

RESEARCH UPDATES

Performance evaluation of the Zenit PRO, a new automated system for indirect immunofluorescence: a preliminary study

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Aim of the study

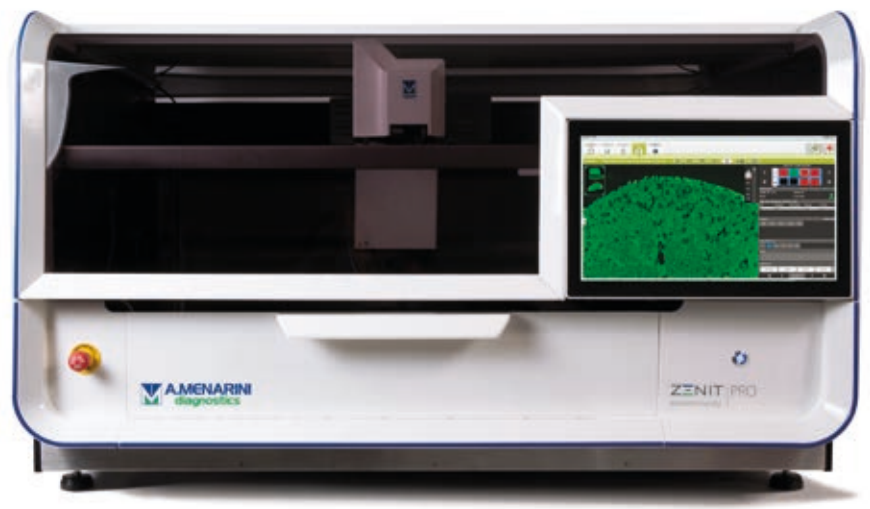
The newly developed Zenit PRO system (A. Menarini Diagnostics) is a fully automated instrument performing indirect immunofluorescence (IIF) assays that streamlines the complete IIF protocol, from slide processing to reading and interpretation of results (Fig. 1).

The aim of this study was a preliminary evaluation of Zenit PRO anti-nuclear antibody (ANA) testing by IIF on HEp-2 cells on a series of routine samples to set the negative/positive cut-offs and to evaluate operating mode, execution time and analytical performance.

Methods

We selected 64 ANA-positive patients with nuclear or cytoplasmic patterns at different titres (from 1:80 to >1:5120), among those 32 with definite diagnoses of autoimmune diseases, either systemic and organ-specific [10 systemic lupus erythematosus (SLE), 9 systemic sclerosis (SSC), 6 Sjögren's syndrome (SjS), 1 undifferentiated connective tissue disease (UCTD), 1 polymyositis, 5 primary biliary cholangitis (PBC)]. In particular, 59 ANA-positive patients (mean age 55±14; 50F/9M) with coverage of all the major nuclear patterns at different titres and five ANA-positive patients (mean age 62±11; 4M/1F) with cytoplasmic patterns

Figure 1: The Zenit PRO IIF automatic analyser



(three mitochondria-like, one diffuse fine speckled, one Golgi-like) at different titres (from 1:80 to 1:640).

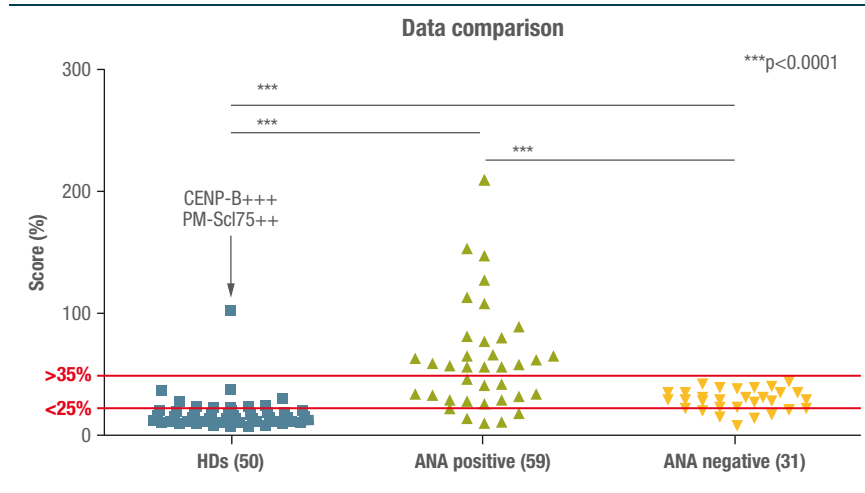
Thirty-one ANA-negative patients and 50 age/sex matched blood donors (HDs) were selected as a control series. We carried out three complete sessions on three different days. Eight positive samples (two homogeneous, two centromere, two fine speckled, two coarse speckled) at high (1:1280) and low (1:160) titre, were chosen for between-run (five runs in total) and

within-run repeatability tests and titrations. The Zenit PRO expresses the fluorescence intensity index score as a percentage (0 to 100% of the sensor saturation), so we compared % versus standard titrations observed in the same sera using the in-house automatic method for IIF HEp-2 analysis (Inova Diagnostics, CA).

Results

Overall, ANA-positive samples disclosed higher % scores than the HDs ($p < 0.0001$)

Figure 2: Raw data comparison between the fluorescence index scores obtained by the Zenit PRO in the HDs, ANA-positive and ANA-negative samples



and ANA-negative samples ($p < 0.0001$; Fig. 2). Of note, the only high positive sample among HDs finally disclosed high titre anti-centromere antibodies and anti-PM-Scl75 antibodies when tested by line blot (Euroimmun, Germany). When comparing ANA-positive samples versus HDs, the

ROC curve analysis (Fig. 3) identified the fluorescence intensity index score $< 25\%$ as the negative cut-off with a sensitivity of 85% and a specificity of 88% (LR 5.8), a grey zone between 25% and 35% and a positive cut-off $> 35\%$, with a sensitivity of 70% and a specificity of 94% (LR 3). When

these cut-offs were applied to the three series (Fig. 2), all the samples reported as ANA-negative using the in-house IIF HEp-2 analytical method, remained below 25% or in the grey zone, with a moderate-to-good final concordance between the two automatic IIF analysers (overall concordance 0.779; K of Cohen 0.563), that appeared in line with previous experiences as regards automated ANA IIF methods comparison^{1,2}. As shown in Table 1, the agreement between the two ANA IIF automatic methods increased from 50-75% for low-positive ANA samples (1:80 to 1:160) to 96-100% for high positive ANA samples (1:320 to $\geq 1:1280$).

As concerns the cytoplasmic patterns, the overall concordance was 83.3% (4/5); the only discordant result was at low titre 1:80 (diffuse fine speckled with anti-SRP antibodies).

As illustrated in Table 2A, between-run repeatability tests disclosed quite good performances in all the major ANA patterns, either at high or at low titre, with a mean CV of $18 \pm 6\%$. Even better results were obtained by the within-run repeatability tests (Table

Figure 3: ROC curve analysis and cut-off settings

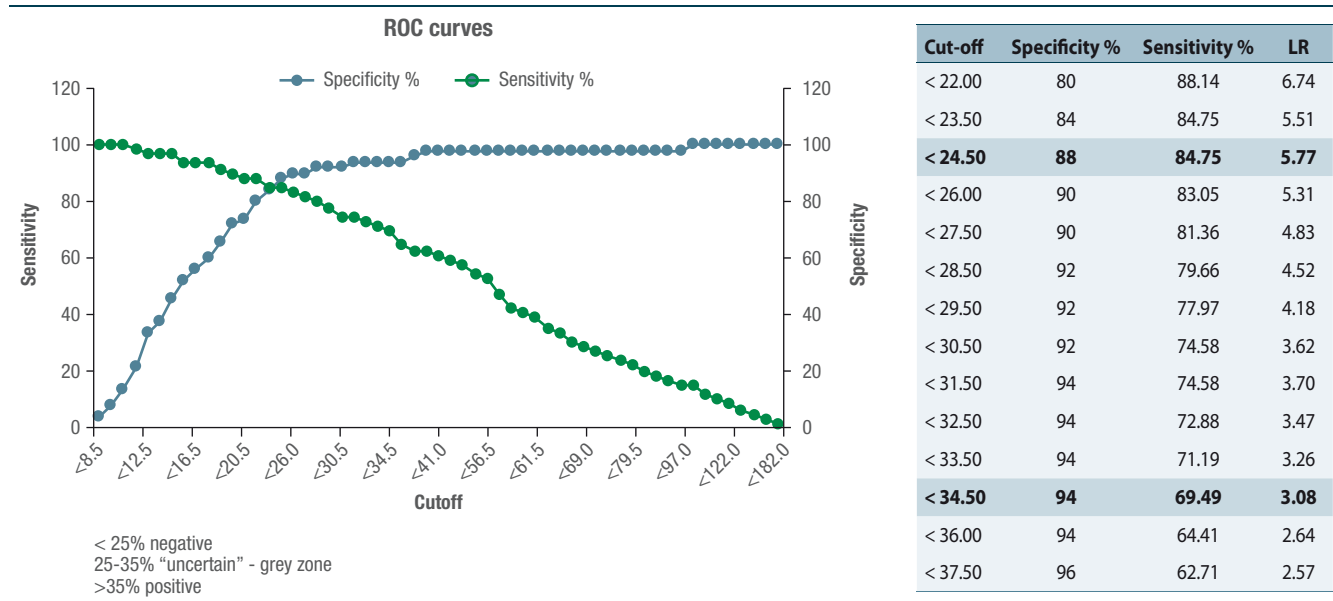


Table 1: Comparison between ANA results and interpretation by the in-house IIF method and the Zenit PRO. ANA-positive samples (nuclear patterns) are displayed by increasing ANA titre (from 1:80 to 1:5120). Samples with low titre (1:80 to 1:160) showed 50% to 75% of concordance, while high positive ANA samples (1:320 to \geq 1:1280) revealed very high concordance (96%-100%). Legend: pos= positive; neg= negative; unc: uncertain (grey zone)

Patient ID	Age	Sex	ANA pattern	Titre	ANA specificity	Diagnosis	ANA results in-house method	Zenit PRO index score	Zenit PRO interpretation
5569348604	50	F	DFS70-like	80	DFS70+		Pos	28%	Unc
5566647704	36	F	Nuclear Matrix	80			Pos	15%	Neg
5570797303	66	F	Homogeneous	80			Pos	12%	Neg
5567962906	74	F	Homogeneous	80	anti-dsDNA+++	SLE	Pos	42%	Pos
5548116907	29	F	Homogeneous	80	SSA-Ro52 / dsDNA	SLE	Pos	19%	Neg
5562825707	30	M	Coarse Speckled	80	Sm/RNP+		Pos	59%	Pos
5569375506	21	F	DFS70-like	160	DFS70+		Pos	20%	Neg
5567457703	51	F	DFS70-like	160	DFS70++ / SSA-Ro60+		Pos	32%	Unc
5570945205	67	M	Mitotic Fuse and Homog.	160			Pos	18%	Neg
5568824703	50	F	Nuclear Matrix	160			Pos	58%	Pos
5569442603	56	F	Multiple Nuc. dots	160	SP100++	PBC	Pos	23%	Neg
5568597703	56	F	Multiple Nuc. dots	160	PML++	PBC	Pos	25%	Unc
5570856103	39	F	Homogeneous	160			Pos	29%	Unc
5569544103	70	F	Homogeneous	160			Pos	27%	Unc
5545609707	78	M	Homo and cyto. diffuse fine sp.	160	OJ+		Pos	35%	Unc
5559429704	74	M	Homog. and nucleolar	160			Pos	47%	Pos
5548557603	55	F	Homog. and nucleolar	160			Pos	30%	Unc
5566787603	55	F	Fine speckled	160	SSA-Ro60++	SjS	Pos	43%	Pos
5569472904	63	F	Fine speckled	160	SSA-Ro60+++		Pos	15%	Neg
5569934405	65	M	Fine speckled	160			Pos	11%	Neg
5571915007	52	F	Fine speckled	160	SSA-Ro60+++	SjS	Pos	66%	Pos
5570371503	54	F	DFS70-like	320	DFS70+++		Pos	47%	Pos
5567404005	55	F	DFS70-like	320	DFS70+++		Pos	56%	Pos
5567791804	34	F	DFS70-like	320	DFS70+++		Pos	75%	Pos
5568612505	61	F	DFS70-like	320	DFS70+++		Pos	40%	Pos
5568504904	75	F	Homogeneous	320			Pos	37%	Pos
5570150604	43	M	Homogeneous	320	dsDNA++	SLE	Pos	35%	Unc
5571155406	53	F	Homogeneous	320	SSA-Ro60++		Pos	35%	Unc
5569373001	17	F	Homogeneous	320			Pos	33%	Unc

Table 1 Continued

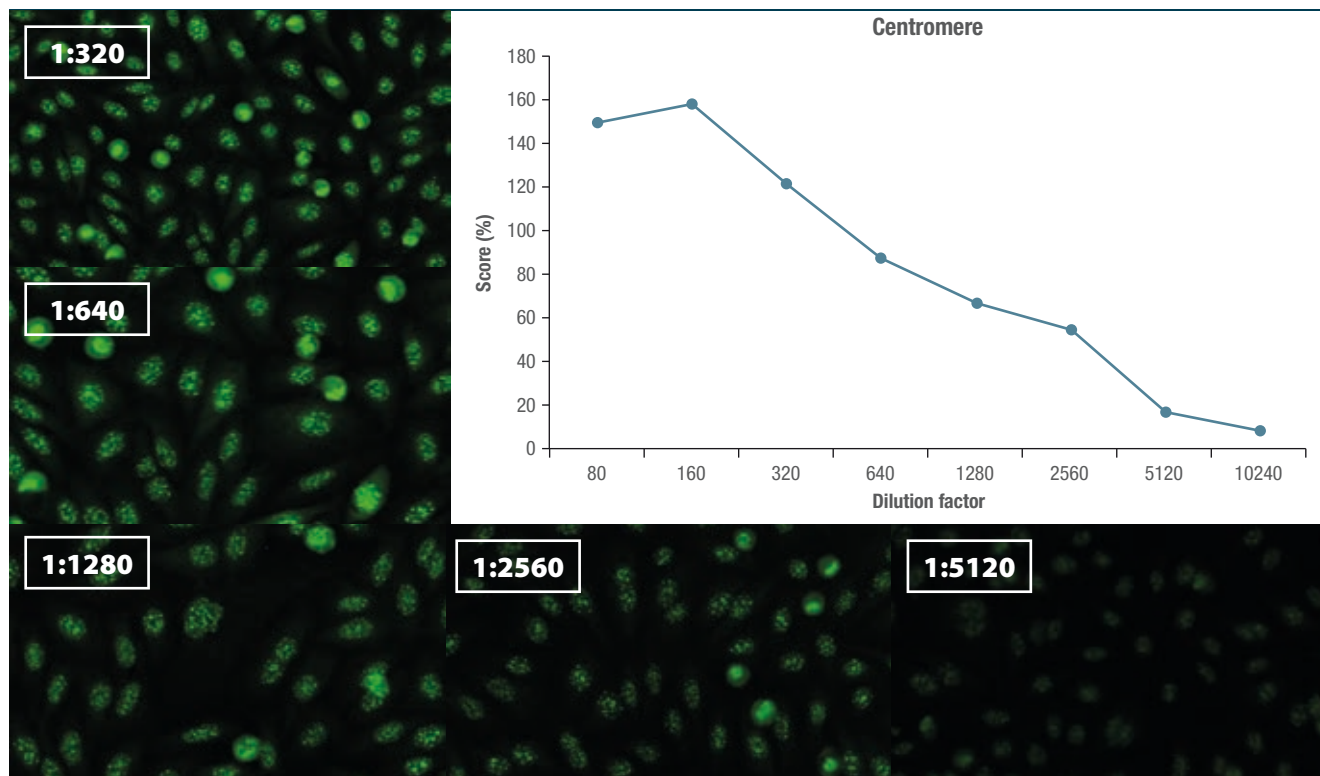
Patient ID	Age	Sex	ANA pattern	Titre	ANA specificity	Diagnosis	ANA results in-house method	Zenit PRO index score	Zenit PRO interpretation
5569536403	31	F	Homogeneous	320			Pos	57%	Pos
5570388704	51	F	Fine speckled	320	SSA-Ro60+++		Pos	64%	Pos
5563277005	62	F	Coarse Speckled	320	SSA-Ro60+++ / Sm/RNP+	UCTD	Pos	34%	Unc
5568098708	38	F	Homogeneous	640	dsDNA++	SLE	Pos	148%	Pos
5570450604	53	F	Homogeneous	640	SCL70+++	SSC	Pos	57%	Pos
5567973207	65	F	Homogeneous	640	SSA-Ro60+++	SJS	Pos	90%	Pos
5569079306	45	F	Homogeneous	640	dsDNA+++	SLE	Pos	210%	Pos
5568303503	46	F	Homogeneous	640			Pos	58%	Pos
5568573405	70	F	Homogeneous	640			Pos	60%	Pos
5559434806	47	F	Homogeneous	640	SCL70+++	SSC	Pos	81%	Pos
5552921708	62	F	Homogeneous	640	SCL70+++	SSC	Pos	23%	Neg
5532668303	50	M	Homogeneous	640	SCL70+++	SSC	Pos	57%	Pos
5532253606	69	F	Homogeneous	640	SCL70+++	SSC	Pos	82%	Pos
5563085904	70	F	Coarse Speckled	640	Sm/RNP+++		Pos	154%	Pos
5556853206	56	F	Centromere and fine speckled	640	CENP-B+++ / SSA-Ro52+	SSC	Pos	30%	Unc
5569494005	62	F	Centromere	1280	CENP-B+++	SSC	Pos	116%	Pos
5570420906	55	F	Centromere	1280	CENP-B+++	SSC	Pos	76%	Pos
5569340804	51	F	DFS70-like and few dots	1280	DFS70+++ / SP100+++		Pos	109%	Pos
5555702503	49	F	Nucleolar	1280	PM-Scl100+		Pos	63%	Pos
5545732305	41	F	Nucleolar	1280	dsDNA+		Pos	71%	Pos
5571349706	68	M	Homogeneous	1280	dsDNA+++	SLE	Pos	67%	Pos
5570426103	64	F	Homogeneous	1280		SLE	Pos	66%	Pos
5558073804	37	F	Homogeneous	1280	Nucleosome+++ / Histone++	SLE	Pos	78%	Pos
5568908104	69	F	Fine speckled	1280	SSA+++ / SSB+++	SJS	Pos	109%	Pos
5561229703	67	F	Centromere	2560		SJS	Pos	58%	Pos
5570407406	50	F	Homogeneous	2560	SSA+++	SJS	Pos	91%	Pos
5566259607	66	F	Homogeneous	2560	dsDNA+++	SLE	Pos	63%	Pos
5569975504	79	F	Mitotic Fuse and fine speckled	2560	SSA+++		Pos	128%	Pos
5569076504	69	M	Centromere	5120		SSC	Pos	142%	Pos
5568095106	76	F	Homogeneous	5120	dsDNA+++	SLE	Pos	114%	Pos

Table 2: Raw data of the between-run repeatability tests (A) and of the within-run repeatability tests (B)

A - Between-run											
Sample ID	Pattern	Run 1	Run 2	Run 3	Run 4	Run 5	Mean	SD	CV%		
5569076504	CENP +++	142	113	92	118	132	119	17.11	14.33%		
5571349706	Homog +++	67	59	76	82	71	71	7.82	11.02%		
5568908104	Fine Sp+++	109	203	176	144	142	155	32.10	20.73%		
5563085904	Coarse Sp+++	154	132	107	158	155	141	19.45	13.77%		
5562825707	Coarse Sp+	59	30	54	48	50	48	9.85	20.43%		
5570150604	Homog +	35	34	56	71	40	47	14.27	30.24%		
5570388704	Fine Sp+	64	80	52	50	55	60	11.00	18.27%		
5569494005	CENP +	116	76	116	116	116	108	16.00	14.81%		
										Mean	18%
										SD	6%

B - Within-run												
Sample ID	Pattern	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Mean	SD	CV%
5569076504	CENP +++	138	182	178	162	161	169	177	186	169	15.5	0.09
5571349706	Homog +++	111	75	96	108	101	102	109	95	100	11.6	0.12
5568908104	Fine Sp+++	144	152	146	140	162	136	142	179	150	14.1	0.09
5563085904	Coarse Sp+++	158	172	174	201	167	187	190	192	180	14.6	0.08
											Mean	10%
											SD	1%

Figure 4: Titration performance in a sample with high titre centromere pattern



2B), which revealed a mean CV around 10% for all the patterns investigated. Also the titrations run very well, as we observed a linear response along titrations for all the different patterns (see Figure 4 for the centromere sample).

Each session (comprising about 150 samples) was run completely (from slide processing to reading) in about four hours. The touch-screen monitor incorporated in the processing unit provides an intuitive and simple interface, displaying high reso-

lution images with >3000 cells per well, and offering the possibility to navigate inside the well as a virtual microscope. In addition, a mitotic gallery is always available to discriminate critical cases.

Conclusions

The Zenit PRO automatic IIF analyser gave the impression of being a highly promising instrument. It showed good agreement with the in-house automatic method and good analytical performances. Its unique features

(end-to-end management of the overall IIF analytical process) will allow maximizing the “walk-away” time and improve the standardization of the entire process.

Consolidation of the preliminarily identified negative/positive cut-offs is underway using larger series as is the optimization of the software for pattern recognition and other IIF substrate automatic analyses. This will finally close the gap in standardization by reducing significantly the variability of subjective interpretation.

References

- 1 Bizzaro N, Antico A, Platzgummer S, Tonutti E, et al. Automated antinuclear immunofluorescence antibody screening: a comparative study of six computer-aided diagnostic systems. *Autoimmun Rev* 2014;13(3):292-8.
- 2 Infantino M, Meacci F, Grossi V, et al. The burden of the variability introduced by the HEP-2 assay kit and the CAD system in ANA indirect immunofluorescence test. *Immunol Res* 2017;65(1):345-354

COMPANY PINBOARD

Latest Marketing & Scientific Events

SPML Congress, Figueira da Foz (Portugal)

In mid April 2018, about 350 participants attended an Autoimmunity Symposium organized by **A. Menarini Diagnostics** Portugal during the 10th SPML Meeting of Laboratory Medicine, held in Figueira da Foz.

Prof. Abreu (Nova University, Lisbon), Dr. Ramos (Unilabs) and Dr. Daria Picchioni (Visia Imaging) presented and discussed the following topics:

- Is there still a role for IIF in the Autoimmune Serology Laboratory?
- Laboratory challenges: traceability, quality control and workflow
- An all-in-one workstation for IIF automated procedures.



The speakers with part of the Menarini Diagnostics team at the SPLM meeting

2018 International Congress on Autoimmunity (16th-20th May 2018)

The 11th International Congress on Autoimmunity will take place in Lisbon, Portugal at the Lisbon Congress Center.

Organized by Professor Yehuda Shoenfeld, the Congress will see the participation of the main international leaders in Autoimmune diseases.

Participants are welcome to take advantage of the following contributions

provided by **A. Menarini Diagnostics**, a Gold Sponsor of the meeting:

- The A. Menarini Diagnostics 64 sqm booth will display the latest technical achievements in IFA. Visitors will have the opportunity to stop by and discuss specific topics;
- A Parallel Session (PL28) in Auditorium I (18th May, 14:00-16:00) "Dilemmas in the diagnosis of autoimmune

diseases, detection and standardization", will feature Nicola Bizzaro (Italy), Xavier Bossuyt (Belgium) and Guy Serre (France) as Chairmen

- A Short Oral Discussion (SO15, 19th May, 13:15-13:20) "Zenit PRO, a fully automated indirect immune fluorescence analyser: a preliminary evaluation of the analytical performance" by Dr. Martina Fabris (Italy).

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