

12th International Autoimmunity Seminar

October 14-15, 2025 • Vilnius



Diagnostic performance of particle-based multi-analyte technology (PMAT) compared to indirect immunofluorescence (IFA) in screening for ANA in patients with autoimmune rheumatic diseases

Nicola Bizzaro

Study Group on Autoimmune Diseases

**Italian Society of Clinical Pathology and
Laboratory Medicine**

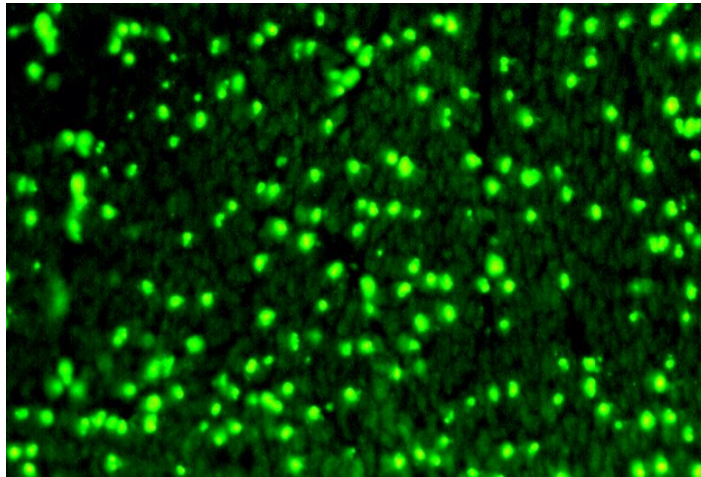


ANA-IIF: a very special test

First description by Friou in 1957

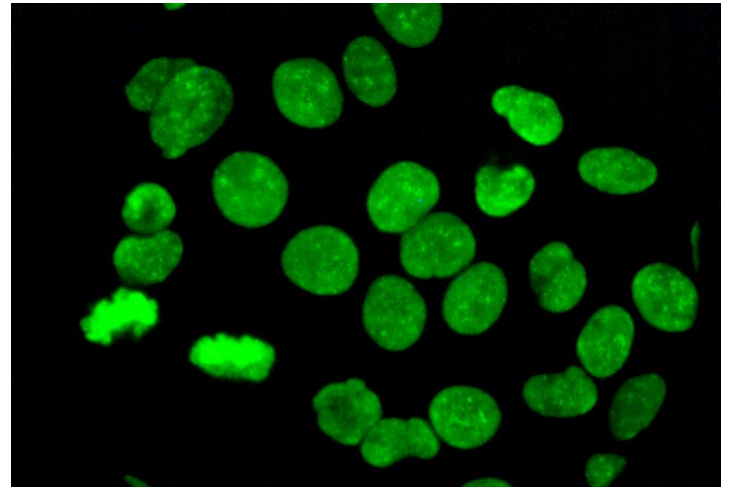
For over 68 years survived huge technological evolution

Murine tissues

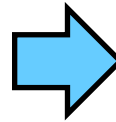


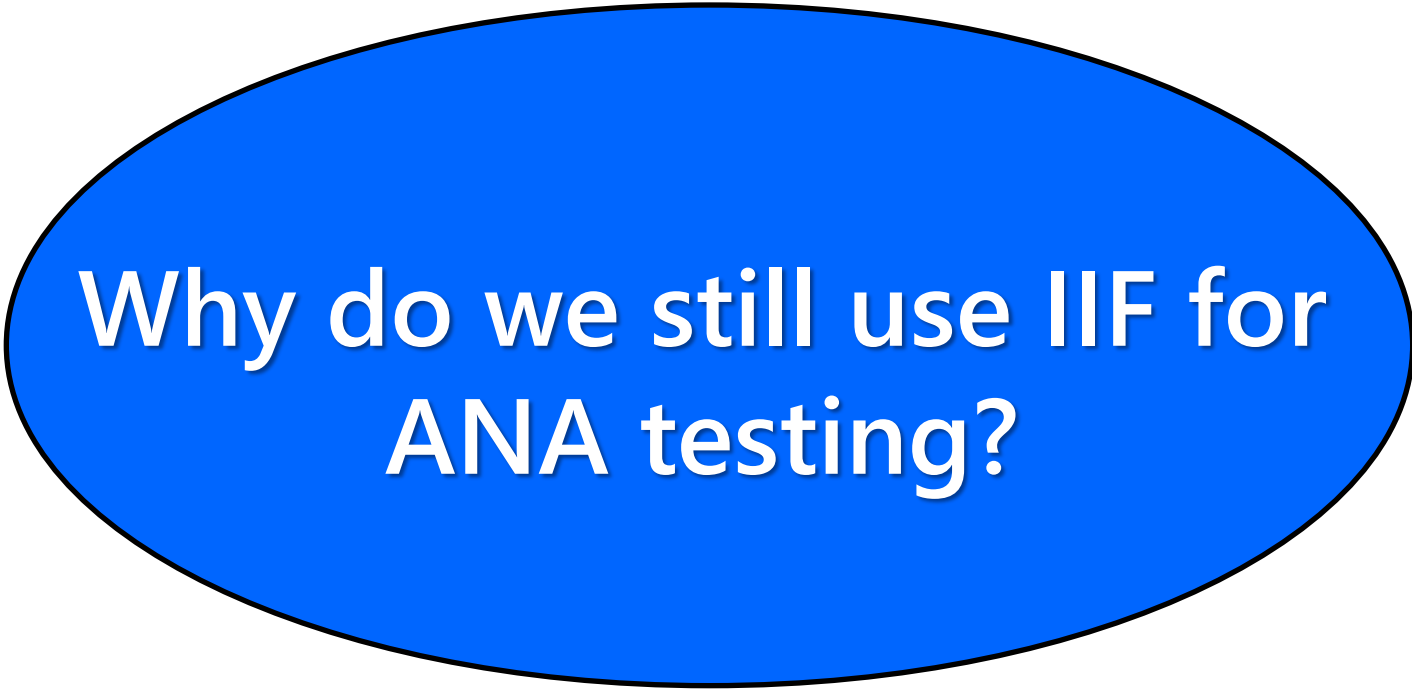
1957- 1984

HEp-2 cells



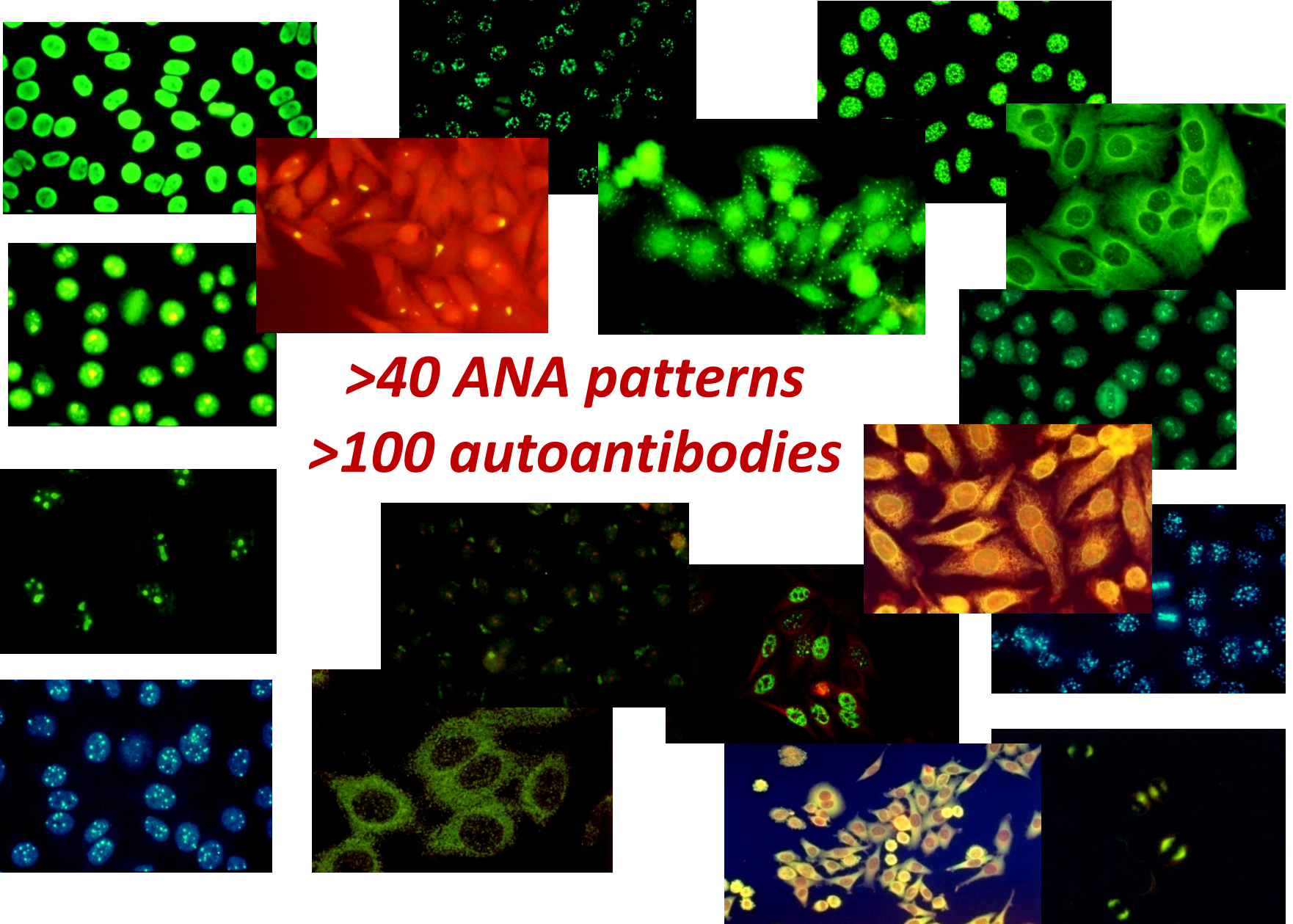
1985 - 2025





Why do we still use IIF for
ANA testing?

High sensitivity of the IIF-HEp2 method



AMERICAN COLLEGE OF RHEUMATOLOGY

POSITION STATEMENT

SUBJECT: Methodology of Testing for Antinuclear Antibodies

PRESENTED BY: American College of Rheumatology

FOR DISTRIBUTION TO: Members of the American College of Rheumatology
Medical Societies
Managed Care Organizations/Third Party Carriers
Laboratories

SUMMARY:

- 
- The immunofluorescence antinuclear antibody (ANA) assay is the gold standard for ANA testing with greater sensitivity than solid phase assays.
 - HEp-2 cells have approximately 100 to 150 possible autoantigens. These cells are used to detect ANAs by the immunofluorescence (IF) method, in which both pattern and titer can be described, and to display a variety of autoantigens not present in multiplex ANA tests.
 - Many commercial laboratories and some hospital laboratories have switched their ANA screening test to solid phase immunoassays, such as a multiplex platform. The latter technique can screen and process large volumes of clinical specimens more quickly and at less cost than the traditional immunofluorescence ANA test using fixed HEp-2 cells as substrate.
 - These multiplex assays can detect only the specific autoantibodies directed against the limited number (typically 8-10) autoantigens that are displayed.
 - Laboratories should indicate the method used when reporting ANA results.

ANA

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

Nancy Agmon-Levin,^{1,2} Jan Damoiseaux,³ Cees Kallenberg,⁴ Ulrich Sack,⁵ Torsten Witte,⁶ Manfred Herold,^{7,8} Xavier Bossuyt,⁹ Lucille Musset,¹⁰ Ricard Cervera,¹¹ Aresio Plaza-Lopez,¹² Carlos Dias,¹³ Maria José Sousa,¹⁴ Antonella Radice,¹⁵ Catharina Eriksson,¹⁶ Olof Hultgren,¹⁷ Markku Viander,¹⁸ Munther Khamashta,¹⁹ Stephan Regenass,²⁰ Luis Eduardo Coelho Andrade,²¹ Allan Wiik,²² Angela Tincani,²³ Johan Rönnelid,²⁴ Donald B Bloch,²⁵ Marvin J Fritzler,²⁶ Edward K L Chan,²⁷ I Garcia-De La Torre,²⁸ Konstantin N Konstantinov,²⁹ Robert Lahita,³⁰ Merlin Wilson,³¹ Olli Vainio,³² Nicole Fabien,³³ Renato Alberto Sinico,³⁴ Pierluigi Meroni,³⁵ Yehuda Shoenfeld^{1,2,36}

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.

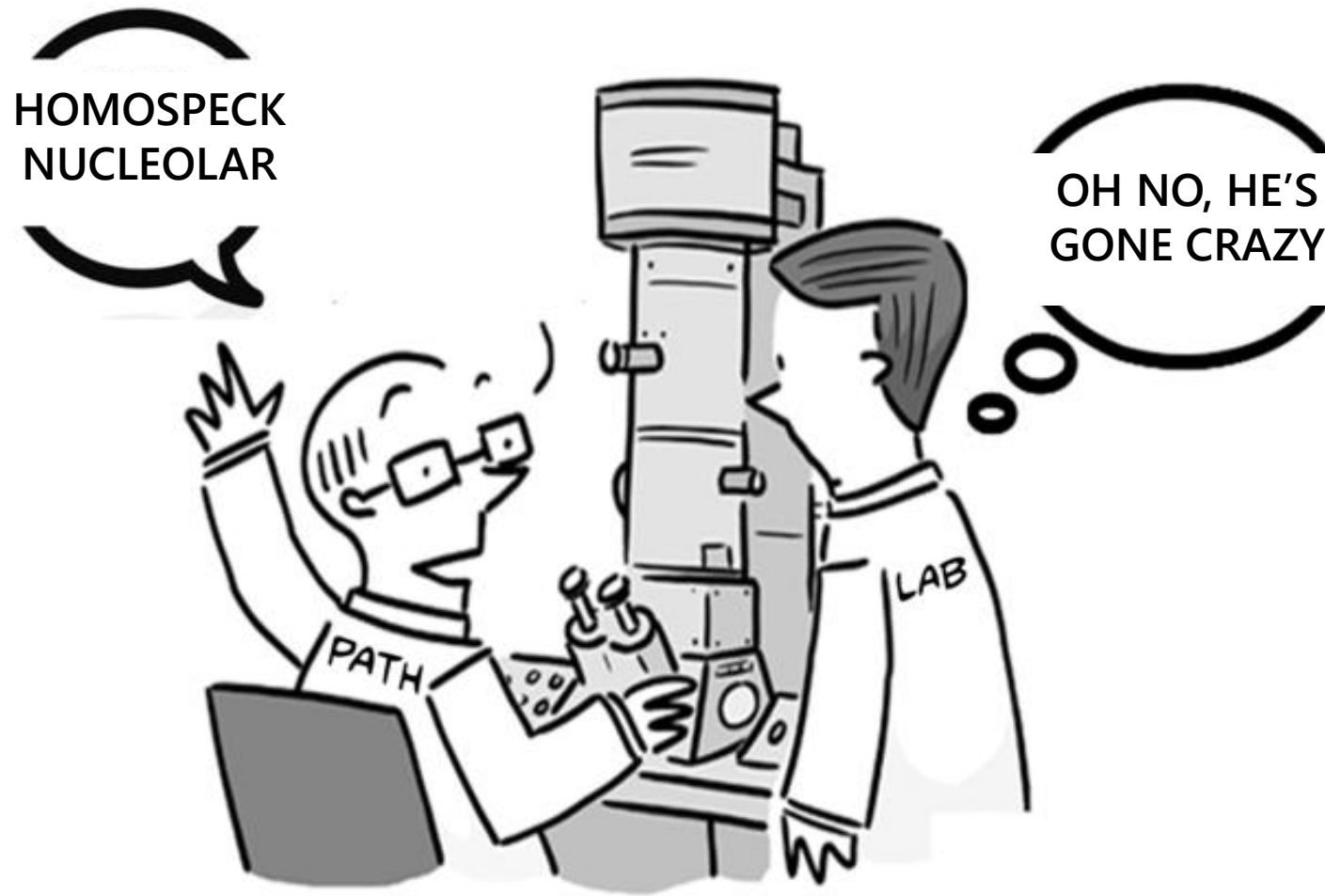
- ✓ The detection of ANA is the **first level test** for lab diagnosis of SARD.
- ✓ IIF is the **reference method** for ANA screening.
- ✓ IIF **limitations** are acknowledged.

Shortcomings of the manual IIF assay

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

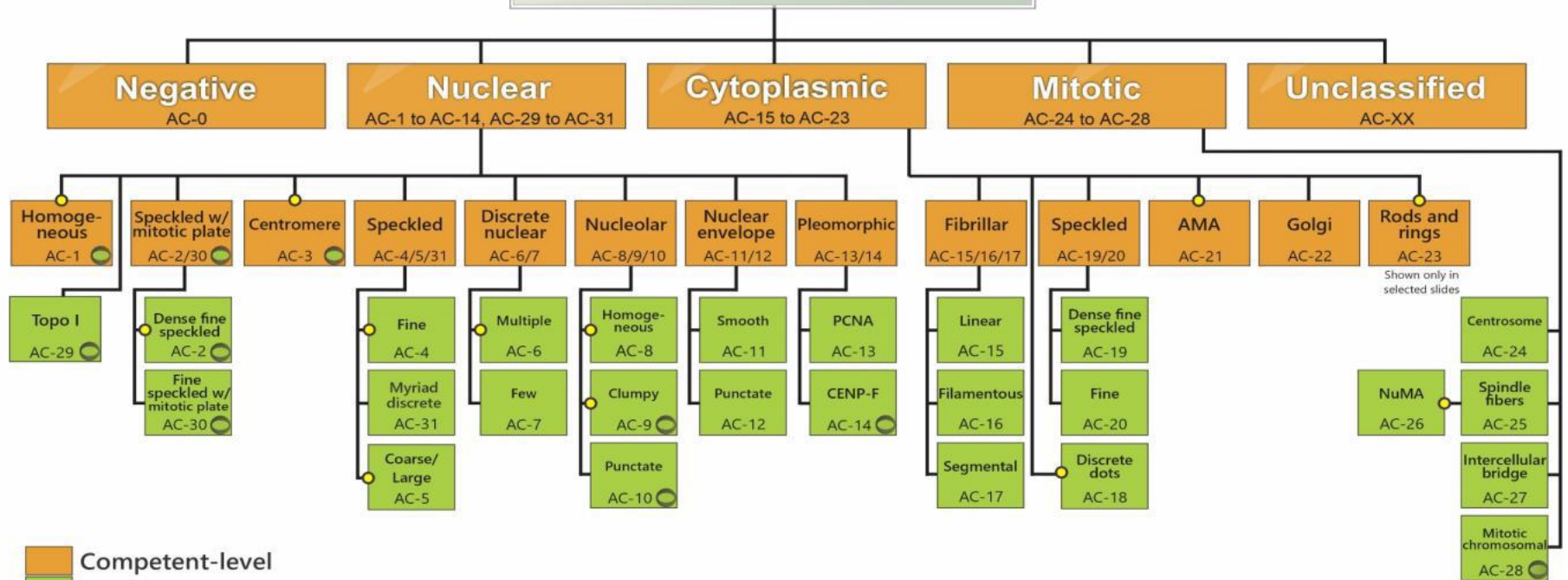


SUBJECTIVITY IN PATTERN INTERPRETATION



ICAP Classification

HEp-2 cell patterns



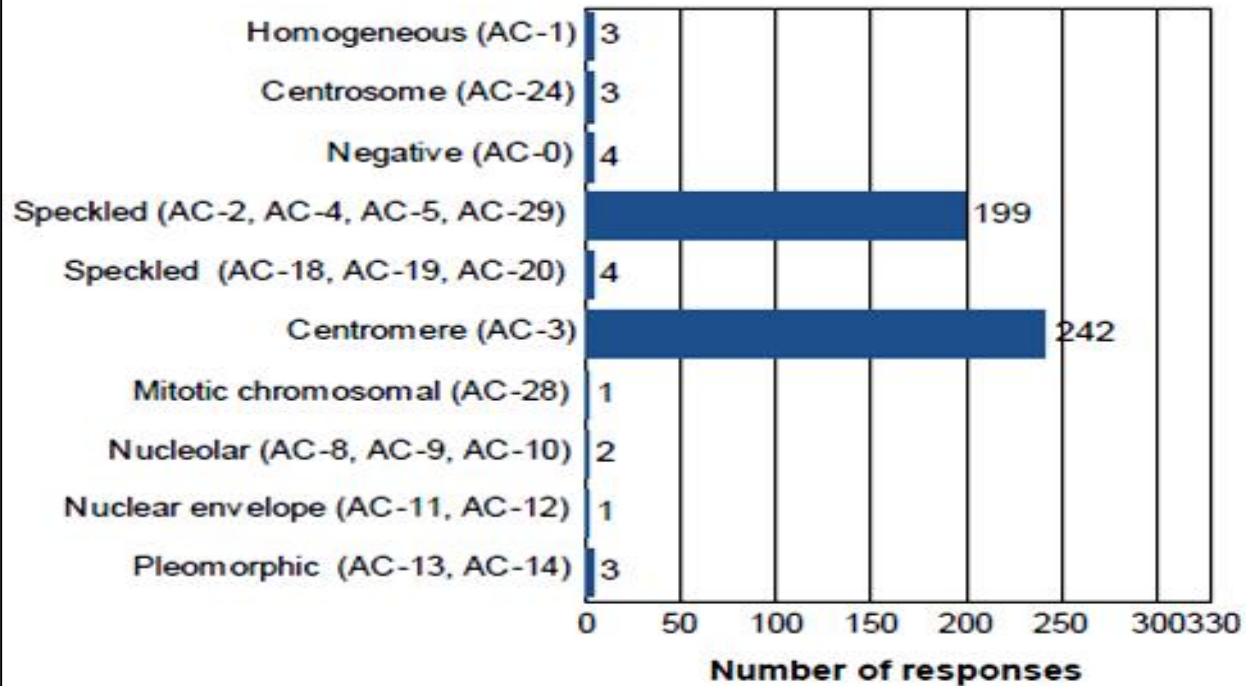
- Competent-level
 - Expert-level report
 - Metaphase plate is stained
 - Reference materials available at www.AutoAb.org
- *Classification tree updated February 2024

Distribution : 215

September 2021

Participant : INFO

ANA- IIF Staining patterns



Reading subjectivity is an important cause in
titer variability



RESEARCH

Open Access

Sex and vision II: color appearance of monochromatic lights

Isr

Murray IJ, et al. **Sex-related differences in peripheral human color vision: A color matching study.** *J Vision* 2012; 12:18.

“Females have more cones than males”.

“Males display a bias toward the green”.

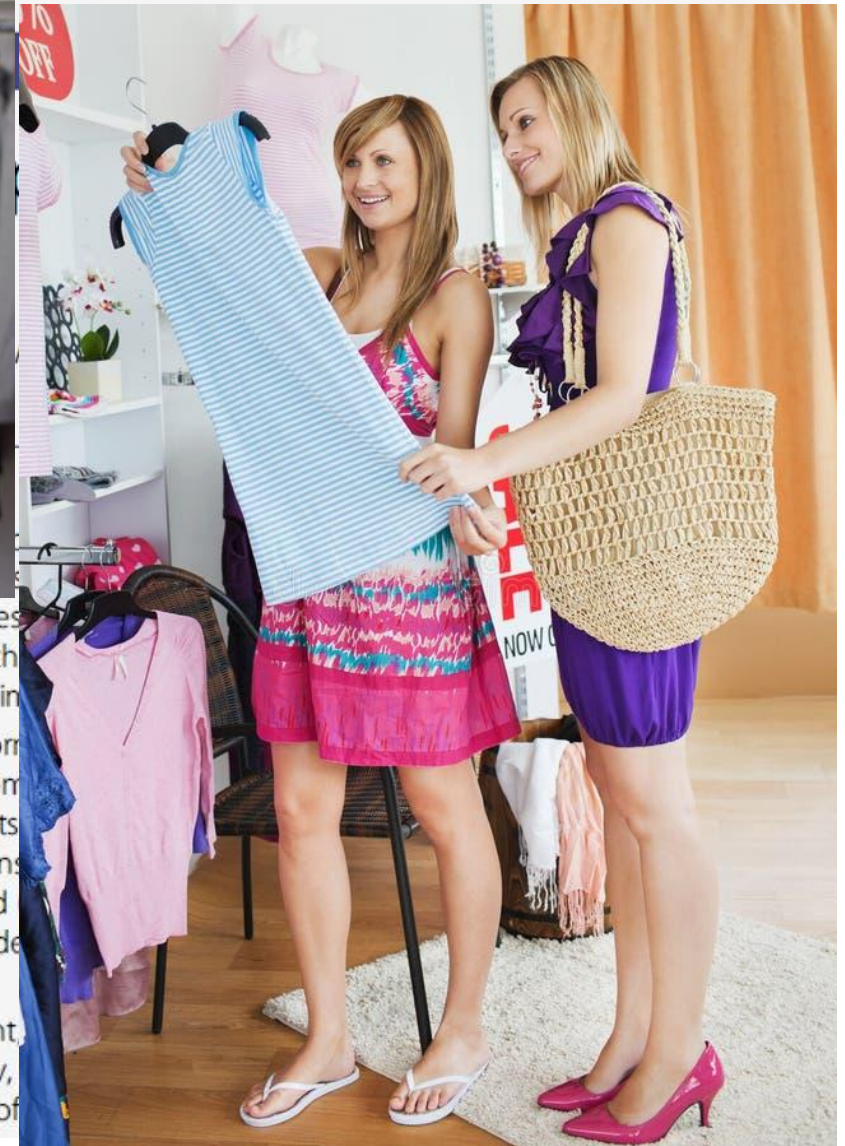
“Women perceive more chromatic bands than normal males”.

possible
of men
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objects.

tion,

and stereopsis. Based on standard color-screening and anomaloscope data, we excluded all color-deficient observers. Stimuli were equi-luminant monochromatic lights across the spectrum. They were foveally-viewed flashes presented against a dark background. The elicited sensations were measured using magnitude estimation of hue and saturation. When the only permitted hue terms are red (R) yellow (Y), green (G), blue (B), alone or in combination, such hue descriptions are language-independent and the hue and saturation values can be used to derive a wide range of color-discrimination functions.

Results: There were relatively small but clear and significant differences between males and females in the hue sensations elicited by almost the entire spectrum. Generally, males required a slightly longer wavelength to experience the same hue as did females. The spectral loci of the unique hues are not correlated with



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Results:

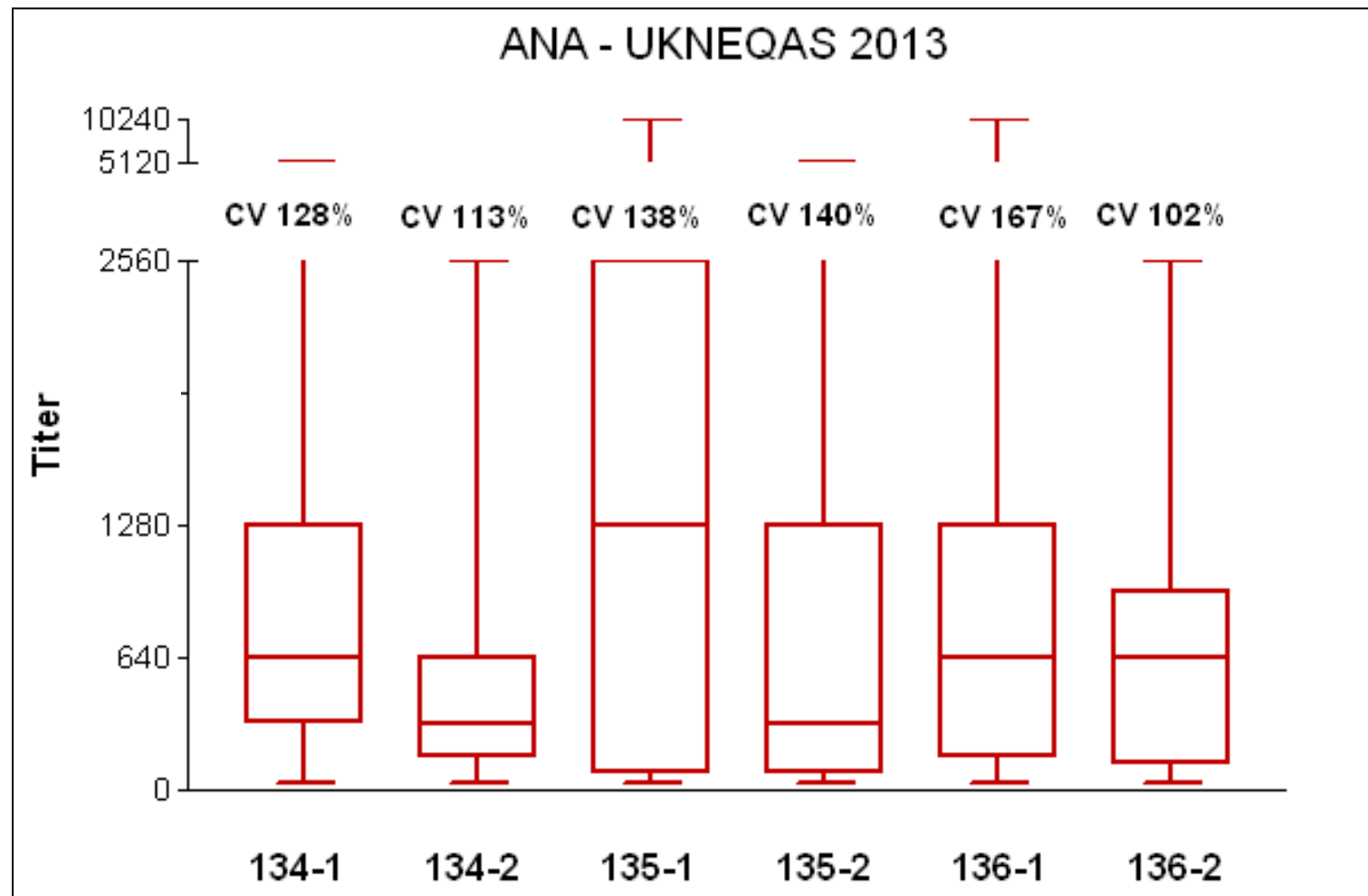
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and significant
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spectral loci of

Imprecision of ANA IIF method



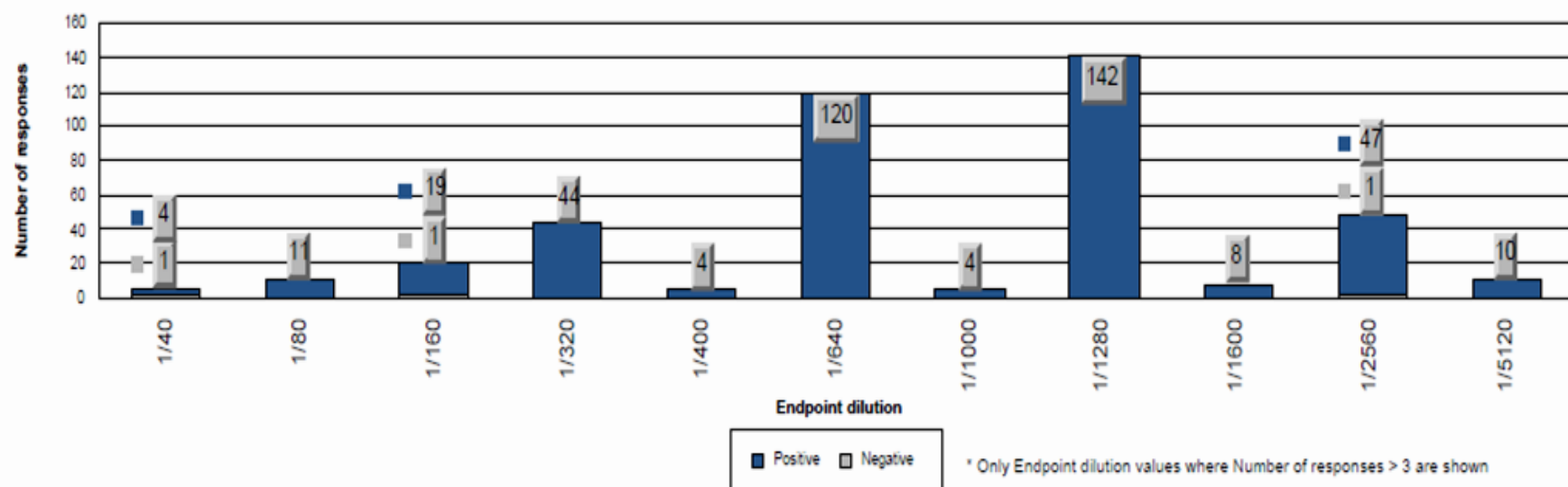
ANA (nuclear, cytoplasmic, mitotic) Responses for Sample 224-1

Distribution : 224

July 2022

Participant : INFO

Endpoint dilution - all IIF methods



Shortcomings of the manual ANA-IIF assay

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



Can solid-phase assays **replace** the immunofluorescence method for ANA screening?



SPECIAL ARTICLE

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

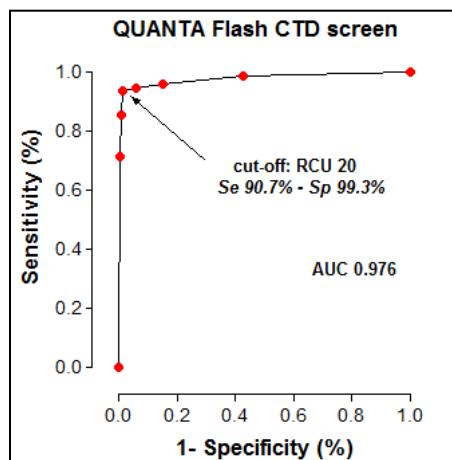
Use ANA of $\geq 1:80$ as an entry criterion.

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

New solid-phase assay for ANA screening

QUANTA Flash CTD Screen CLIA IgG

(Inova Diagnostics)

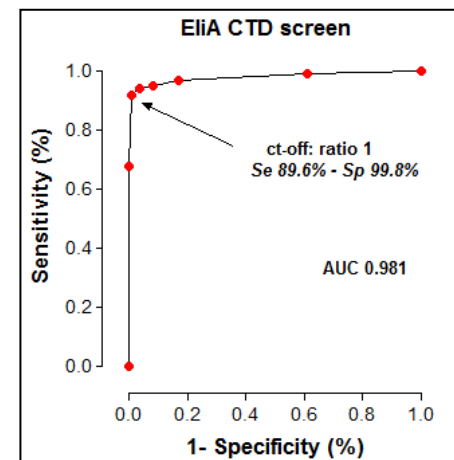


antigenic panel
16 CLIA 15 FEIA

Antigen	QUANTA Flash ANA Screen	EliA CTD Screen
dsDNA	X	X
RNP	X	X
Sm	X	X
Ro60	X	X
Ro52	X	X
SS-B	X	X
Jo-1	X	X
Scl-70	X	X
CENP-B	X	X
RNA Pol III	X	X
PM/ScI	X	X
Mi-2	X	X
PCNA	X	X
Th/To	X	
Fibrillarin		X
Ku	X	
Ribosomal P	X	X

EliA CTD Screen FEIA IgG

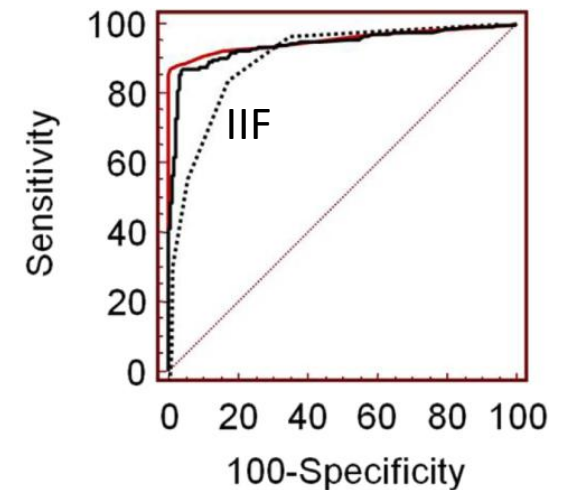
(Thermofisher)



Performance characteristics

(5043 samples from the routine)

	ANA-IIF		CTD screen	
	95%CI		95%CI	
Sensitivity	89.2 %	(87.2 - 91.0)	87.1 %	(84.8 - 89.2)
Specificity	64.6 %	(63.1 - 66.1)	98.0 %	(97.5 - 98.4)
PPV	40.6 %	(39.5 - 41.7)	90.7 %	(88.7 - 92.3)
NPV	95.7 %	(94.9 - 96.3)	97.1 %	(96.6 - 97.6)
Accuracy	69.9 %	(68.6 - 71.1)	96.0 %	(95.4 - 96.5)
LR+	2.52	(2.41 - 2.64)	43.2	(34.9 - 53.6)
LR-	0.17	(0.14 - 0.20)	0.13	(0.11 - 0.16)
OR	15.1	(12.3 - 18.5)	329.3	(246.3 - 440.2)



Performance of the ANA-IIF test and of the CTD screen test in identifying ANA-associated autoimmune rheumatic diseases

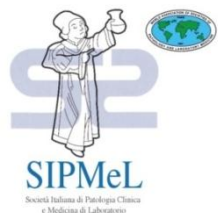
Disease	no.	ANA-IIF positive	CTD screen positive	<i>p</i>
Sjögren's syndrome	128	100 (87.0%)	111 (99.1%)	0.0001
SLE	123	104 (94.6%)	91 (69.1%)	<0.0001
Systemic sclerosis	66	59 (96.7%)	47 (81.0%)	0.004
Autoimmune myositis	39	23 (74.2%)	25 (89.3%)	0.086
MCTD	12	11 (91.7%)	8 (66.7%)	0.139
Total	368	297 (80.7%)	282 (76.6%)	0.175

Performance characteristics

(5043 samples from the routine)

	ANA-IIF		CTD screen		ANA-IIF and CTD screen combined	
	95%CI		95%CI		95%CI	
Sensitivity	89.2 %	(87.2 - 91.0)	87.1 %	(84.8 - 89.2)	97.4 %	(96.3 - 98.3)
Specificity	64.6 %	(63.1 - 66.1)	98.0 %	(97.5 - 98.4)	98.0 %	(98.0 - 98.8)
PPV	40.6 %	(39.5 - 41.7)	90.7 %	(88.7 - 92.3)	94.1 %	(92.7 - 95.3)
NPV	95.7 %	(94.9 - 96.3)	97.1 %	(96.6 - 97.6)	99.3 %	(99.0 - 99.5)
Accuracy	69.9 %	(68.6 - 71.1)	96.0 %	(95.4 - 96.5)	98.2 %	(97.8 - 98.6)
LR+	2.52	(2.41 - 2.64)	43.2	(34.9 - 53.6)	61.7	(48.5 - 78.6)
LR-	0.17	(0.14 - 0.20)	0.13	(0.11 - 0.16)	0.03	(0.02 - 0.04)
OR	15.1	(12.3 - 18.5)	329.3	(246.3 - 440.2)	2327	(1486 - 3643)

RACCOMANDAZIONI E LINEE GUIDA



Updating the Italian Society of Clinical Pathology and Laboratory Medicine guidelines on the use of autoantibody tests in the diagnosis and monitoring of systemic rheumatic autoimmune diseases: analytical methods for the screening of antibodies to cellular antigens (ANA)

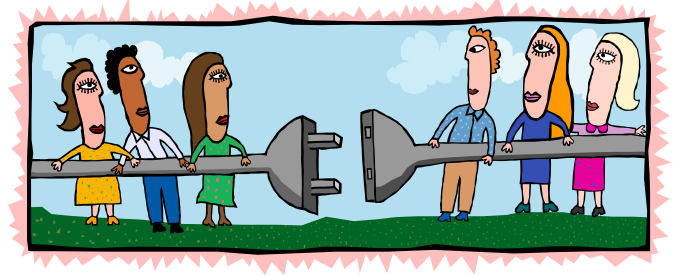


Luigi CINQUANTA¹ *, Maria INFANTINO², Mariangela MANFREDI²,
Massimo DAVES³, Antonella RADICE⁴, Danilo VILLALTA⁵,
Maria Concetta SORRENTINO⁶, Brunetta PORCELLI⁷, Lucia TERZUOLI⁷, Marcello BAGNASCO⁸,
Ignazio BRUSCA⁹, Nicola BIZZARO¹⁰ a nome del Gruppo di Studio in Autoimmunologia
della Società Italiana di Patologia Clinica e Medicina di Laboratorio

“For the detection of ANA, the use of the HEp-2 cell indirect immunofluorescence method is recommended, **combined with solid phase immunometric tests characterized by the presence of no less than 15-16 intracellular antigenic specificities related to the main systemic rheumatic autoimmune diseases”.**

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

FIRMA – APTIVA STUDY



Particle-based multi-analyte technology (PMAT)

Simultaneous detection of **29 autoantibodies**

1241 subjects clinically well-characterized:

782 CTD (SLE, pSS, SSc, UCTD, IIM) e **459** controls



PMAT-Aptiva

 **Inova
Diagnostics**
A Werfen Company

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	TIF1 γ
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%

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

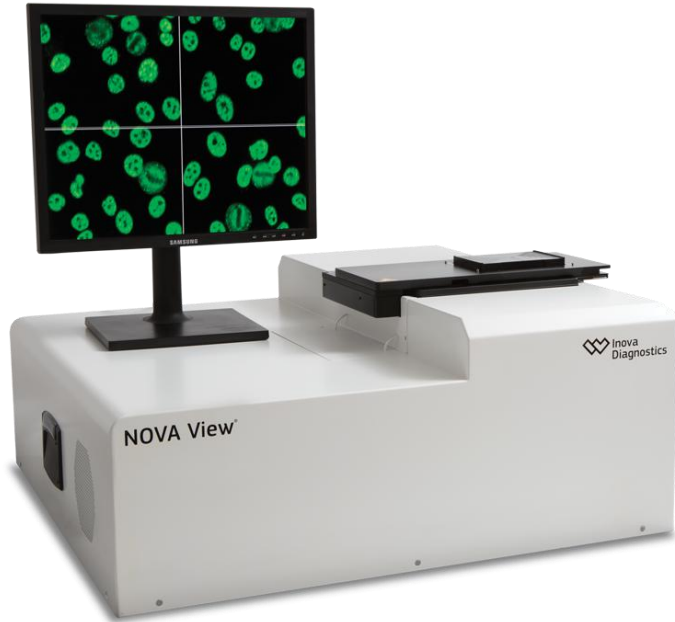
	Cutoff (FDA appr)	SP %
dsDNA	5	93.7
RNP	5	98.0
Sm	5	99.8
Ro52	5	98.5
Ro60	5	94.8
La	5	100
Scl-70	5	97.6
Jo-1	5	99.8
Centromere	5	97.4
DFS70	5	96.7
Ribo-P	5	98.9

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

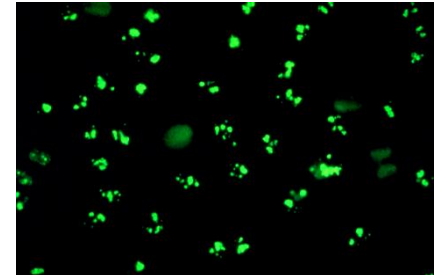
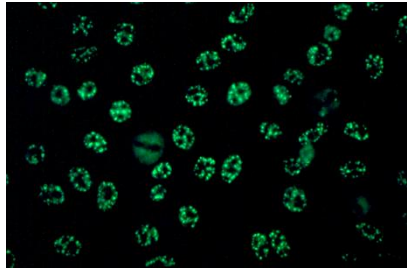
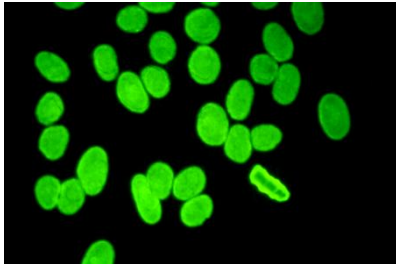
Overall diagnostic specificity of Aptiva PMAT
77.8%

ANA IMMUNOFLUORESCENCE TEST

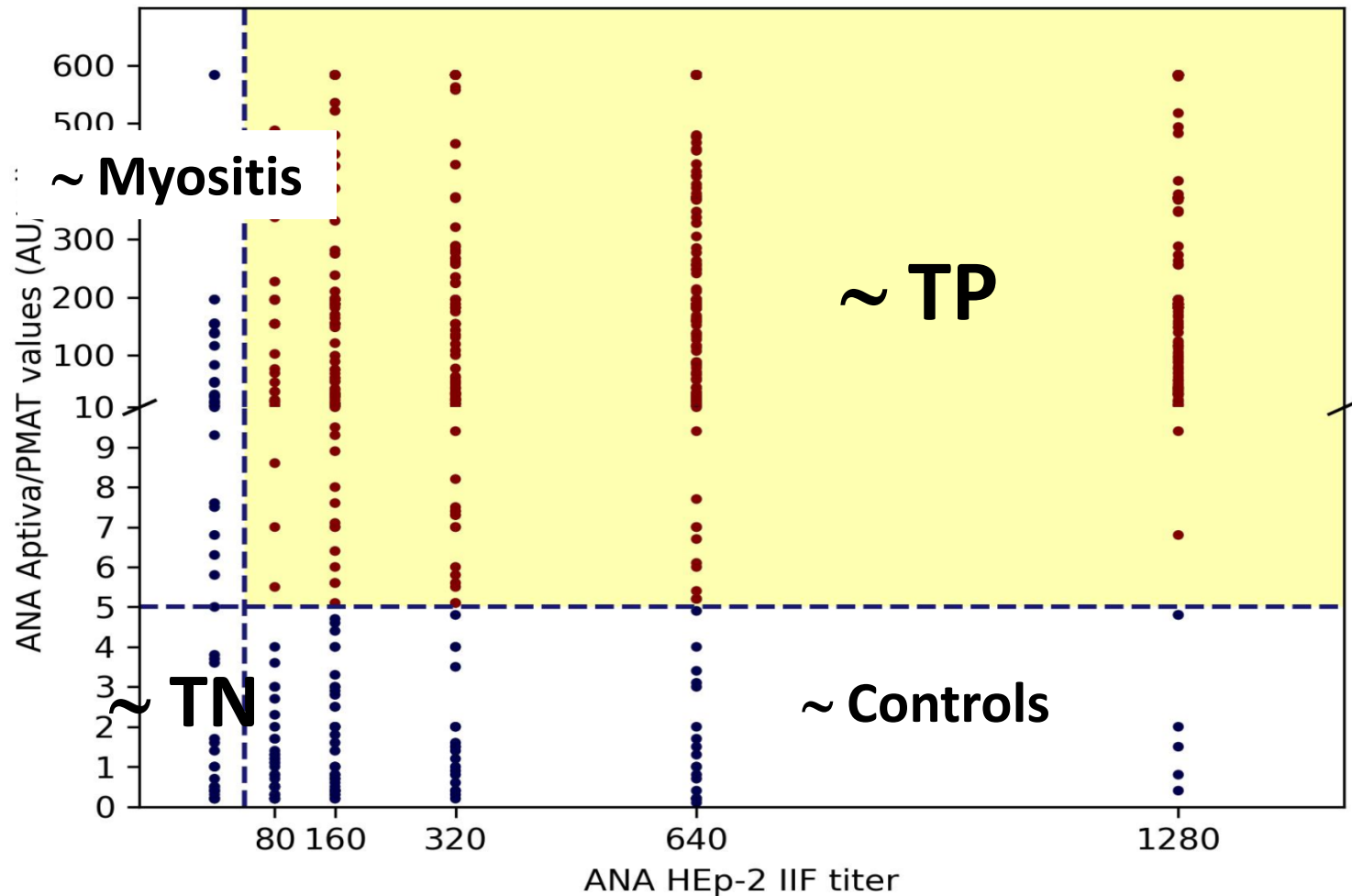


ANAs were detected using an automated HEP-2 IIF method (NOVA LITE, Inova Diagnostics).

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



Plot analysis comparing Aptiva/PMAT (values) and HEp-2 IIF (titer) results (dashed lines show cutoffs)



Since cutoff levels of CTD IgG Essential™ (5 arbitrary units (AU)/ml) and of CTD IgG Comprehensive™ / “Autoimmune Myopathy IgG™ (1 AU/ml) are different, to enable comparison of results, all test values of CTD IgG Comprehensive™ and Autoimmune Myopathy IgG™ have been multiplied 5x.

Results

ARD patients (782)

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

Controls (459)

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

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

(False) positives in other autoimmune/inflammatory disorders

Table IIIa. Number of positive samples detected by HEp-2 IIF and Aptiva/PMAT stand alone, and double positive samples for each single disease included in the control group named 'other autoimmune/inflammatory disorders'.

Disease	n.	Only Aptiva/PMAT positive	Only HEp-2 IIF positive	Double positive	Double negative
AIG	30	2 (6.7%)	13 (43.3%)	6 (20%)	9 (30%)
AITD	25	3 (12%)	10 (40%)	5 (20%)	7 (28%)
CD	25	3 (12%)	6 (24%)	6 (24%)	10 (40%)
FM	17	8 (47%)	2 (11.8%)	2 (11.8%)	5 (29.4%)
OA	3	0	1 (33.3%)	0	2 (66.7%)
PBC	30	4 (13.3%)	4 (13.3%)	6 (20%)	16 (53.4%)
PMR	23	3 (13%)	7 (30.4%)	2 (8.7%)	11 (47.9%)
PsoA	17	2 (11.8%)	5 (29.4%)	3 (17.6%)	7 (41.2%)
RA	15	1 (6.7%)	9 (60%)	3 (20%)	2 (13.3%)
AS	36	1 (2.8%)	19 (52.7%)	10 (27.8%)	6 (16.7%)
Total	221	27 (12.2%)	76 (34.4%)	43 (19.5%)	75 (33.9%)

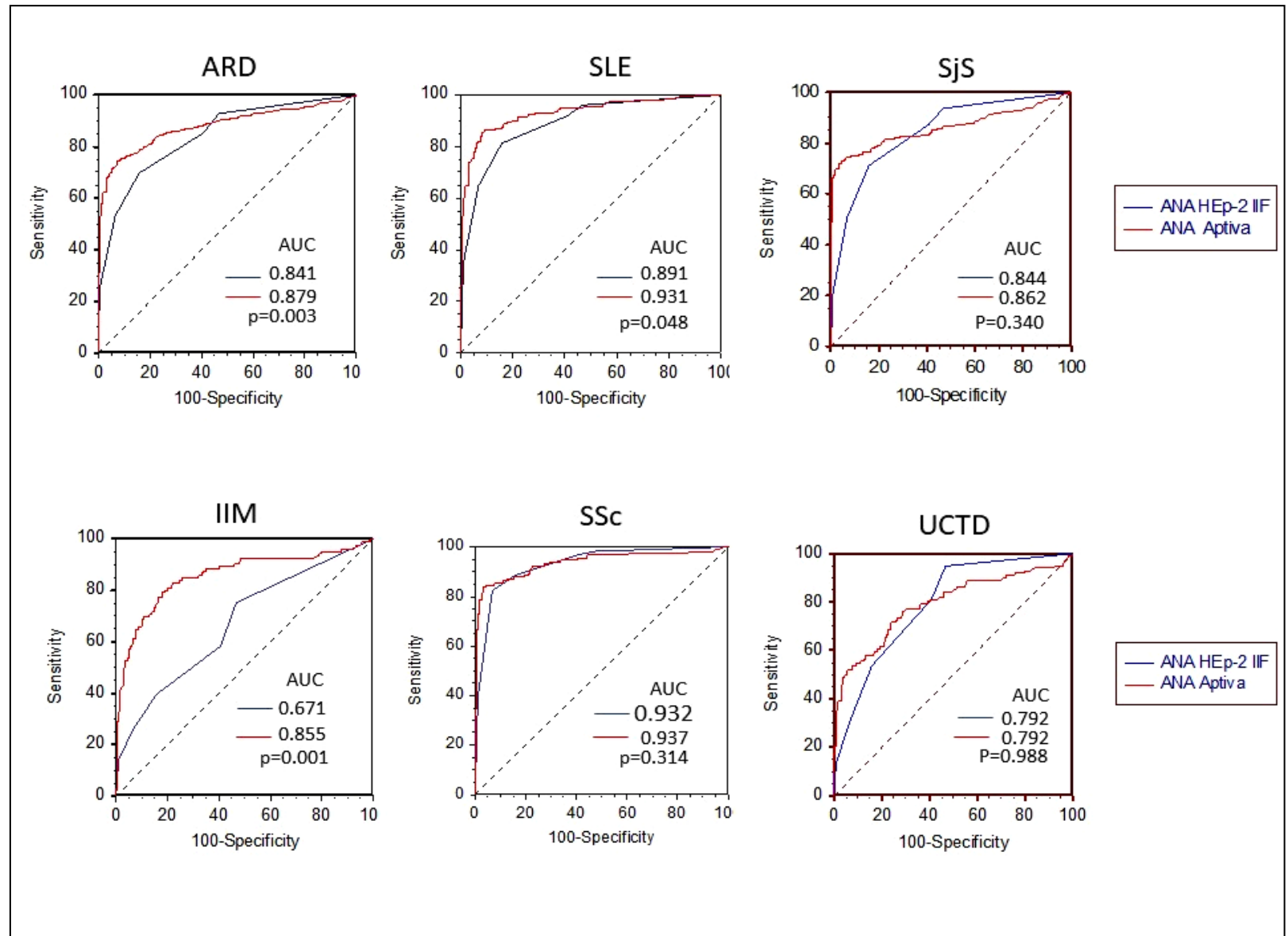
AIG: autoimmune gastritis; AITD: autoimmune thyroid disease; CD: coeliac disease; FM: fibromyalgia; OA: osteoarthritis; PBC: primary biliary cholangitis; PMR: polymyalgia rheumatica; PsA: psoriatic arthritis; RA: rheumatoid arthritis; AS: ankylosing spondylitis.

(False) positives in acute/chronic infections and healthy subjects

Table IIIb. Number of positive samples detected by HEp-2 IIF and Aptiva/PMAT stand alone, and double positive samples for each single disease included in the control group named 'acute/chronic infections'.

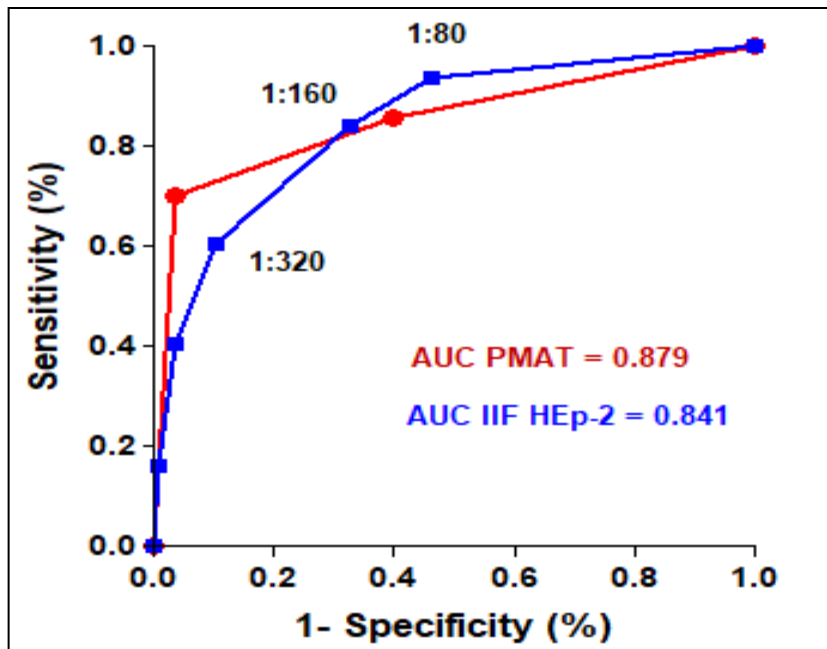
Disease	n.	Only Aptiva/PMAT positive	Only HEp-2 IIF positive	Double positive	Double negative
HBV	25	4 (16%)	11	0	10
CMV	22	2 (9.1%)	11	4	5
EBV	21	4 (19%)	7 (33.3%)	0	10 (47.7%)
HCV	27	2 (7.4%)	9 (33.3%)	2 (7.4%)	14 (51.9%)
SYP	23	1 (4.3%)	11 (47.8%)	2 (8.7%)	9 (39.2%)
Total	118	13 (11.1%)	49 (41.5%)	8 (6.8%)	48 (40.6%)
HD	120	11 (9.1%)	23 (19.2%)	6 (5%)	80 (66.7%)

ROC curves of HEp-2 IIF and Aptiva/PMAT for ARD patients overall, SLE, SjS, IIM, SSc, UCTD.



PMAT-APTIVA AS A SCREENING TEST

	Sensitivity	Specificity	Efficiency
Aptiva	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)

CONCLUDING REMARKS

- To date (2025), no single method is capable of providing absolute diagnostic accuracy.
- When PMAT-Aptiva is used as a screening test, its diagnostic efficiency is higher than the ANA IIF HEp-2 test.
- However, at present, HEp-2 IIF should not be considered outdated, as the sensitivity of this method in some rheumatic diseases is still higher than that of solid-phase methods (either CTD screen or PMAT).
- Similarly to what has already been proposed for the CTD screen, for optimal diagnosis, the combined use of the two methods (IIF and PMAT) is recommended.

