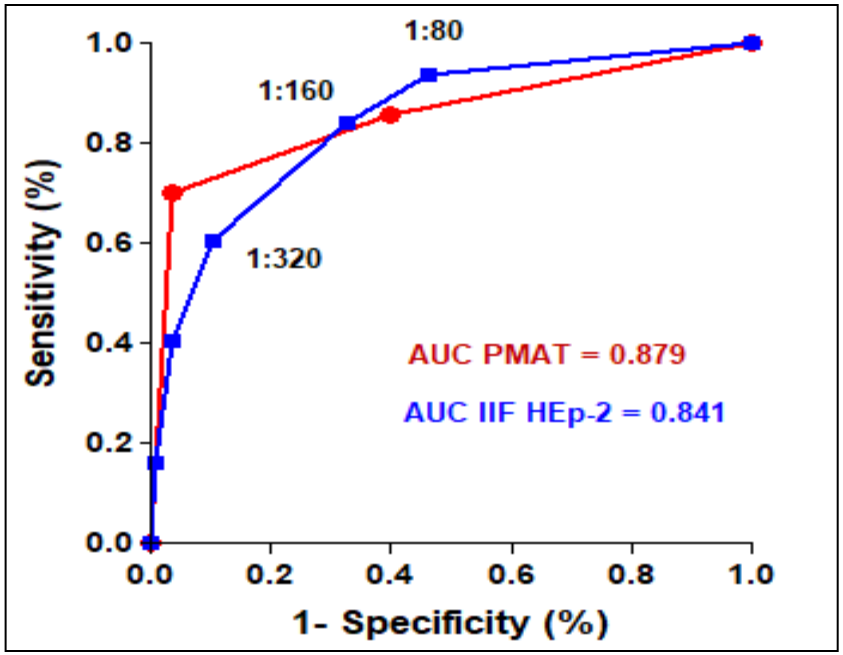
Sensitivity, specificity and likelihood ratios for HEp-2 IIF and Aptiva-PMAT in SARD patients

		SE % (95%CI)	SP% (95%CI)	LR+	LR-
AARDs (n=782)	HEp-2 IIF	92.8 (90.8 – 94.5)	54.0 (48.1 – 57.5)	1.97	0.14
	Aptiva/PMAT	82.6 (79.8 – 85.2)	77.8 (73.7 – 81.7)	3.81	0.22
	Double POS	78.0 (74.9 – 80.9)	87.6 (84.2 – 90.5)	6.31	0.25
SLE (n=164)	HEp-2 IIF	92.0 (86.7 – 95,7)	59.0 (54.2 - 63.7)	2.24	0.14
	Aptiva/PMAT	90.2 (84.5 – 94.3)	77.9 (73.7 – 81.7)	4.08	0.13
	Double POS	89.0 (83.1 – 93.3)	87.6 (84.1 – 90.5)	7.15	0.13
SjS (n=277)	HEp-2 IIF	87.4 (82.9 – 91.0)	59.0 (54.2 – 63-7)	2.13	0.21
	Aptiva/PMAT	80.9 (75.7 – 85.3)	77.9 (73.7 – 81.7)	3.66	0.25
	Double POS	78.7 (73.4 – 83.4)	87.6 (84.1 – 90.5)	6.33	0.24
SSc (n=132)	HEp-2 IIF	97.0 (92.4 – 99.1)	59.0 (54.2 – 63.7)	2.36	0.05
	Aptiva/PMAT	90.9 (84.7 – 95.2)	77.9 (73.7 – 81.7)	4.11	0.12
	Double POS	89.4 (82.8 – 94.1)	87.6 (84.1 – 90.5)	7.18	0.12
IIM (n=106)	HEp-2 IIF	58.5 (48.5 – 69.0)	59.0 (54.2 – 63.7)	1.43	0.70
	Aptiva/PMAT	82.1 (73.4 – 88.8)	77.9 (73.7 – 81.7)	3.71	0.23
	Double POS	57.5 (47.6 – 67.1)	87.6 (84.1 – 90.5)	4.84	0.44
UCTD (n.=03)	HEp-2 IIF	81.4 (72.4 – 88.4)	59.0 (54.2 – 63.7)	1.98	0.32
	Aptiva/PMAT	65.7 (55.6 – 74.8)	77.9 (73.7 – 81.7)	2.96	0.38
	Double POS	59.8 (49.6 – 69.4)	87.6 (84.2 – 90.5)	4.81	0.46

PMAT-APTIVA *vs*. IIF AS SCREENING TEST

	Sensitivity	Specificity	Efficiency
ANA PMAT	82.6 %	77.8 %	81.8 %
ANA IIF 1:80	92.8 %	54.0 %	77.0 %
ANA IIF 1:160	82.5 %	65.6 %	75.7 %
ANA IIF 1:320	69.9 %	84.1 %	75.2 %



(Efficiency: **%** of accurate results, either positive or negative)

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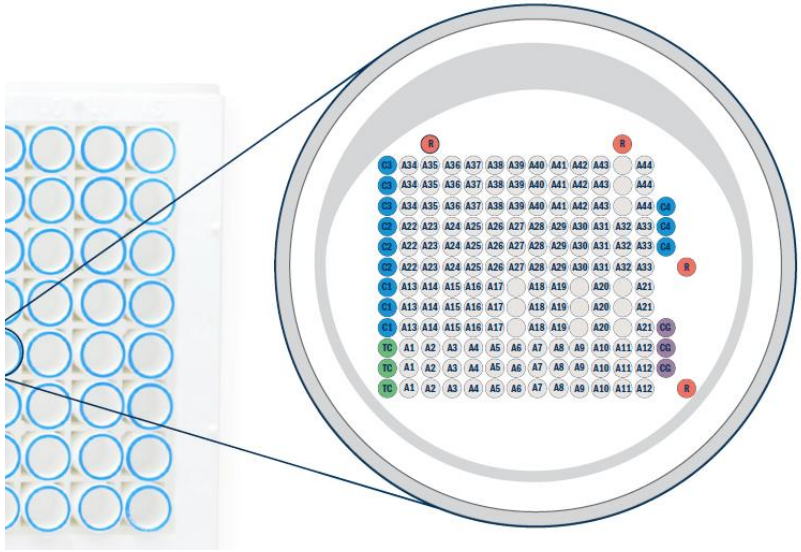
BioVendor Group MBA

Microblot array



- **R** – Riferimenti
- **TC** – Controllo del Test
- **CA** – Controllo Conj IgA
- **CG** – Controllo Conj IgG
- **CM** – Controllo Conj IgM
- **C1** – CAL 1
- **C2** – CAL 2
- **C3** – CAL 3
- **C4** – CAL 4

PANNELLO ANA:
LISTA ANTIGENI PRESENTI IN OGNI POZZETTO

No.	Antigene	Descrizione	Probabile associazione con la patologia (Valutazione dell'associazione con la patologia mediante SWI)			
			ANA	Miosite	Sclerodermia	SLE ed altre malattie del tessuto connettivo
1	Jo-1	Hystidyl tRNA synthetase	•	•		
2	PL-7	Threonyl tRNA synthetase	•	•		
3	PL-12	Alanyl tRNA synthetase	•	•		
4	EJ	Glycyl tRNA Synthetase	•	•		
5	OJ	Isoleucyl tRNA synthetase	•	•		
6	KS	Asparaginyl tRNA synthetase	•	•		
7	YARS	Tyrosyl tRNA synthetase (Ha)	•	•		
8	ZoA	Phenylalanyl tRNA synthetase	•	•		
9	ZoB	Phenylalanyl tRNA synthetase	•	•		
10	SAE-1	Small ubiquitin-like modifier activating enzyme	•	•		
11	SAE-2	Small ubiquitin-like modifier activating enzyme	•	•		
12	SRP54	Signal recognition particle	•	•		
13	Mi-2	Helicase protein-nuclear transcription	•	•		
14	TIF1γ	Transcription Intermediary Factor 1	•	•		
15	MDA5	Melanoma differentiation associated protein 5 (CADM-140)	•	•		
16	NXP2	Nuclear matrix protein 2 (p140, Mi)	•	•		
17	PMScl 100	Human exosome complex	•	•	•	
18	PMScl 75	Human exosome complex	•	•	•	
19	Scl70	DNA-topoisomerase I	•		•	
20	CENP A	Centromere A	•		•	
21	CENP B	Centromere B	•		•	
22	POLR3A	RNA polymerase III	•		•	
23	NOR90	Nucleolar transcription factor 1 (Ubtf1)	•		•	•
24	Th/To	Ribonuclease P protein subunit 25 (Rpp25)	•		•	
25	PDGFR β	Platelet-derived growth factor receptor beta	•		•	
26	Fibrillarin	U3 RNP - fibrillarin	•		•	
27	Ro52	TRIM21	•	•	•	•
28	Ro60	Sjögren's-syndrome-related antigen A (SS-A)	•			•
29	La	Sjögren's-syndrome-related antigen B (SS-B)	•			•
30	RNP A	U1 small nuclear ribonucleoprotein A	•		•	•
31	RNP 68/70	U1 small nuclear ribonucleoprotein 68/70 kDa	•		•	•
32	RNP C	U1 small nuclear ribonucleoprotein C	•		•	•
33	Sm B	Smith antigen B	•			•
34	Sm D	Smith antigen D	•			•
35	PCNA	Proliferating cell nuclear antigen	•			•
36	P0	Ribosomal protein P0	•			•
37	Ku	Ku (p70/p80)	•	•	•	•
38	Nucleolin	Nucleolin	•			•
39	Histons	Histone	•			•
40	Nucleosome	Nucleosome	•			•
41	dsDNA	Double-stranded DNA	•			•
42	M2	Mitochondrial M2 (AMA-M2)	•		•	
43	DFS70	Dense fine speckled 70 antigen	•			

• - antigeni supplementari, LES - Lupus eritematoso sistemico

MBA profile : 43 antigens

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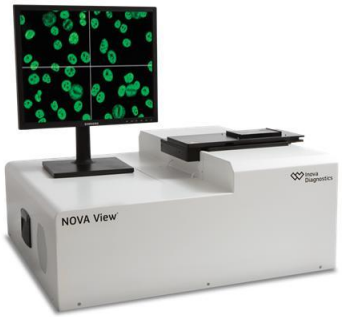
ANA IIF *vs*. ANA MBA
A Pilot Study

152 Systemic autoimmune diseases (SARD)

33	SLE
20	MCTD
20	Autoimmune myositis
29	Sjögren's syndrome
50	Systemic sclerosis

ANA IMMUNOFLUORESCENCE TEST



ANAs were detected using an automated HEp-2 IIF method (QUANTA LITE, Inova Diagnostics).

Sera were diluted to 1:80 with phosphate-buffered saline (PBS), and positive samples were titrated to a maximum dilution of 1:1280.

230 Controls

77	Viral infections (CMV, HCV, HBV, EBV)
107	Patients hospitalized (no SARD)
46	Various diseases

ANA IIF *vs*. ANA MBA as screening tests

SARD (152)

		MBA +	MBA -
IIF	+	133	3
	-	16	0

Agreement 87.5%

CONTROLS (230)

		MBA +	MBA -
IIF	+	14	36
	-	42	138

Agreement 66.1%

Overall agreement 74.6%

ANA IIF *vs*. ANA MBA as screening tests

SARD (152)			
		MBA	
		+	-
IIF	+	133	**3**
	-	**16**	0

2 Sjögren -1 SSc

(2 titer 1:80)

12/16 autoimmune myositis

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ANA IIF *vs*. ANA MBA as screening tests

CONTROLS (230)			
		MBA	
		+	-
IIF	+	**14**	**36**
	-	**42**	138

13/36 (36%) viral infection
23/36 various diseases (SARD excluded)

11/14 (79%) viral infection

25/42 (60%) viral infection

56 FP MBA

19	Ro60/TROVE2
7	RNP-A
7	Nucleosome
6	Ro52/TRIM21
4	dsDNA
4	Histones
1-3	Other 12 antibodies

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Diagnostic specificity of MBA

Jo-1	PL-7	PL-12	EJ	OJ	KS	YARS	ZoA	ZoB
100%	100%	100%	99.1%	100%	100%	99.6%	100%	100%
SAE-1	SAE-2	SRP54	Mi-2	TIF1γ	MDA5	NXP2	PMScl 100	PMScl 75
100%	99.6%	98.7%	98.7%	99.1%	99.6%	99.1%	100%	99.6%
M2	DFS70	Scl70	CENP A	CENP B	POLR3A	NOR90	Th/To	PDGFR-β
100%	98.2%	99.6%	99.1%	99.1%	100%	99.1%	100%	100%
Fibrillarin	Ro52	Ro60	La	PCNA	P0	SmB	SmD	Nucleolin
100%	97.3%	91.5%	100%	99.6%	100%	98.7%	99.1%	100%

Nucleosome	Histone	RNP A	RNP 68	RNP C	Ku	dsDNA
96%	98.2%	96%	98.7%	98.2%	99.1%	97.3%

Mean diagnostic specificity 99.0%

Overall diagnostic specificity 75.7%

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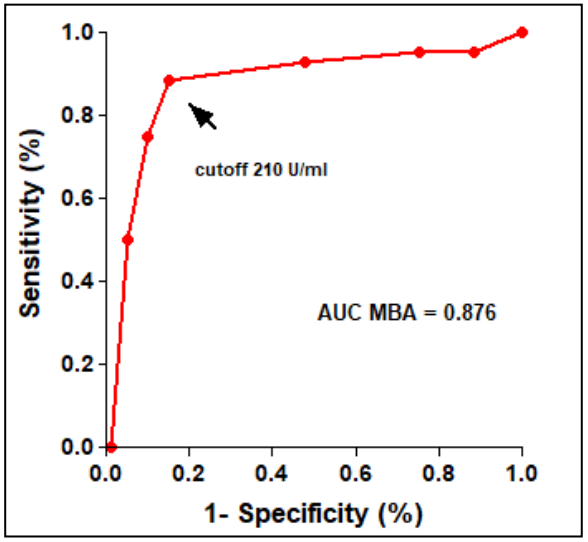
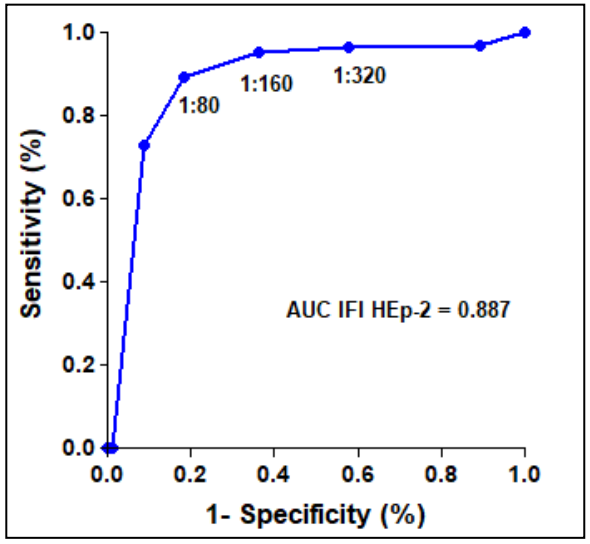
MBA *vs*. IIF AS A SCREENING TEST

	Sensitivity	Specificity	Efficiency
MBA	98.0 %	75.7 %	84.5 %
IIF 1:80	89.5 %	78.3 %	82.7 %





Considering the results in relation to the number of antigens

Method	Antigens	Sensitivity %	Specificity %
PMAT	29	82.6	77.8
MBA	43	98.0	75.7

Warning: a direct comparison between the two studies cannot be made because the study populations are different.

Buzzulini F. et al. Clin Exp Rheumatol 2025
Barrale M. et al. 15° Int. Cong. Autoimmunity. Prague. 2026

Results in relation to the number of antigens SPA *vs*. IIF

Method	Antigens	Sensitivity %	Specificity %
ANA - PMAT	29	82.6	77.8
ANA IIF HEp-2 1:80	~100	92.8	54.0
ANA - MBA	43	98.0	75.7
ANA IIF HEp-2 1:80	~100	89.5	78.3

Buzzulini F, et al. Clin Exp Rheumatol 2025
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Results in relation to the number of antigens SPA *vs*. IIF

Method	Antigens	Sensitivity %	Specificity %	Efficiency %
PMAT	29	82.6	77.8	81.2
ANA IIF HEp-2 1:80	~100	92.8	54.0	77.0
MBA	43	98.0	75.7	84.5
ANA IIF HEp-2 1:80	~100	89.5	78.3	82.7

Buzzulini F. et al. Clin Exp Rheumatol 2025
Barrale M. et al. 15° Int. Cong. Autoimmunity. Prague. 2026

Final remarks

1. Multiparametric immunometric methods can be used to identify the antibodies responsible for positive screening tests.

2. The more antigens are included in the assays, the closer they get to the performance of the IIF HEp-2 method.

3. The overall low specificity, which represents a particular feature of multiparametric systems, leads us to consider that the ideal would be to set multiparametric systems so that the specificity of the individual antibodies is always 100%.

4. If you decide to use them as screening tests for ANA, they provide equal or slightly superior performance to the IIF HEp-2 methods. Costs should be considered.


Thank you !