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The new EliA Symphony^{S}

Diagnostic Performance of the novel EliA Symphony^{S}
Increased Sensitivity for Antibodies against SmD and Scl-70

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Editorial

As an innovative company committed to providing high quality autoimmunity tests, Thermo Fisher Scientific is excited to introduce EliA™ Symphony^{S}.

EliA Symphony^{S} represents a further development/ enhancement of the well-established EliA™ Symphony screening test for autoantibodies against extractable nuclear antigens (ENA) associated with connective tissue diseases (CTD). The superscript S in EliA Symphony^{S} is an acronym for “sensitive”.

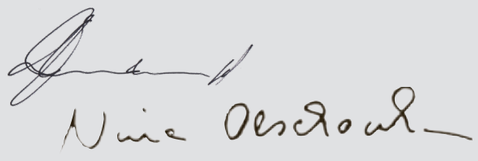
We are the first company to deliver an ENA screen which uses only human recombinant antigens in combination with a synthetic SmD peptide. Performance of both the old and the new EliA Symphony tests was demonstrated in an internal study including over 1,000 clinically defined samples. The Erasmus MC, University Medical Center Rotterdam was also approached to do an extensive evaluation of EliA Symphony^{S} compared to the current EliA Symphony, the findings of which are shared on pages 3 to 7.

We thank the following people for their cooperation and contribution to this ImmunoDiagnostics Journal; Marco W.J. Schreurs, PhD, Medical Immunologist, José Huybers, Jac Kuijpers-Entrup, Roseri Roelofsen-de Beer, PhD, Clinical Chemist i.t. and Pieter van der Pol, PhD, Medical Immunologist.

On page 3 you can find: Diagnostic performance of the novel EliA Symphony^{S} for screening antibodies against extractable nuclear antigens (ENA) by Marco W.J. Schreurs Medical Immunologist of the Erasmus MC, University Medical Center Rotterdam.

On page 7 you can find our internal study: EliA Symphony^{S} - An integral part of your diagnostic screening algorithm for connective tissue diseases.

Enjoy reading,
Gerben Zuiderveld and Nina Olschowka



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Diagnostic performance of the novel EliA Symphony^{S} for screening antibodies against extractable nuclear antigens (ENA)

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Objective

Anti-nuclear antibodies (ANA) are the hallmark of systemic autoimmune rheumatic diseases (SARD), including Systemic Lupus Erythematosus (SLE), Sjögren´s Syndrome (SjS), Systemic Sclerosis (SSc), Mixed Connective Tissue Disease (MCTD) and Polymyositis/ Dermatomyositis (PM/DM). A subset of ANA are specifically associated with these SARD and the respective antibody targets are collectively referred to as extractable nuclear antigens (ENA). The most common ENA include SS-A/Ro, SS-B/La, U1RNP, Sm, Scl-70, Centromere B and Jo-1. Approximately 15 years ago, TFS/Phadia developed a fluoroenzymeimmunoassay (FEIA), the EliA Symphony, that enables simultaneous screening for antibodies against these ENA in a single test performed on their random access instrument.

TFS/Phadia recently developed a novel version of the EliA Symphony, the Symphony Sensitive (S). The EliA Symphony^{S} wells are coated with human recombinant U1RNP (RNP70, A, C), SS-A/Ro (60 kDa, 52 kDa), SS-B/La, Centromere B, Scl-70 and Jo-1 proteins, and synthetic SmD_3 peptide. Compared to the original EliA Symphony, the Scl-70 substrate has been biotinylated and is bound to solid phase streptavidin, and the purified Sm substrate has been replaced by synthetic SmD_3 peptide. Both modifications are intended to improve overall sensitivity of screening antibodies against extractable nuclear antigens (ENA). In addition, the antigen substrate of EliA Symphony^{S} is now harmonized with that of the specific EliA tests for anti-Scl-70 and anti-Sm in which the before mentioned modifications have been executed previously.

The current study evaluates the diagnostic performance of EliA Symphony^{S} for screening antibodies against ENA, prospectively in patients suspected of SARD and retrospectively in patients previously diagnosed with SARD. Sensitivity and specificity of EliA Symphony^{S} is directly compared with that of EliA Symphony.

Patients and methods

The study included an unselected prospective study population of 247 patients suspected of SARD and submitted for routine ANA testing to the Laboratory Medical Immunology of the Erasmus MC (secondary/ tertiary care center) over the course of two months. Afterwards, the medical records of the subjects were evaluated for SARD diagnosis. In this study, SARD was

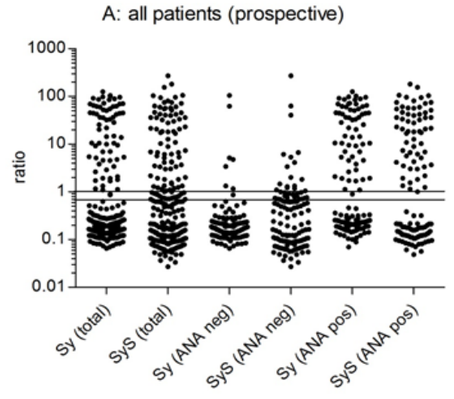

Figure 1A: EliA Symphony^{S} (SyS) test results compared to EliA Symphony (Sy) in a cohort of prospectively included patients suspected of systemic autoimmune rheumatic disease (SARD). Panel A shows all patients. Lines represent the borderline area of the test (0.7-1.0 ratio). ANA: anti-nuclear antibody, determined by indirect immunofluorescence (IIF) on HEp-2 cells.

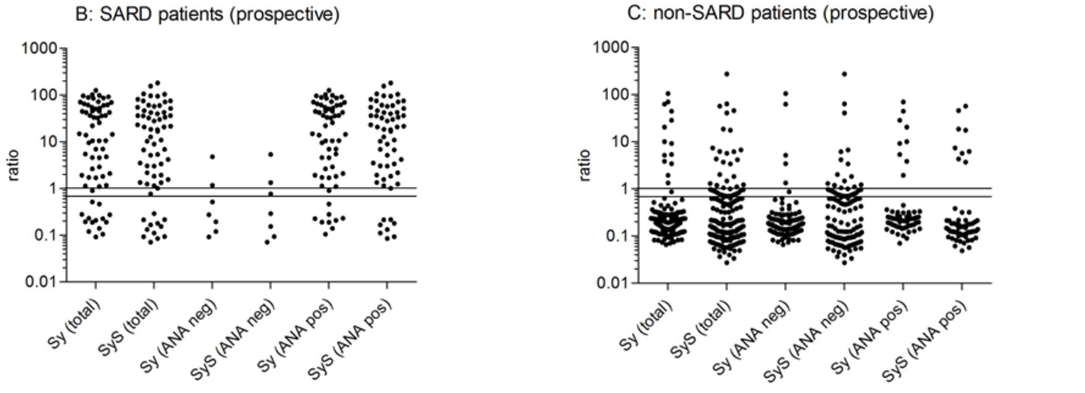

Figure 1B,C: EliA Symphony® (SyS) test results compared to EliA Symphony (Sy) in a cohort of prospectively included patients suspected of systemic autoimmune rheumatic disease (SARD). Panel B shows SARD diagnosed patients, panel C shows non-SARD diagnosed patients. Lines represent the borderline area of the test (0.7-1.0 ratio). ANA: anti-nuclear antibody, determined by indirect immunofluorescence (IIF) on HEp-2 cells.

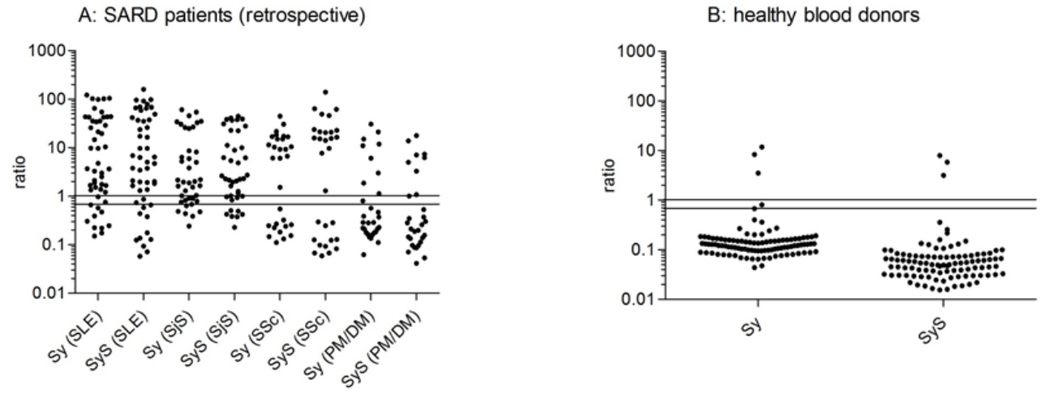

Figure 2A: EliA Symphony® (SyS) test results compared to EliA Symphony (sy) in a cohort of retrospectively included patients previously diagnosed with Systemic Autoimmune Rheumatic Diseases (SARD, Panel A). SLE: Systemic Lupus Erythematosus, SjS: Sjögren syndrome , SSc: Systemic Sclerosis, MCTD: Mixed Connective Tissue Disease, PM/DM: Polymyositis/Dermatomyositis.

Figure 2B: EliA Symphony® (SyS) test results compared to EliA Symphony (sy) in a cohort of healthy blood donors (Panel B).

defined as SLE, SjS, SSc, MCTD or PM/DM. SARD in remission was not considered as SARD. In addition, a second study population of 150 patients previously diagnosed with SARD were retrospectively included, consisting of patients diagnosed with SLE (n=50), SSc (n=30), SjS (n=40) or PM/DM (n=30). Samples were obtained from patients as part of routine screening for autoantibodies in the laboratory. There was informed consent for this study. The (retrospective) control group consisted of apparently healthy blood donors (n=100).

All 247 sera prospectively included were tested for ANA by indirect immunofluorescence (IIF) using NOVA Lite HEp-2 cells (Inova Diagnostics). The assay was performed according to the manufacturer's instructions, using a screening serum dilution of 1:80. All prospectively included sera, retrospective SARD sera and healthy blood donor derived sera were tested in parallel with both EliA Symphony and EliA Symphony® on a Phadia™ 250 instrument, according to the manufacturer's instructions. Results were expressed as ratio, using reference values

All prospective patients					
	total	**Sy neg**	**Sy pos**	**SyS neg**	**SyS pos**
SARD (n)	68	15	53	13	55
non-SARD (n)	179	165	14	155	24
sensitivity (%)		77.9		80.9	
specificity (%)		92.2		86.6	
ANA positive prospective patients					
	total	**Sy neg**	**Sy pos**	**SyS neg**	**SyS pos**
SARD (n)	61	10	51	8	53
non-SARD (n)	60	51	9	51	9
sensitivity (%)		83.6		86.9	
specificity (%)		85.0		85.0	
ANA negative prospective patients					
	total	**Sy neg**	**Sy pos**	**SyS neg**	**SyS pos**
SARD (n)	7	5	2	5	2
non-SARD (n)	119	114	5	104	15
sensitivity (%)		28.6		28.6	
specificity (%)		95.8		87.4	

Table 1: Diagnostic performance of EliA Symphony® (SyS) compared to EliA Symphony (Sy) in a cohort of prospectively included patients suspected of Systemic Autoimmune Rheumatic Disease (SARD). ANA: anti-nuclear antibody, determined by indirect immunofluorescence (IIF) on HEp-2 cells.

<0.7 negative; 0.7-1.0 borderline; >1.0 positive. Sensitivity and specificity were calculated for the prospective cohort based on diagnosis SARD versus non-SARD. Results obtained with sera from patients with previous SARD diagnosis were combined from results obtained with healthy blood donors to calculate sensitivity and specificity for the retrospective cohort. For all sensitivity and specificity calculations, borderline EliA results were considered negative. Reproducibility of EliA Symphony® was determined by calculating % coefficient of variation (CV) of the results obtained from single samples tested seven times within the same run on the same day (intra-test variation) and tested once in separate runs on seven consecutive days (inter-test variation).

Results

In total 247 patients submitted for ANA IIF testing based on SARD suspicion were prospectively included. The results of EliA Symphony and EliA Symphony® testing for all included patients is depicted in figure 1A. An EliA Symphony positive result was obtained in 27% (67/247) whereas 32% (79/247) was EliA Symphony® positive. In total, 49% (121/247) of the patients was ANA IIF positive of which 50% (60/121) was EliA Symphony positive and 51% (62/121) EliA Symphony® positive. Within the remaining 51% (126/247) ANA IIF negative patients 0.06% (7/126) was EliA Symphony positive and 0.13% (17/126) was EliA Symphony® positive. These initial results suggest an increase in overall sensitivity of EliA Symphony®. When all prospectively included patients had received final diagnosis, they were subsequently divided in SARD (28%, 68/247) and non-SARD (72%, 179/247). The SARD group consisted of 41 SLE, 16 SjS, 4 SSc, 3 MCTD and 4 PM/DM patients and showed 90% (61/68) ANA IIF positivity. The remaining 10% (7/68) ANA IIF negative SARD patients consisted of 4 SjS and, interestingly, 3 SLE patients. The non-SARD group showed 34% (60/179) ANA IIF positivity. EliA Symphony and EliA Symphony® results of SARD and non-SARD patients are shown in figure 1B and 1C, respectively.

Within the SARD group 78% (53/68) was EliA Symphony positive and 81% (55/68) was EliA Symphony® positive. This increase consists of 2 patients, 1 SLE and 1 SSc, both of them ANA IIF positive. As a result, a similar increase is observed in the ANA IIF positive SARD group, i.e. 84% (51/61) to 87% (53/61). The 8 ANA IIF positive SARD patients that tested negative for both EliA Symphony and EliA Symphony® were all SLE patients with the exception of 1 SSc patient. Within the ANA IIF positive non-SARD group 0.15% (9/60) was positive for both EliA Symphony and EliA Symphony®, in some cases as a result of SS-A antibodies. Interestingly, some of the patients within this group were classified as "incomplete SARD" and eligible for clinical follow-up. The 7 ANA IIF negative SARD patients showed both a positive EliA Symphony and EliA Symphony® result in 2 cases (29%), 1 SLE and 1 SjS. However, within the ANA IIF negative non-SARD group EliA Symphony® showed increased positivity when compared to EliA Symphony, i.e. 0.13% (15/119) versus 0.04% (5/119), affecting the specificity of EliA Symphony®. Based on the results described above an increase in sensitivity of EliA Symphony® for screening the presence of SARD associated anti-ENA antibodies

Retrospective patients and controls					
	total	Sy neg	Sy pos	SyS neg	SyS pos
SLE (n)	50	13	37	13	37
SjS (n)	40	12	28	11	29
SSc (n)	30	12	18	12	18
PM/DM (n)	30	21	9	21	9
SARD (n)	150	58	92	57	93
controls (n)	100	97	3	97	3
sensitivity (%)		61.3		62.0	
specificity (%)		97.0		97.0	

Table 2: Diagnostic performance of EliA Symphony^{S} (SyS) compared to EliA Symphony (Sy) in a cohort of retrospectively included patients previously diagnosed with systemic autoimmune rheumatic disease (SARD) and controls (healthy blood donors). SLE: Systemic Lupus Erythematosus, SjS: Sjögren´s syndrome , SSc: Systemic Sclerosis, MCTD: Mixed Connective Tissue Disease, PM/DM: Polymyositis/ Dermatomyositis.

Intra-test variation SyS		
	sample 1	sample 2
mean (n=7)	0.77	0.92
SD	0.14	0.11
CV (%)	17.7	11.6
Inter-test-variation SyS		
	sample 1	sample 2
mean (n=7)	0.78	1.1
SD	0.11	0.09
CV (%)	14.8	8.1

Table 3: Reproducibility of EliA Symphony^{S} (SyS), determined with single samples tested seven times within the same run on the same day (intra-test variation) and tested once in separate runs on seven consecutive days (inter-test variation). SD: standard deviation, CV: coefficient of variation.

is observed. This increase appears to be mediated partly by the use of modified Scl-70 and Sm substrates in EliA Symphony^{S}, as illustrated by 1 SSc and 1 SLE patient, respectively. In addition, the overall sensitivity appears to be increased as well. However, a decrease in specificity is observed simultaneously and selectively in ANA IIF negative patients suspected of SARD. This decrease in specificity can be eliminated by using the EliA Symphony^{S} anti-ENA screen only in ANA IIF positive patients suspected of SARD, as is the case in many laboratories performing SARD serology. The results of EliA Symphony and EliA Symphony^{S} performance in the cohort of prospectively included patients are summarized in table 1.

For the second, retrospective part of this study 150 SARD patients were included, previously diagnosed with SLE, SjS, SSc or PM/DM. The results of EliA Symphony and EliA Symphony^{S} testing these patients is depicted in figure 2A. In the SLE group 74% (37/50) tested positive for both EliA Symphony and EliA Symphony^{S}. In the SjS group 70% (28/40) tested positive for EliA Symphony and 73% (29/40) tested positive for EliA Symphony^{S}. In the SSc group 60% (18/30) tested positive for both EliA Symphony and EliA Symphony^{S}. In the PM/DM group 30% (9/30) tested positive for both EliA Symphony and EliA Symphony^{S}. Based on these results, the sensitivity of EliA Symphony^{S} is slightly higher when compared to EliA Symphony, however based on a single SjS patient that showed only a minor increase in ratio (0.89 to 1.02).

Figure 2B shows the results obtained with EliA Symphony and EliA Symphony^{S}in the included healthy blood donors. Only 0.03% (3/100) tested positive for both EliA Symphony and EliA Symphony^{S}, in all cases due to the presence of SS-A antibodies. This result indicates similar specificity of EliA Symphony and EliA Symphony^{S}. The results of EliA Symphony and EliA Symphony^{S} performance in the cohort of retrospectively included patients and controls are summarized in table 2.

In the final part of this study the reproducibility of EliA Symphony^{S} test results was determined. For this purpose single samples approximate to the negative/borderline cut-off (0.7 ratio) and borderline/positive cut-off (1.0 ratio) were selected and repetitively tested in a single test run (intra-test variation) and tested on consecutive days (inter-test variation). The results, expressed as %CV, are shown in table 3 and indicate robust reproducibility in the relevant (cut-off) region of the test. Both intra- and inter-variation are within the expected range for FEIA based testing of autoantibodies (<20%).

Conclusions

Collectively, the results of our study indicate increased diagnostic sensitivity of the novel EliA Symphony^{S} anti-ENA antibody screening test. When EliA Symphony^{S} is employed only for ANA IIF positive patients suspected of SARD, a cascade strategy employed by most laboratories involved in SARD serology, diagnostic specificity is not affected when compared to EliA Symphony. However, without initial ANA IIF testing EliA Symphony^{S} may show decreased specificity when compared to EliA Symphony, as illustrated by our prospectively included (secondary/ tertiary care) patient population. Depending on the local patient population, i.e. primary, secondary or tertiary care, the diagnostic performance of EliA Symphony^{S} may vary and thus warrants local verification of its diagnostic performance. Finally, EliA Symphony^{S} shows robust reproducibility in the relevant (cut-off) region of the test.

Acknowledgements

The author thanks José Huybers, Jac Kuijpers-Entrup, Roseri Roelofsen-de Beer and Pieter van der Pol for their significant contributions to this study.

Addendum

The Laboratory Medical Immunology of the Erasmus MC serves as a national reference center for SARD serology in the Netherlands and coordinates the national EQA for SARD serology (ANA/anti-ENA/anti-dsDNA), organized by the Dutch Foundation for Quality Assessment in Medical Laboratories (SKML).

Of note

Shortly after completion of this study, during routine SARD serology, 2 anti-SmD positive serum samples that initially tested negative using EliA Symphony were identified using EliA Symphony^{S}.

EliA Symphony^{S} - An integral part of your diagnostic screening algorithm for connective tissue diseases

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Connective Tissue Diseases (CTD) represent classical models of systemic autoimmune diseases. They are a heterogeneous group of diseases characterised by abnormal structure or function of one or more of the elements of connective tissue, i.e. collagen, elastin or the mucopolysaccharides. Differential diagnosis of CTD is mainly based on clinical findings, but is complicated because of the similarity of their symptoms. Therefore, autoantibodies are useful markers to support the diagnosis or exclusion of CTD. The most prominent CTD are systemic lupus erythematosus (SLE; potentially affecting all organs), Sjögren's syndrome (SS; characterised by diminished lacrimal and salivary gland secretion), scleroderma (systemic sclerosis, SSc; a chronic, progressive dermatosis), limited systemic sclerosis (a scleroderma formerly known as CREST syndrome, with a more benign disease course), polymyositis/dermatomyositis (PM/DM; an acute or chronic inflammatory disease of muscle and skin), and mixed connective tissue disease (MCTD; a syndrome with features of scleroderma, rheumatoid arthritis, SLE and PM/DM).

Why a new EliA Symphony test?

With our mission "We enable our customers to make the world healthier, cleaner, and safer" we want to offer you (the laboratory) the best and most reliable test, so that you can provide the physician (requester) with the right test results which will help them to make the correct diagnosis and start appropriate treatment.

Cohort n=633	EliA SmD^{P}-S	Supplier 1	Supplier 2	Supplier 3
Sensitivity	14.4%	19.6%	19.6%	16.5%
Specificity	98.3%	95.9%	96.1%	95.5%
Sensitivity at stratified specificity of 98%	14.4%	13.4%	11.3%	7.2%
Positive Likelihood Ratio	8.5%	4.8%	5.0%	3.7%
Positive Predictive Value	60.9%	46.3%	47.3%	40.0%

Table 1: Performance data of EliA SmD^{P}-S compared with three automated tests for anti-Sm (SmD3) from other suppliers using 97 sera from SLE patients and 536 disease controls.

Background – Most innovative Sm test

Sm antibodies against SmD protein are a highly specific marker for SLE, and are included in the ACR 1997 and SLICC 2012 criteria for systemic lupus erythematosus (SLE). While most extractable nuclear antigens can be produced recombinantly (preferentially in eukaryotic cells like Sf9 insect cells), this is not possible in case of SmD. Compared to native SmD, recombinant SmD_3 lacks the antigenicity for Sm autoantibodies to bind. Therefore, most tests for Sm antibodies use native Sm purified from animal material. However, SmD is part of the larger multi-subunit U1-snRNP complex, and native Sm preparations can contain not only SmD but also other subunits that can interact with other autoantibodies and in consequence to lower test specificities.

To avoid these false positive test results, we identified a SmD_3 peptide as antigen for Sm antibodies that met all requirements for an antigen to be used in a high quality diagnostic test [1,2]. This peptide is used in EliA™ SmD^{P}-S that replaces EliA Sm using native Sm.

When comparing with tests using native Sm from other manufacturer (table 1), EliA SmD^{P}-S showed a lower sensitivity but the highest specificity, positive likelihood ratio and positive predictive value. When comparing sensitivity at a stratified specificity of 98% (the specificity of EliA SmD^{P}-S), EliA SmD^{P}-S had the highest sensitivity (table 1).

Disease group	Amount
SLE	97
Disease Controls:	
Scleroderma	87
Sjogrens' Syndrome	96
Rheumatoid arthritis	85
Poly-/Dermatomyositis	78
MCTD	46
Infections (bacterial & viral)	119
Tumor	25
Total	536

Table 2: Serum panel used for the development of EliA SmD^{P} [8].

Need for specificity

Systemic lupus erythematosus (SLE), like all connective tissue diseases, is a rare disease. Still, diagnostic markers for SLE are often ordered in the immunology laboratory as doctors want to rule out SLE when patients present with unspecific symptoms such as fatigue, fever, pain, skin irritations, joint pain or others. Sm antibodies are present only in about a fifth of SLE patients [9,10,11], which makes them unsuitable for ruling out SLE. On the other hand, they are highly specific for SLE. Most clinicians assume that a positive Sm antibody is a clear sign for SLE. However, different tests have different clinical specificity for SLE. Some tests include not only SmD but also SmBB'. Since SmBB' and the U1snRNP antigens A and C share a cross-reactive epitope, antibodies against SmBB' are considered less specific for SLE (see table 2).[1,3,4] Therefore, it is of utmost importance to use the right antigen in an Sm test, to avoid false positives and provide high clinical usefulness.

EliA Symphony^{S}	U1RNP (RNP70, A, C), SS-A/Ro (60kDa, 52kDa), SS-B/La, CENP B, Scl-70, Jo-1, SmD peptide
EliA Symphony	U1RNP (RNP70, A, C), SS-A/Ro (60kDa, 52kDa), SS-B/La, CENP B, Scl-70, Jo-1, SmD
EliA CTD Screen	U1RNP (RNP70, A, C), SS-A/Ro (60kDa, 52kDa), SS-B/La, CENP B, Scl-70, Jo-1, SmD, dsDNA, Rib-P, Fibrillarin, RNA Polymerase III, PM-Scl, PCNA and Mi-2
Supplier 1	U1RNP (RNP70, A, C), SS-A/Ro (60kDa, 52kDa), SS-B/La, Scl-70, Jo-1, Sm (no CENP)
Supplier 2	U1RNP (RNP70, A, C), SS-A/Ro (60kDa, 52kDa), SS-B/La, CENP B, Scl-70, Jo-1, Sm, dsDNA
Supplier 3	U1RNP (RNP70, A, C), SS-A/Ro (60kDa, 52kDa), SS-B/La, CENP B, Scl-70, Jo-1, Sm, dsDNA, Rib-P, Chromatin, U1RNP-Sm complex

	EliA Scl-70^{S}	EliA Scl-70	Scl-70 Supplier 1	Scl-70 Supplier 2
Sensitivity	30.7%	26.7%	28.7%	28.7%
Specificity	99.5%	99.5%	98.0%	99.5%
PPV	96.9%	96.4%	87.9%	96.7%
NPV	74.2%	73.1%	73.3%	73.6%
LR (+)	61.4	53.4	14.4	57.4
LR(-)	0.7	0.7	0.7	0.7

Table 4: Performance of EliA™ Scl-70^{S} vs EliA™ Scl-70 and Scl-70 tests from other suppliers[8]

Disease group	Amount
Scleroderma	101
CREST*	33
Disease Controls	
CTD (SLE,SS,MCTD PM/DM)	102
Rheumatoid arthritis	30
Infections (bacterial & viral)	50
Tumor	20
Total	336

Table 5: Serum panel used for the development of EliA Scl-70^{S}.
** CREST-samples were only used to calculate agreements between the tests but not for sensitivity nor specificity, as there is no clear association with Scl-70 antibodies.

	SLE	MCTD	others
U1RNP(A,C,70)	30-40%	>95%	RA, PM/DM, SSc
SmD	20-30%		

Table 2: Frequency of U1snRNP and Sm antibodies in SLE, scleroderma and mixed connective tissue disease[1,3,4]

Benefits of EliA SmD^{P}-S

- High confidence in the identification of SLE patients

Background – Highly sensitive Scl-70 test

In 2013, a joint committee of the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) developed new classification criteria for systemic sclerosis (SSc).[5] One of these eight new criteria was SSc-specific autoantibodies, namely anti-Scl-70 (anti–topoisomerase I), anti-centromere, and anti–RNA polymerase III. Scl-70 antibodies are an indication for progressive systemic sclerosis.[5] In the same year, we launched an improved Scl-70 test on EliA, with a new way of coating the antigen to the well, which resulted in better antigen presentation, better accessibility of epitopes and therefore a higher sensitivity (see table 4). The new EliA Scl-70^{S} Well (14-5637-01) was evaluated with 336 clinically defined samples, see table 5.

Benefits of EliA Scl-70^{S}

- Aids in clear differentiation between systemic sclerosis and other connective tissue diseases
- Supports early diagnostic guidance

The EliA SmD^{P}-S and EliA Scl-70^{S} tests have an excellent clinical performance indicated by a high sensitivity and specificity. Both antigens are important members of the antigen-specific screening test EliA Symphony^{S} test. A screening test should not only be aligned with the corresponding single antigen tests but should also have as high a sensitivity as possible (without losing specificity). Therefore, the alignment of EliA Symphony with the EliA SmD^{P}-S and EliA Scl-70^{S} test was a logical and necessary consequence.

EliA Symphony^{S}

EliA Symphony^{S} is the first ENA screen to use only human recombinant antigens in combination with a synthetic peptide. Therefore, the test has all the advantages of recombinant antigens – pure antigens with no contamination, leading to a high specificity; controlled production of all test ingredients, leading to a high consistency over time; antigen lots which last over several years, leading to low lot-to-lot variation. The result is a clinically relevant, sensitive and highly specific screening assay. This makes it an excellent aid for clinical decisions and, therefore, maximizes the usefulness in a diagnostic setting.

As an intact three-dimensional structure of the antigens (conformation) is crucial for recognition by antibodies,

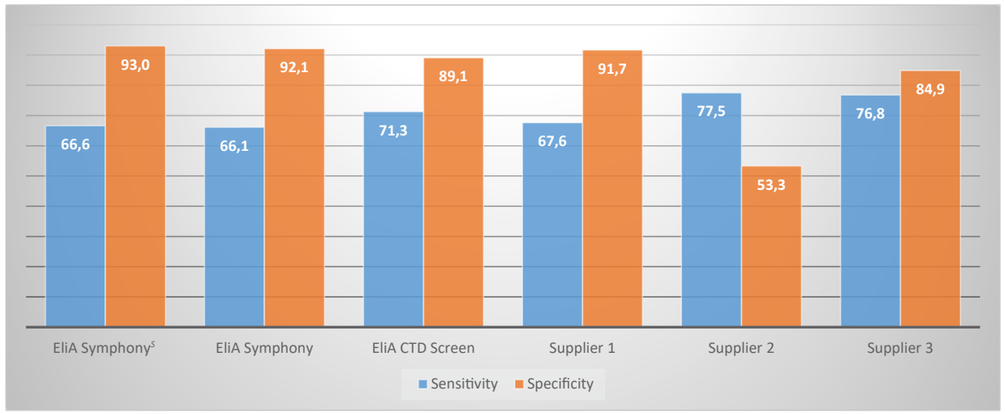

Figure 1: Sensitivity & specificity of EliA Symphony^{S}, EliA Symphony, EliA CTD Screen and ENA screen tests of 3 other suppliers[8].

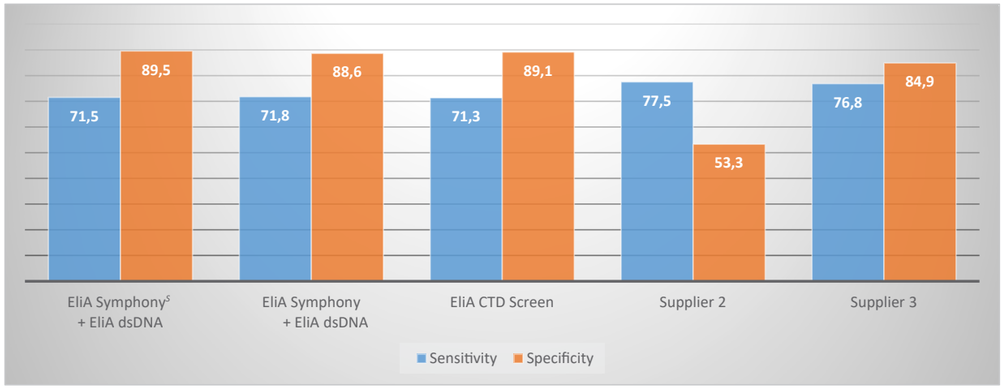

Figure 2: Sensitivity and specificity of EliA Symphony^{S} plus EliA dsDNA, EliA Symphony plus EliA dsDNA, and of three different dsDNA-containing screening tests [8].

Disease	N = 404	Disease Controls	N = 229
SLE	97	Rheumatoid Arthritis	85
Sjögrens Syndrome	96	HBV	36
Scleroderma	87	HCV	36
Poly-/ Dermatomyositis	78	HIV	27
MCTD	46	Tumor	25
		Bacterial Infection	20

(monospecific anti-CENP B samples were not included due to supplier 1 where this antigen is not included)

Table 6: Serum panel used for the development of EliA Symphony^{S}. It is not known whether these were "diagnostic samples" (first measurement, before treatment) or follow-up samples. Details on disease activity or treatment are also not known [8].

most of our human recombinant antigens are produced in the eukaryotic baculovirus/insect cell system. This system, in contrast to bacterial systems, is capable of expressing the antigens in the correct conformation, and performing the complex posttranslational modifications necessary to ensure that the protein is antigenically identical to the human native form.

The natural SmD protein consists of three parts: SmD_1, D_2 and D_3. Mahler et al. demonstrated that one particular peptide of SmD_3 represents the relevant epitopes for Sm and is a more sensitive and more reliable substrate for the detection of anti-Sm antibodies.[1,2] Both EliA Symphony^{S} and EliA SmD^{P}-S use SmD_3 peptide, as it was shown to be the most specific and sensitive antigen for SLE.[2]

Clinical performance

The use of antigens and antigen coating methods as described above should be matched by an improvement of the diagnostic performance. Therefore, the diagnostic performance of EliA Symphony^{S} was not only compared to EliA Symphony but also to other screening assays (EliA™ CTD Screen and 3 ANA Screening tests from different suppliers). All six screening tests include U1RNP, SS-A/Ro, SS-B/La, Scl-70, Jo-1 and Sm (purified Sm or SmD_3 peptide in the case of EliA Symphony^{S}. All but one (supplier 1) include Centromere protein B. EliA CTD Screen as well as the tests from supplier 2 and 3 include dsDNA, and EliA CTD Screen and the test from supplier 3 include further markers for connective tissue diseases (see box, according to the suppliers' websites).

Manufacturer	Sensitivity in%	Specificity in %	Positive likelihood ratio	Negative likelihood ratio
EliA Symphony^{S}	66.6%	93.0%	9.53	0.36
EliA Symphony	66.1%	92.1%	8.41	0.37
EliA CTD Screen	71.3%	89.1%	6.53	0.32
Supplier 1 ENA screen	67.6%	91.7%	8.14	0.35
Supplier 2 ENA screen	77.5%	53.3%	1.66	0.42
Supplier 3 ENA screen	76.8%	84.9%	5.10	0.27

Table 7: Performance of EliA Symphony^{S} vs EliA Symphony, EliA CTD Screen and ENA screen tests from other suppliers[8].

The assays were compared by using 404 clinically defined samples from patients with different connective tissue diseases, as well as 229 patients with different non-autoimmune diseases as controls. (See table 6) Here it should be mentioned that, at a ratio of 404:229, the proportion of CTD patients versus non-CTD patients in this cohort is much higher than in any routine situation. A proportion of 0-5% of connective tissue disease patients is more realistic in a routine diagnostic cohort. The more non-CTD patients are included, the more obvious is the relevance of specificity, even when used as first line testing.

The data show that EliA Symphony^{S} has a slightly increased sensitivity compared to EliA Symphony, due to the use of an improved coating method which resulted in better antigen presentation and better accessibility of epitopes. As expected, the main improvements in sensitivity were observed in the Systemic Lupus Erythematosus and Scleroderma cohort (see table 8).

	EliA Symphony^{S}	EliA Symphony
Sensitivity in SLE	59.8%	58.8%
Sensitivity in Scleroderma	67.8%	64.4%

Table 8: Sensitivity of EliA Symphony^{S} and EliA Symphony in an SLE cohort (n=97) and a Scleroderma cohort (n=87).

Improved sensitivity for SmD antibodies

In our cohort of 404 samples from patients with connective tissue diseases, there were only three samples which were monospecific positive for SmD antibodies. Most SmD samples also contain other antibodies like Ro52, Ro60, U1RNP or La. However, these three samples were negative in the current EliA Symphony but clearly positive for EliA Symphony^{S} due to the improved sensitivity for these antibodies (see table 9).

Sample	EliA Symphony^{S} ratio cut-off 1.0	EliA Symphony ratio cut-off 1.0	EliA SmDP in U/ml cut-off 10
1	6.73	0.45	137.7
2	1.47	0.41	14.1
3	1.06	0.23	11.8

Table 9: 3 samples with SmD antibodies, positive in EliA Symphony^{S} but negative in EliA Symphony.

EliA Symphony^{S} is used in combination with EliA dsDNA

Of course, the three tests which do not include dsDNA (EliA Symphony^{S}, EliA Symphony and the test from supplier 1) have a lower sensitivity than the three tests including dsDNA (EliA CTD Screen and the tests from suppliers 2 and 3), but on the other hand, all non-dsDNA tests are clearly superior in terms of specificity, with EliA Symphony^{S} having the highest specificity (93%). Of the dsDNA-containing screening tests, only EliA CTD Screen has a good specificity of almost 90%.

Bearing in mind the routine approach of an immunology laboratory, the results of EliA Symphony^{S} and EliA Symphony were combined with a specific dsDNA test (see figure 2). Unfortunately, the results of the screening test from supplier 2 could not be combined with a specific anti-dsDNA test as the sera were not available in sufficient volume.

This internal study reflects the expected improvement in the performance of EliA Symphony^{S} in routine. Both, EliA CTD Screen and the combination of EliA Symphony^{S} with

EliA™ dsDNA showed the highest specificity and highest positive likelihood ratio.

Is specificity important for a screening test?

In the diagnosis of connective tissue diseases, screening tests are used to rule out autoimmune diseases. Therefore, doctors expect high sensitivity from a screening test in order not to miss any patient with connective tissue diseases, while the specificity is usually seen as unimportant. However, this approach is risky, particularly in rare diseases such as the connective tissue diseases. As the pre-test probability is often less than 1%, a non-specific screening test is far more often falsely positive than correctly positive (low positive predictive value). Although a screening test is not meant to be decisive for the diagnosis of any disease, it is often used as such, which leads to a high number of false diagnoses. Up to 50% of patients diagnosed with SLE because of ANA-IIF positivity do not have SLE.[6, 7]

In addition to the 633 clinically defined samples listed above, 400 healthy blood donors were tested with EliA Symphony®. Seven out of the 400 samples gave a positive result. In further analysis, all of these samples contained specific antibodies, as shown in table 10. Therefore, the results were technically not false positive, as the blood donors really did have autoantibodies. However, without clinical symptoms, a single positivity of antinuclear antibodies is not diagnostically significant. It remains to be studied, whether individuals with (high titre and persistent) antinuclear antibodies will develop a connective tissue disease in the long-term follow-up.

7 samples positive	EliA Symphony® [Ratio]	Result
1	1.2	EliA Ro52 positive
2	2.4	EliA U1RNP positive
3	33.7	EliA Ro52 and Ro60 positive
4	11.4	EliA Ro60 positive
5	1.1	EliA U1RNP positive
6	23.0	EliA U1RNP and Ro60 positive
7	25.9	EliA CENP positive

Table 10: Results of seven samples from apparently healthy blood donors positive in EliA Symphony®[8].

EliA Symphony® Conclusions

- Well-known EliA quality, first fully recombinant ENA screening test
- High specificity (higher than competitor tests) and therefore high clinical accuracy
- EliA Symphony® and single EliA ENA are perfectly aligned
- Increased sensitivity and maintained specificity
- Fully automaded. Can be run on:
  - Phadia™ 100 instrument
  - Phadia™ 250 instrument
  - Phadia™ 2500 instrument
  - Phadia™ 5000 instrument

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