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Clinical performance of three automated screening assays for antibodies against nuclear antigens

Gerben Zuiderveld[1], Jérémie Gautier[2] and Christian Konrad[1]
[1]Thermo Fisher Scientific, Freiburg, Germany; [2]Cerba Xpert, Saint-Ouen l'Aumône, France

BACKGROUND

Connective tissue disease (CTD) is a synonym for a group of systemic autoimmune diseases with many similar clinical features. The analysis of patient samples for autoantibodies against anti-nuclear antigens (ANA) and extractable nuclear antigens (ENA), a subset of ANA, represent an important aid in the diagnoses of CTD, e.g. Sjögren's Syndrome (SjS), Systemic Lupus Erythematosus (SLE), Systemic Sclerosis/Scleroderma (SSc), Poly-/Dermatomyositis (PM/DM) and Mixed Connective Tissue Disease (MCTD) [1].

Solid phase assays for screening patient serum samples for autoantibodies against ANA and ENA are commercially available and include antigens, e.g. Ro52 and Jo-1, to detect autoantibodies that have been reported to be frequently missed by immuofluorescence assays (IFA) [2]. Besides in their antigen composition, commercially available solid phase assays also differ in the source of the included antigens (native vs recombinant vs peptides).

The Thermo Scientific EliA™ Symphony^S assay is a newly developed ENA screening assay that differs form other ANA/ENA screening assays by comprising only recombinant human proteins and a synthetic SmD_3 peptide but no antigens from native sources [3, 4]. Previous studies reported that using recombinant proteins and the synthetic SmD_3 peptide improved the clinical performance of antibody assays by leading to an increased specificity [3, 10, 11]

AIMS

Using a defined serum panel of patients clinically diagnosed with various CTD as well as various disease controls, this study aimed to analyze the clinical performance of the EliA Symphony^S assay [3, 4]. Additionally, its clinical performance was compared to two assays for the detection of ANA/ENA from different manufacturers that have a similar antigen composition (Table 1). The INOVA Diagnostics QUANTA Flash™ ENA7 assay is comprised of recombinant and native antigens but excludes CENP-B in its analyte composition [5], while the A.Menarini Zenit RA™ ANA Screen assay has the same analyte composition as the EliA Symphony^S assay plus dsDNA but their source (recombinant or native) were not indicated in the instructions for use [7].

METHODS

To analyze and compare the clinical performance, a serum panel comprising 404 samples from patients diagnosed with SLE, SjS, SSc, PM/DM and MCTD and 229 disease controls (table 2) was analyzed with the EliA Symphony^S assay and the two screening assays from the other manufacturers (table 1). All samples were measured according to the manufacturers' instructions. The reported results were analyzed using the commercial software Microsoft Excel and Analyze-it, Graphpad Prism 4.

Antigen	EliA Symphony^S assay	QUANTA Flash ENA7 assay	Zenit RA ANA Screen assy
SS-A/Ro52	Human recombinant	Recombinant, species not stated	Source not indicated
SS-A/Ro60	Human recombinant	Recombinant, species not stated	Source not indicated
SS-B/La	Human recombinant	Recombinant, species not stated	Source not indicated
Scl-70	Human recombinant	Recombinant, species not stated	Source not indicated
Jo-1	Human recombinant	Recombinant, species not stated	Source not indicated
CENP-B	Human recombinant	- not included -	Source not indicated
RNP	Human recombinant (RNP70,A,C)	Calf thymus	U1RNP (RNP70, A, C); source not indicated
Sm	SmD_3 peptide	Calf thymus	Source not indicated
dsDNA	- not included -	- not included -	Source not indicated
Measuring range	Ratio 0.09 - 60	3.6 – 429.4 CU	Ratio; range not indicated
Result interpretation / reference range	< 0.7 – negative 0.7 – 1.0 – equivocal > 1.0 – positive	< 20 – negative ≥ 20 – positive	< 1 – negative ≥ 1 – positive

Table 1: Overview of the assays analyzed in this study. The included analytes and their source (recombinant, native or peptide) as well as key features are indicated as mentioned in the respective instructions for use [4 – 7].

Connective Tissue Disease (CTD)	Number (n=404)	Disease controls (DC)	Number (n=229)
Systemic Lupus Erythematosus	97	Rheumatoid Arthritis	85
Sjögren's Syndrome	96	Viral Infection	99
Systemic Sclerosis	87	Bacterial Infection	20
Poly -/ Dermatomyositis	78	Tumor	25
Mixed Connective Tissue Disease	46		

Table 2: Overview of the serum panel analyzed in this study.

RESULTS I – CLINICAL PERFORMANCE

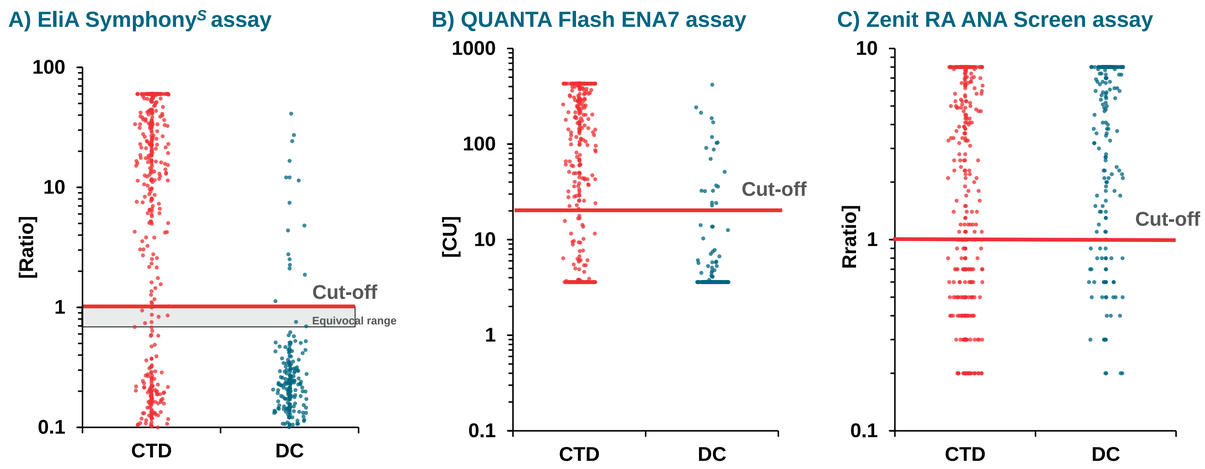

Figure 1: Performance analysis of **A)** EliA Symphony^S assay, **B)** QUANTA Flash ENA7 assay and **C)** ZENIT RA ANA Screen assay using the sample cohort from table 2. For better visualization, results reported outside of the test specific measuring ranges were set to the respective lower and upper limits. CTD = connective tissue disease; DC = disease controls

	Sensitivity	Specificity	TP	FP	TN	FN	PPV	NPV
EliA Symphony^S assay (equiv = neg)	66.6%	93.0%	269	16	213	135	94.4%	61.2%
EliA Symphony^S assay (equiv = pos)	68.3%	92.6%	276	17	212	128	94.2%	62.4%
QUANTA Flash ENA7 assay	67.8%	91.3%	274	20	209	130	93.2%	61.7'%
Zenit RA ANA Screen assay	80.0%	49.3%	323	116	113	81	73.6%	58.2%

Clinical performance at stratified specificity 93%*	Cut-off	Sensitivity	Specificity	TP	FP	TN	FN	PPV	NPV
EliA Symphony^S assay	1	66.6%	93.0%*	269	16	213	135	94.4%	61.2%
QUANTA Flash ENA7 assay	32 CU	64.4%	93.0%*	260	16	213	144	94.2%	59.7%
Zenit RA ANA Screen assay	Not possible to calculate with obtained ROC data								

Table 3: Clinical performance of EliA Symphony^S assay, Quanta Flash ENA7 assay and Zenit RA ANA Screen assay analyzing the sample cohort from table 2. Results were interpreted applying the manufacturer recommended cut-offs. EliA Symphony^S assay differ from the other assays by having an equivocal range. Therefore, samples found as equivocal (equiv) with the EliA Symphony^S assay were either considered negative (neg) or positive (pos) when calculating diagnostic accuracy. For better comparison of the three assays, the Receiver Operating Characteritic (ROC) curve data were used to analyze sensitivities at a defined (stratified) specificity. TP: true positives; FP: false positives; TN: true negatives; FN: false negatives; PPV: positive predictive value; NPV: negative predictive value; *stratified specificity

RESULTS II – Comparison of EliA Symphony^S assay and Quanta Flash ENA7 assay

The measurement of antibodies against CENP aids in the diagnosis of systemic sclerosis patients [8]. In contrast to EliA SymphonyS, QUANTA Flash ENA7 lacks CENP-B [4, 5]. Therefore, both assays were compared in more details. Due to its low specificity in this study, Zenit RA ANA Screen was not included in further analyses.

	EliA Symphony^S assay (equiv = neg)	QUANTA Flash ENA7 assay
Total agreement	95.1%	
Positive agreement	93.1%	
Negative agreement	96.8%	
Samples above measuring range	25.7%	43.1%

Table 4: Agreement between EliA Symphony^S assay and QUANTA Flash ENA7 assay for the serum panel described in table 2. In the lack of an international standard for antigen composition and titer for ENA screening assays, only the agreement and not the correlation was determined. The upper limit of the measuring range from the respective test manuals were applied [4, 6].

	Sensitivity (Systemic Sclerosis)
EliA Symphony^S assay (equiv = neg)	67.8%
EliA Symphony^S assay (equiv = pos)	72.4%
QUANTA Flash ENA7 assay	66.7%

Clinical performance at stratified specificity 93%	Sensitivity (Systemic Sclerosis)
EliA Symphony^S assay (cut-off 1)	67.8%
QUANTA Flash ENA7 assay (cut-off 32 CU)	62.1%

Table 5: Clinical performance (sensitivity) of EliA Symphony^S assay and QUANTA Flash ENA7 assay as described in table 3 but considering only the samples from systemic sclerosis patients (table 2). The sensitivity at the stratified specificity of 93% (table 3) was also determined for better comparison.

SUMMARY and CONCLUSIONS

- EliA Symphony^S assay has a good clinical performance and aids in the diagnosis of CTD.
- Among the assays included in this study, the EliA Symphony^S assay had the highest specificity, produced the lowest number of false positive test results and showed the highest positive predictive value (PPV).
- EliA Symphony^S assay is distinct by including only recombinant proteins and a synthetic peptide as antigens. Its higher specificity is in line with previous studies reporting that recombinant proteins and synthetic peptides can increase the specificity of an antibody assay [3, 9 - 11].
- At the same (stratified) specificity, the EliA Symphony^S assay showed the highest sensitivity in this study.
- The inclusion of human recombinant CENP-B in the antigen composition of the EliA Symphony^S assay provides the laboratory with the capability to screen patient samples, e.g. from systemic sclerosis patients, where the predominant autoantibody is against CENP [8].

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TRADEMARKS

CONTACT: christian.konrad@thermofisher.com