INVESTIGATION OF ANA SCREENING METHODS IN THE DIAGNOSIS OF AUTOIMMUNE DISEASES

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KEY MESSAGES

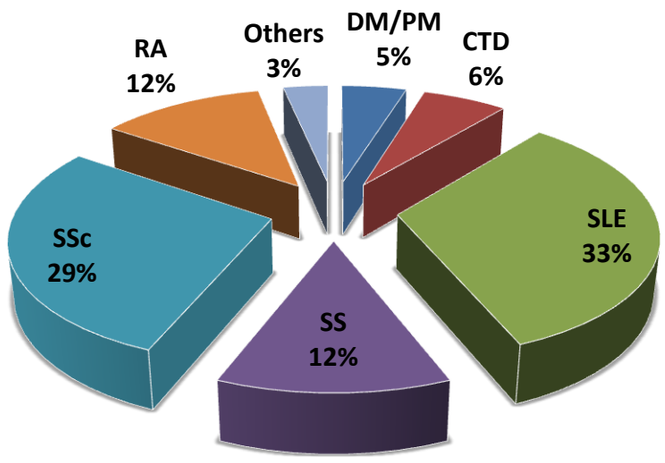
- HEp-2 IIF is still recommended for ANA screening
- Limitations of the assay are acknowledged
- Good performance was found for QUANTA Flash CTD Screen SPA *vs*. IIF

INTRODUCTION

Antinuclear antibodies (ANA) detection is important in the diagnosis of several autoimmune diseases and indirect immunofluorescence (IIF) on HEp-2 cells is the most commonly used method, which is however affected by several pitfalls. Automated ANA readers have been recently proposed, but some limitations still endure and new ANA solid phase assays (SPA) on automated closed platforms are evolving. The aim of this multi-center study was to evaluate ANA screening in patients with a diagnosis of systemic autoimmune diseases and regular follow-up by a novel SPA compared to IIF.

METHODS

271 well defined autoimmune patients were recruited (Figure 1). Control groups included 33 non-autoimmune pathological controls (PC) and 62 healthy donors (HC).



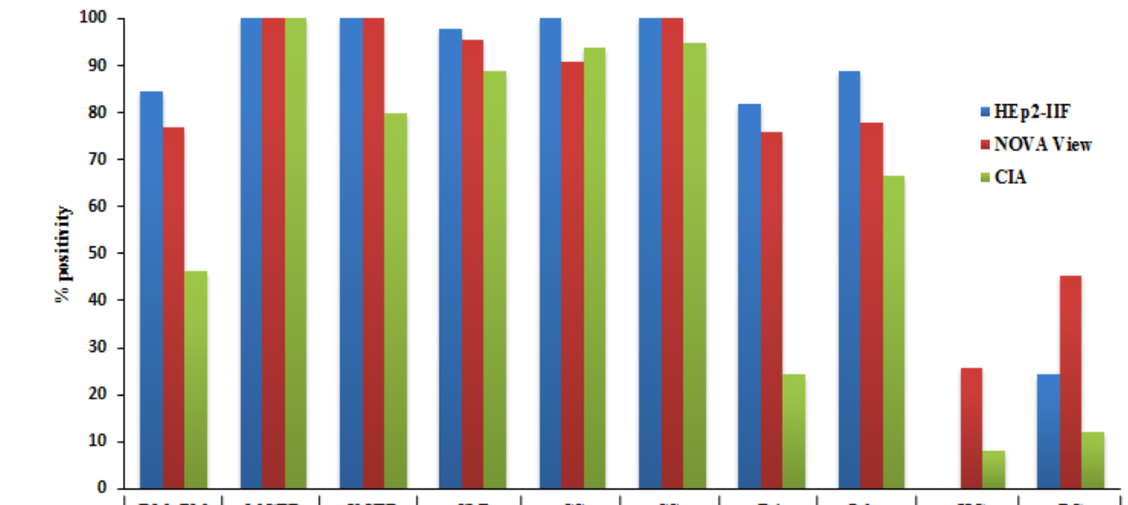
Abbreviations:
RA=rheumatoid arthritis
SSc=systemic sclerosis
SS=Sjögren's syndrome
SLE=systemic lupus erythematosus
CTD= connective tissue disease
DM/PM=dermatomyosytis/polymyosytis
others= overlap syndromes

Figure 1 Distribution of autoimmune diseases within patients cohort

ANA screening was performed using the chemiluminescence assay (CIA) QUANTA Flash CTD Screen Plus (BIO-FLASH instrument, Inova Diagnostics, San Diego, USA) which includes the most relevant nuclear/cytoplasmic antigens: dsDNA, Sm/RNP, Ro52, Ro60, SS-B, Scl-70, centromere, Mi-2, Ku, ThTo, RNAPol III, Pm/Scl, PCNA, Jo-1 and ribosomal-P protein. ANA were also detected by HEp-2 IIF with both visual and automated interpretation (NOVA View, Inova Diagnostics).

RESULTS

The percentage and the absolute numbers of samples positive for ANA detected by HEp-2 IIF or SPA are showed in Figure 2.



	DM+PM	MCTD	UCTD	SLE	SS	SSc	RA	Others	HC	PC
HEp2-IIF	11	12	5	87	33	77	27	8	0	8
NOVA View	10	12	5	85	30	77	25	7	16	15
CIA	6	12	4	79	31	73	8	6	5	4

Figure 2 Percentage and numbers of ANA-positive samples detected by HEp-2 IIF or CIA.

The comparison between SPA and IIF showed a good overall (83.1%) and negative agreement (88.8%) with a slightly lower positive concordance (81.0%). The agreement markedly improved after RA patients were excluded, as the SPA does not include RA specific antigens Table 1).

Table 1 Overview of the concordance between HEp-2 IIF and CIA.

Agreement	SPA vs. IIF	SPA vs. IIF (excluding RA)
% Positive Agreement (95% CI)	81.0% (73.4-83.0%)	87.1% (82.9-91.4%)
% Negative Agreement (95% CI)	88.8% (82.5-95.0%)	89.1% (82.8-95.5%)
% Overall Agreement (95% CI)	83.1% (76.6-84.9%)	87.7% (84.2-91.2%)
Cohen's *kappa*	0.62	0.71

Looking at diagnosis, the SPA displayed comparable specificity and lower sensitivity *vs.* IIF (Table 2). ROC curves confirmed the improved performance of the SPA after the exclusion of RA patients (Figure 3).

Table 2 Comparison between HEp-2 IIF and CIA.

INCLUDING RA	HEp-2 IIF % (95% CI)	QF CTD Screen % (95% CI)
Accuracy	94.8 (92.5 – 97.1)	83.3 (79.5 – 87.2)
Sensitivity	95.9 (93.6 – 98.3)	80.8 (76.1 – 85.5)
Specificity	91.6 (86.0 – 97.2)	90.5 (84.6 – 96.4)
PPV	97.0 (95.0 – 99.1)	96.1 (93.5 – 98.6)
NPV	88.8 (82.5 – 95.0)	62.3 (54.2 – 70.4)
LR+/LR-	11.39 / 0.04	8.53 / 0.21
OR	257 (100 – 660)	40 (19 – 85)

EXCLUDING RA	HEp-2 IIF % (95% CI)	QF CTD Screen % (95% CI)
Accuracy	96.1 (94.0 – 98.2)	89.2 (85.9 – 92.5)
Sensitivity	97.9 (96.1 – 99.7)	88.7 (84.6 – 92.7)
Specificity	91.6 (86.0 – 97.2)	90.5 (84.6 – 96.4)
PPV	96.7 (94.4 – 98.9)	95.9 (93.3 – 98.5)
NPV	94.6 (89.9 – 99.2)	76.1 (68.2 – 84.0)
LR+/LR-	11.63 / 0.02	9.36 / 0.13
OR	507 (161 - 1591)	75 (34 - 165)

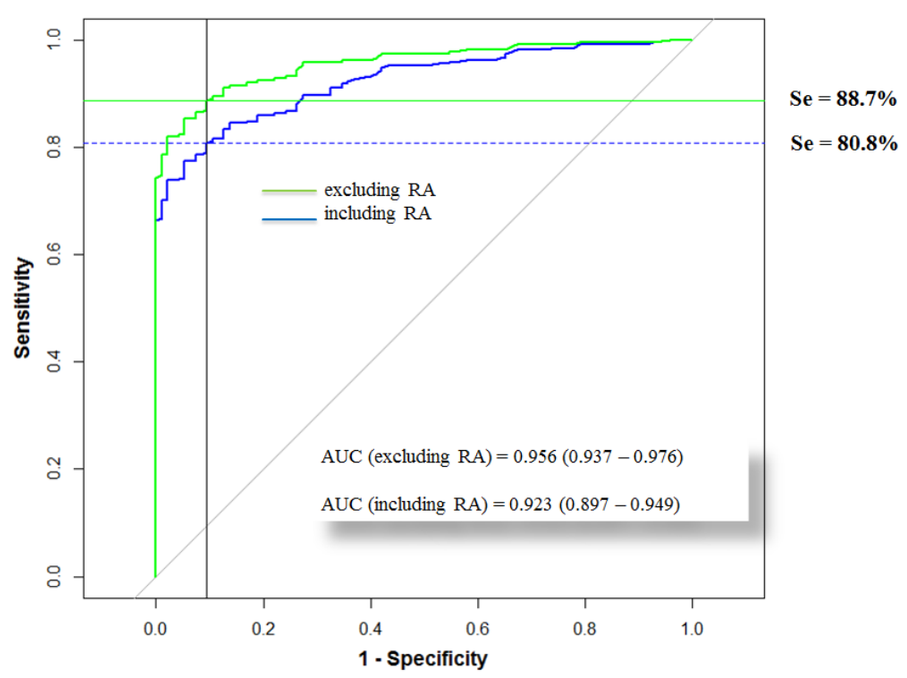


Figure 3 ROC analysis of QUANTA Flash CTD Screen *vs.* diagnosis

CONCLUSION

The QUANTA Flash CTD Screen Plus shows good agreement with IIF especially when RA patients are not considered. Further studies are needed, preferably on diagnostic samples, to define the potential position of the new SPA in ANA testing algorithms.

REFERENCES

1. Meroni PL, Shur P. ANA screening: an old test with new recommendations. *Ann Rheum Dis* 2010, 69:1420-22.
2. Bossuyt X, Fieuws S. Detection of antinuclear antibodies: added value of solid phase assay? *Ann Rheum Dis* 2014, 73:e10.

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