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Journal ImmunoDiagnostics

Scientific news, opinions and reports

8th International Congress on Autoimmunity

The most beautiful city Granada was the venue of the 8th International Congress on Autoimmunity which took place from May 9th to 13th. According to the organizers, more than 2000 participants came to this worldwide biggest congress on Autoimmunity. The congress boasted 81 sessions featuring 500 speakers. Thermo Fisher Scientific is one of the main sponsors of this congress. A high number of posters and oral presentations were about EliA products. These posters are reprinted in this issue of the Immuno-Diagnostics Journal.

EliA CTD Screen and EliA dsDNA

EliA ANA Single Assays

EliA CCP, EliA RF

EliA Celikey, EliA Gliadin^{DP}

Thermo SCIENTIFIC

Superlatives in Autoimmunity

This year's congress on Autoimmunity was a congress of superlatives. It took place in the most beautiful city of Spain (according to local tourist guides) with the most fantastic weather and the most breath-taking scenery with the snowy mountains of the Sierra Nevada. With more than 2000 participants, 500 speakers and 81 sessions, it was the biggest congress on autoimmunity ever.

As before, we were one of the main sponsors of the congress, but this was the first year that we appeared as a Thermo Fisher Scientific company. We had a new booth with a new appearance. And, on the theme of superlatives: many visitors certified that the coffee at the Thermo Fisher booth was the best of the congress.

One of the best attended sessions of the congress was the EASI conference. The chairmen Yehuda Shoenfeld, Eckart Mummert and Jan Damoiseaux led through the fascinating program of "ANCA-ASSOCIATED DISEASES – A DIAGNOSTIC CHALLENGE". The program and the abstracts of the presentations are accessible on www.easi-network.com.

A large number of posters and oral presentations at the congress were about EliA products, such as our EliA CTD Screen or the EliA assays which allow the measurement of the single antinuclear antibodies which are included in the EliA CTD Screen, such as EliA Rib-P or EliA Fibrillarin. The posters involving EliA products are summarized in this special edition of our new ImmunoDiagnostics Journal. The posters come from independent labs and do not always reflect our opinion. However, our products seemed to convince with their excellent performance, and the results confirmed our strategy of producing specificity-focused, high-quality products.

Enjoy reading

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A new strategy to detect ANA: IIF HEp-2 cells at second level after the EliA CTD Screen test. Is the algorithm correct?

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Objective: To discuss the possibility to apply a new strategy to detect ANA, based on an algorithm in which IIF on HEp-2 cells test can be positioned at second level after the new EliA CTD Screen (Thermo Fisher Scientific), assessing the diagnostic performance obtained in routine samples as well as clinically well-defined serum samples.

Patients and Methods: 157 sera from outpatients referred to Rheumatology, Pavia Hospital, 144 sera from connective tissue diseases (CTD) inpatients of Rheumatology, Siena Hospital, and 150 sera from disease controls were analyzed. All samples were tested using ANA IIF on HEp-2 cells and ENA (EliA Symphony and EliA CTD Screen, Thermo Fisher Scientific) and anti-dsDNA (EliA dsDNA, Thermo Fisher Scientific), performed on the Phadia250 analyzer. Single subspecificities were analyzed in positive sera using the EliA single ANA analytes and EliA research methods (both Thermo Fisher Scientific) for rare specificities.

EliA CTD Screen	Clinical diagnosis		
positive ratio >1	**pos**	**neg**	**Total**
pos	115	4	119
neg	29	146	175
Total	144	150	294

EliA CTD Screen	Clinical diagnosis		
positive ratio >0.7	**pos**	**neg**	**Total**
pos	124	6	130
neg	20	144	164
Total	144	150	294

Table 2: Agreement of clinical diagnosis and EliA CTD Screen result at different cut offs.

	HEp-2			EliA Symphony	EliA CTD Screen	
cut off	1:80	1:160	1:320	pos > 1.0 ratio	pos > 0.7 ratio	pos > 1.0 ratio
Sensitivity	92.8%	84.3%	71.1%	67.5%	80.7%	75.9%
Specificity	48.4%	67.1%	82.0%	95.7%	84.5%	87.6%
LR +	1.08	2.56	3.95	15.52	5.20	6.11
LR -	0.15	0.23	0.35	0.34	0.23	0.28
PPV	48.1%	56.9%	67.0%	88.9%	72.8%	75.9%
NPV	92.9%	89.3%	84.6%	85.1%	89.5%	87.6%

Table 1: Comparison of the performance of IIF, EliA Symphony and EliA CTD Screen.

Results: 117/144 clinically defined "true positive" CTD patients were IIF positive and 8 (5 SLE, 2 SjS, 1 DM/PM) were negative, but positive for some autoantibodies by other methods; 13/17 EliA CTD Screen negative/ IIF positive patients remained negative for any identifiable antibodies, whatever the method used.

Conclusions: The new EliA CTD Screen showed a good correlation with ANA IIF. Moreover, the wider antibody profile offered a good sensitivity for CTD diagnosis. This allows us to apply a new strategy to detect ANA, based on an algorithm in which IIF HEp-2 cells test can be positioned at the second level.

Potential advantages of applying a new strategy for ANA screening:
Reduction of the number of IIF and reduction of final identification assays to be performed. The combined use of both tests with the suggested algorithm should allow to identify, through the fluoroscopic pattern, the most probable specificity.

Potential disadvantages of applying a new strategy for ANA screening:
The algorithm does not identify patients affected by diseases different from CTD, or CTD patients with rare antigens not included in the assay, or CTD patients with antibodies directed against unknown antigens which usually display granular pattern in IIF.

For these reasons HEp-2 IIF ANA should be considered the reference method, nevertheless the data showed that the new EliA CTD Screen as first level test doesn't seem to lead to relevant mistakes.
This algorithm could be useful in particular situation, i.e. laboratories with elevated number of assays (automatisation suggested !) or without expert pathologist in IIF ANA pattern interpretation.

Diagnostic algorithm for ANA screening and characterization of specificities

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Objective: To establish an adequate sequence of techniques to an effective evaluation of ANA and their specificities.

Patients and Methods: IIF-ANA (HEp-2 cells, Immunoconcepts; cut-off< 1/40) and EliA CTD Screen (Thermo Fisher Scientific) were used to test ANA in sera from 479 patients with different pathologies. Sera with EliA CTD≥1 Ratio (cut-off recommended by manufacturer), were tested by Immunoblot (ANA-, Systemic sclerosis- and Myositis-profile; Euroimmun) and EliA dsDNA (Thermo Fisher Scientific). Statistical analysis was performed with SPSS 11.5 program.

Results: An own cut-off was established for EliA CTD Screen values at 1.2 Ratio by comparing the EliA CTD Screen with the IIF and the presence of specificities in confirmatory techniques.

Conclusions: IIF-ANA is still the more suitable and cheap method for testing ANA as initial screening. Also, it allows us to detect important patterns that, although not suggestive of connective tissue diseases, can be associated with other important pathologies (Sp-100 nuclear dots, nuclear

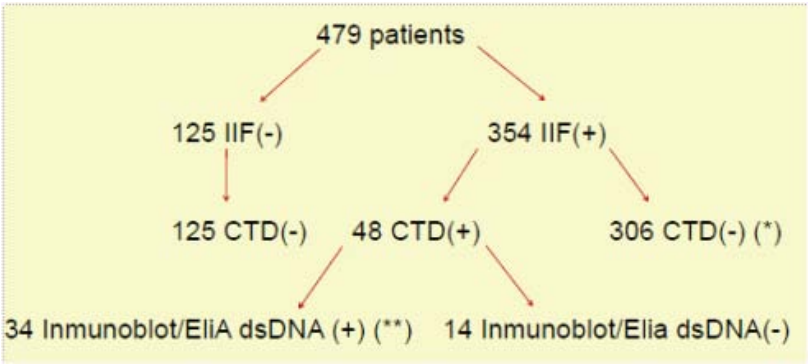

Figure 1: distribution of patients. (*) 18 of them had patterns not suggestive of connective tissue diseases: Sp-100 nuclear dots, nuclear membranous, nuclear membrane pores, midbody, NuMA, centriole, Golgi complex. (**) On samples with characterized specificity, 12 had titres ≤1/80.

membranous…). If other patterns are found, EliA CTD Screen can be used as a sensible technique in a second step in order to detect the most relevant specificities in connective tissue diseases. On positive samples, the fluorescence patterns observed can help again to decide to use the confirmatory and more specific test.

It would be desirable that each laboratory establishes its own diagnostic algorithm with their available techniques.

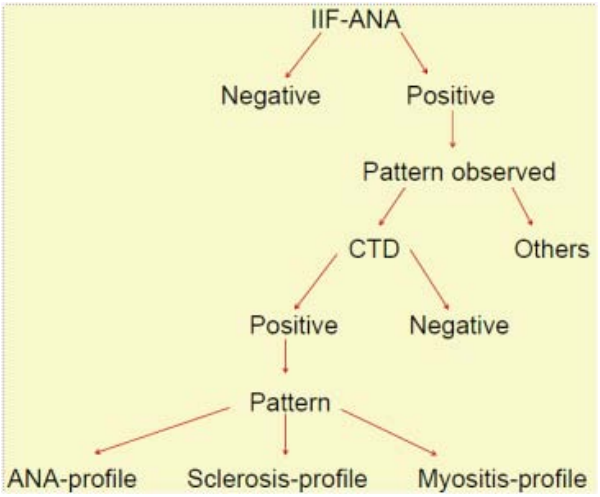

Figure 2: Algorithm of ANA detection.

EliA CTD Screen serum levels in colorectal cancer patients

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Objective: This poster studies the presence of autoantibodies in a cohort of patients with colorectal cancer using a new commercial autoantibody screening method (EliA CTD Screen, Thermo Fisher Scientific). The specificity of the method was assessed by comparison with indirect immunofluorescence (IIF) test in the same patients.

Patients and Methods: The follow-up cohort included 186 patients which were consecutively selected between June 2008 and July 2010. All patients underwent diagnostic colonoscopies. The analysis included 45 patients with histological confirmed CRC and diagnosed by the Gastroenterology Service. Following surgery, the excised specimens were transferred to the pathology department; each patient with CRC was classified according to the histological grade and clinical reports. Clinical staging was assessed according to TNM score.

Results: Overall, 45 patients with a diagnosis of CRC [mean age 68.9 years (range 38-92), 31.1% females] were included in the analyses.

Colorectal cancer	n	EliA CTD Screen positive	IIF positive
TNM	**45**	**4**	**23**
Stage 0	2	0	1
Stage I	8	1	1
Stage II	12	0	8
Stage III	16	2	9
Stage IV	7	1	4
T Class			
TX	1	0	0
Tis	2	0	1
T1	2	0	0
T2	11	1	3
T3	23	2	14
T4	6	1	5
Nodal staus			
N0	23	1	10
N1	14	2	8
N2	5	0	2
N3	3	1	3
Metastases status			
MX	8	1	3
M0	30	2	16
M1	7	1	4

Table: Tumor characteristics, EliA CTD Screen and IIF results of the 45 colorectal cancer patients.

Four cases were positive for EliA CTD Screen (8.89%) and 23 for IIF (51.1%; 9 samples with a titer 1:80; 3 with 1:160; 2 with 1:320; 2 with 1:640; 6 with 1:1280 and one with 1:2560). Of the four positive by EliA CTD Screen, two were positive and one indeterminate for dsDNA. Most positive by IIF appeared in TNM advanced stages; this weak relationship is also seen in the EliA CTD Screen positive patients. By raising the IIF cut-off from 1:80 to 1:160 the agreement between both methods improve from 53.3% to 68.9%.

All patients with positive EliA CTD Screen died (n=4); on the contrary, of the twenty patients positive by IIF (excluding the three positive for CTD), only six died (30%).

Conclusions: IIF shows much higher positivity rates among patients with CRC than EliA CTD Screen. CRC patients with IIF positive may be falsely suspected or diagnosed for connective tissue diseases.

EliA CTD Screen: enzyme fluoroimmunoassay for ANA detection

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Objective: To evaluate the EliA CTD Screen, a new enzyme fluoroimmunoassay (Thermo Fisher Scientific) for detection of anti-nuclear antigen auto-antibodies (ANA) in human sera for the differential diagnosis of CTDs. To correlate the EliA CTD Screen with the INNO-LIA ANA (Innogenetics).

Patients and Methods: Thermo Fisher Scientific recently developed an EliA CTD Screen based on enzyme linked immunofluorescence on the Phadia250 instrument (EliA IgG method). The EliA antigen panel contains 17 ANA-targeted antigens: dsDNA, Sm-D, Rib-P, PCNA, U1-RNP (70kDa, A, C), SS-A/Ro52, SS-A/Ro60, SS-B/La, Scl-70, CENP, Fibrillarin, RNA-Polymerase III, Jo-1, Mi-2 and PM-Scl. The results are expressed in ratio's, where < 0.7 indicates a negative result, 0.7-1.0 an equivocal result and > 1.0 a positive result.
INNO-LIA ANA is a line immunoassay (Auto-LiPA, Innogenetics) for detection and semi-quantitative measurement of 13 ANA. The antigen strip contains Sm-B, Sm-D, RNP70, RNP-A, RNP-C, Ro52, Ro60, SSB, CenpB, TopoI, Jo-1, RiboP and histones. The test result is expressed as a relative intensity and after comparison to a cut-off intensity, translated to a positive, equivocal or negative result.

In the evaluation study, a total of 55 sera from ANA-positive patients (n=10), ANA-negative patients (n=14), healthy blood donors (n=20) and external UKNEQAS and WIV controls (n=11) were determined with EliA CTD Screen and compared to the results of INNO-LIA ANA.
Further, the measuring range and linearity was evaluated with NIBSC non-WHO reference material Anti-Nuclear Factor Serum.
We controlled the reference values by analysing 20 samples of healthy blood donors.

Results:

	INNO-LIA ANA		
EliA CTD Screen	**positive**	**negative**	**Total**
positive	16	3	19
negative	2	34	36
Total	18	37	55

Table 1: Comparison results obtained with EliA CTD Screen and INNO-LIA ANA (n=55). Two-tailed Fisher exact test with p<0.0001 (highly significant association PPV: 84,2%, NPV: 94,4%, sensitivity: 88,9%, specificity: 91,9%.

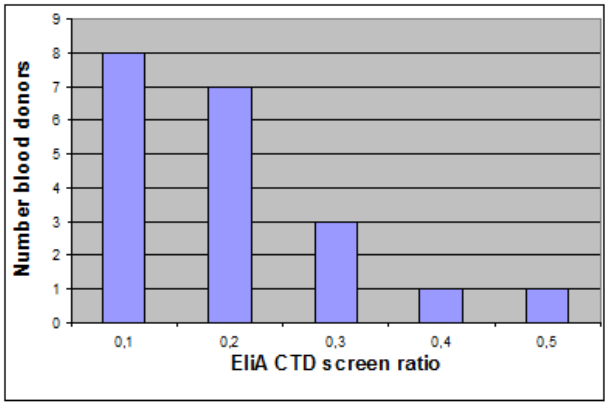

Figure 1: Distribution histogram of the EliA CTD Screen ratio's in a healthy population (n=20). Analysis of blood donors (n=20) showed that all EliA CTD Screen ratio's for a healthy population lie between 0.1 - 0.5, far below the cut-off of 0.7.

Dilution	IU/ml	Test result (CTD ratio)	Interpretation
undiluted	100	14	> 1,0: pos
1:10	10	4,3	> 1,0: pos
1:20	5	2,8	> 1,0: pos
1:40	2,5	1,7	> 1,0: pos
1:80	1,25	1,3	> 1,0: pos
1:160	0,625	0,8	0,7-1,0: equivocal
1:320	0,312	0,4	< 0,7: neg
1:640	0,156	0,2	< 0,7: neg

Table 2: Diagnostic measuring range NIBSC non-WHO reference Anti-Nuclear Factor Serum.

Conclusions:

1. The EliA CTD Screen shows a high statistically significant association with INNO-LIA ANA ($p<0.0001$) and good diagnostic performance with PPV 84.2%; NPV 94.4%, specificity 91.1% and sensitivity 88.9%.
2. The cut-off of the EliA CTD Screen lies at a concentration equalling 0.625 IU/ml (Table 2). The method shows a good linearity.
3. The EliA CTD Screen can obviously distinguish a healthy from a diseased population.

It is concluded that EliA CTD Screen is a good performing serological method to support IIF patterns in CTD diagnosis and can be used in a routine cohort.

Evaluation of two automated screening methods to detect extractable nuclear antigens: ANA-8 Screen and dsDNA-G Screen on Chorus Trio System analyser compared to EliA CTD Screen on Phadia100

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Objective: Two novel commercially available automated anti-ENA screening methods, ANA-8 Screen and dsDNA-G Screen on Chorus Trio System analyser (DIESSE) and EliA CTD Screen on Phadia100 (Thermo Fisher Scientific) are evaluated in a three-step-cascade, whereby the ENA-screening method is positioned between IIF and the final anti-ENA identification, in order to reduce the amount of labour-intensive and costly confirmatory antigen specific tests.

Patients and Methods: Analytical evaluation was done by determining performance characteristics between and within run imprecision with +/- QC material in each run and by cut off verification of the 99th one-sided percentile of 10 healthy donors.

Assay comparisons were made in a 3-step-cascade:
1. Screening by Indirect Immunofluorescence IIF (HEp-2000 Fluorescent ANA-Ro Test System (Immuno Concepts));
2. ENA-Screening with EliA CTD Screen on Phadia100 and

ANA-8 Screen and dsDNA-G Screen; 3. Confirmatory tests for anti-ENA with EUROASSAY immunoblot (EUROIMMUN), INNO-LIA ANA Update (Innogenetics) and EliA Single analyte testing (Thermo Fisher Scientific) and for anti-dsDNA with Crithidia luciliae IIF and EliA dsDNA-G (Thermo Fisher Scientific).

Results: Imprecision characteristics: No international analytical criteria are available, but table 1 shows clearly the superior imprecision characteristics of EliA CTD Screen compared to Chorus ELISA tests with inter-run CV (%) of respectively 4.6% and 9.5%.
Cut-off verification: Thermo Fisher Scientific tested 400 healthy blood donors. DIESSE does not specify in the package insert how reference values were calculated. The recalculated cut-off values are slightly lower than the manufacturer's specifications. Only a limited number of samples were tested.

Focussing on the discrepant results, EliA CTD Screen generated three false positive results and ANA-8 Screen produced one false positive and three false negative results compared to the confirmatory immunoblot.Two of the false negatives were strongly SS-A (52 kDa) positive. As the SS-A is one of the most prevalent autoantibodies, this is an inadmissible shortcoming of the ANA-8 Screen. dsDNA-G Screen scored poor by giving too many false positive results compared to IIF based on Crithidia luciliae substrate and immunoblotting (67% false positives) and compared to EliA dsDNA (33% false positives).

Conclusions: In conclusion, the EliA CTD Screen showed to be superior to ANA-8 Screen as a sensitive second-line screening test for anti-ENA antibodies.

Mean	Mean	SD	CV%	Calculated cut-off value	Manufacturer's cut-off value
ANA-8 Screen (OD-ratio)	Intra-run 1.9 Intra-run 1.7	Intra-run 0.1 Intra-run 0.2	Intra-run 3.6 Intra-run **9.5**	0.4	<0.8
dsDNA-G Screen (IU/ml)	Intra-run 1.7 Intra-run 48	Intra-run 0.2 Intra-run 7.1	Intra-run 10.2 Intra-run **14.7**	8.3	<12
EliA CTD Screen (ratio)	Intra-run 1.9 Intra-run 1.9	Intra-run 0.1 Intra-run 0.1	Intra-run 4.6 Intra-run **4.6**	0.3	<0.7

Tabel 1: Imprecision characteristics and cut-off verification

	Chorus analyser ANA-8 Screen/dsDNA-G Screen			
		Positive	Negative	Total
Phadia100 EliA CTD Screen	Positive	17	**6**	23
	Negative	**1**	11	12
	Total	18	17	35

Tabel 2: Discrepant results by means of 2x2 contingency table.

Specificity of EliA CTD Screen in patients with infectious diseases

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Objective: To assess the specificity (number of false positives) of the new ANA screening method EliA CTD Screen in samples of patients chronically infected with different infectious agents: HBV, HCV, HIV, syphilis and toxoplasma. At present, there are few studies that evaluate the specificity of this new method.

Patients and Methods: Sera from 165 patients with different ID were consecutively collected in the Alto Guadalquivir Hospital from July 2009 to August 2011.
EliA CTD Screen (Thermo Fisher Scientific) was assayed and compared to IIF (HEp-2 substrate). Solid phase assay EliA CTD Screen was assessed on the Phadia250 instrument (Thermo Fisher Scientific). Positive or equivocal samples were analysed by blot, EliA Symphony and EliA ANA single assays. Medical records showed that only 10 patients had an autoimmune disease simultaneously.

Results: Twenty-one patients were positive for EliA CTD Screen (14 for dsDNA and 5 for Ro) and 101 patients were positive in IIF. 19 of the 21 patients positive for EliA CTD Screen but 97 of the 101 patients positive for IIF had no autoimmune disease. Many of the 101 IIF positives (85.1%) did not show CTD specific antibodies.
A large proportion of the IIF positives had an antibody titer of only 1:100. Among these the main pattern was (at least partly) fine speckled, which can be caused by Ro, La or Mi-2 antibodies but also by currently not known antibodies against nucleosomal structures. Ro, La or Mi-2 antibodies have only been detected in 2 of the 62 (1:100) fine speckled samples.

Conclusions: IIF shows much higher positivity rates among patients with ID than EliA CTD Screen. Many of the IIF positives (85,1%) did not show connective tissue diseases specific antibodies (EliA CTD Screen positives). IIF positive patients with an ID may be falsely suspected for connective tissue diseases.

	Sensitivity (%)	Specificity (%)	Negative predictive value (%)
EliA CTD Screen	20,0	87,7	94,4
IIF (HEp-2 substrate)	40,0	37,4	90,6

Table 1: Diagnostic characteristics for detection of autoimmune diseases in 165 patients with ID.

Titer	Number of samples	%	Remarks
1:100	60	73,2	53 with (partly) fine speckled pattern
1:320	18	22,0	
1:1000	3	3,7	1 with a pattern (GW/P-bodies) unspecific for CTD
1:10000	1	1,2	IIF pattern for autoimmune liver disease (PBC)

Table 2: Distribution of IIF titers and patterns in the 82 patients being EliA CTD Screen negative and IIF positive.

Antibodies to extractable nuclear antigens in antinuclear antibody negative samples

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Objective: This prospective study was performed to evaluate whether and to what extent IIF on HEp-2 failed to detect ENA-Ab.

Patients and Methods: A total of 3751 samples collected over 6 months (starting December 2011) were tested for ANA on HEp-2 (Euroimmun) by IIF. In order to assess the presence of ENA-Ab, sera were tested by EliA CTD Screen (Thermo Fisher Scientific). All positive ENA-Ab sera were subsequently tested for single ENA antigens by EliA (Thermo Fisher Scientific). Positive ENA-Ab ANA-negative sera were evaluated also with anti-ENA Profile Plus test (Euroimmun).

Results: Out of 3751, samples 3654 were tested for ANA by IIF. 2536 (69.4%) resulted ANA-negative (i.e., ANA and anti-cytoplasmic antibody titers <1:80) and 1118 (30.6%) ANA-positive respectively. Out of 3751 sera, 3025 sera were screened for ENA-Ab by EliA screening, while 726 ANA negative sera were not tested for ENA-Ab because not required by physician. EliA CTD Screen revealed 220 ENA-Ab positive out of 1118 ANA-positive (19,7%) and 52 ENA-Ab positive out of 1837 ANA-negative (2,8%) sera, respectively.

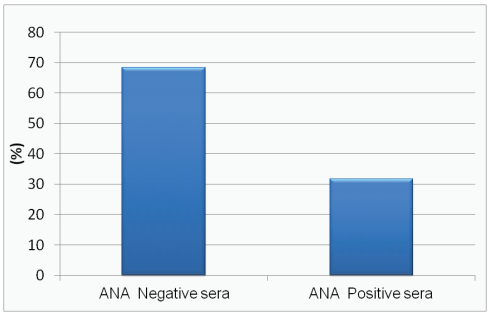

Figure 1: Prevalence of ANA on HEp-2 cells.

The 52 ENA-Ab positive ANA-negative sera were further investigated by EliA for the presence of antibodies to individual antigens: CENP B, Jo1, U1- RNP (70 kDa, A, C), ScL-70, Sm, SSa (52 and 60 kDa) and SSb. 34 sera (65%) were SSa positive, 12 sera were RNP positive (23%), 1 serum was SSb positive (2%), 2 sera were SSa and SSb positive (4%) and 3 sera didn't react with any antigen (6%). The sera were tested also with Anti-ENA Profile Plus (native SSa, SSa 52KDa, RNP/Sm, Scl-70, Jo1) and those that showed a reactivity with SSa antigens were also investigated for SSa Ro52 and SSa Ro60 specificity by EliA.

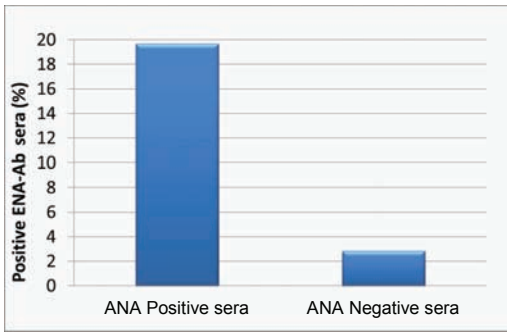

Figure 2: Prevalence of ENA-Ab positivity

Regarding SSa positivity the two methods gave concordant results: 18 sera were SSa-52, 11 sera were SSa-60 and 6 sera were SSa-52 and SSa-60 positive. However, anti-ENA Profile Plus system detected also SSb antibodies in 6 SSa positive sera with EliA, and SSa-60 antibodies in 3 sera out of 11 RNP-positive with EliA. The 3 sera that were ENA-Ab positive by the EliA screening but negative with single antigens EliA resulted negative also with the anti-ENA ProfilePlus.

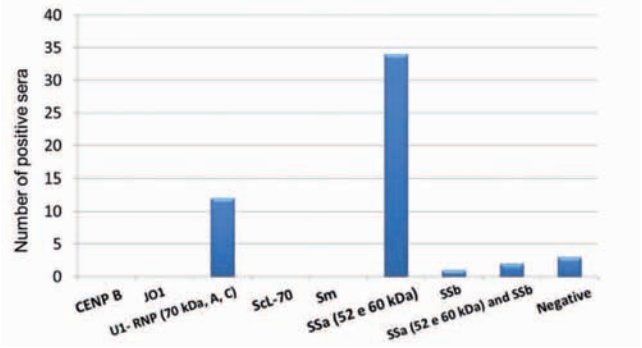

Figure 3: Antigen specificity of ANA negative ENA-Ab positive sera

Conclusions: These preliminary results indicate that ENA-Ab may be overlooked by IF. Studies are ongoing to evaluate these ANA negative ENA-Ab positive subjects after at least six months either serologically and by clinical evaluation.

Clinical evaluation of the EliA assay for detection of anti-PM-Scl 100, anti-RNA Polymerase III and anti-fibrillarin in patients with systemic sclerosis

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Objective: To evaluate the prevalence and the diagnostic specificity of anti-PMScl100, anti-RNA polymerase III (RNAP) and anti-fibrillarin (AFA) antibodies, detected by the new EliA assay, in patients with systemic sclerosis (SSc).

Patients and Methods: Anti-PMScl100, anti-RNAP, AFA, anti-centromere (ACA), and anti-topoisomerase I (ATA) antibodies were measured by the EliA™ assay (Thermo Fisher Scientific) in 143 consecutive SSc patients (100 with limited cutaneous SSc [lcSSc] and 43 with diffuse cutaneous SSc [dcSSc]) and in 95 control patients with other rheumatic diseases (32 with systemic lupus erythematosus [SLE], 25 with Sjögren syndrome [SS], 19 with undefined connective tissue disease [UCTD], 9 with rheumatoid arthritis [RA], and 10 with overlap syndromes [SLE/RA; SLE/SS; RA/SS]). Anti-nuclear antibodies (ANA) were detected in all the patients by the indirect immunofluorescence method.

Results: In SSc patients, the antibody prevalence rates were as follows: ANA, 125/143 (87.4%); ACA 40/143 (27.9%); ATA, 29/143 (20.2%); anti-PMScl100, 8/143 (5.6%); anti-RNAP, 4/143 (2.8%); and AFA, 2/143 (1.4%). The specificity was 100% for all the antibodies tested by EliA. 5/8 anti-PMScl 100 and 2/4 anti-RNAP-positive patients had lcSSc, whereas 2/2 AFA-positive patients had dcSSc. 3/8 (37.5%) of the anti-PMScl100-positive patients were affected by myositis. All antibodies were mutually exclusive.

	Positive	%
ANA	125/143	87,4
ACA	40/143	27,9
ATA	29/143	20,2
PM-Scl100	8/143	5,6
RNAP	4/143	2,8
AFA	2/143	1,4

Table 1: Prevalence of autoantibodies in 143 consecutive SSc patients.

Conclusions: Using the EliA assay, the prevalence of anti-PMScl100, anti-RNAP and AFA is similar to the prevalence previously reported in the European population using different methods, and the specificity is very high. This method, therefore, may be considered a very accurate tool for the detection of this subset of autoantibodies.

A new anti-fibrillarin test detects scleroderma and nothing else

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Objective: To evaluate the specificity of the new EliA anti-fibrillarin test.

Patients and Methods: The new EliA Fibrillarin (Thermo Fisher Scientific) automated test uses a full-length 34 kDa human recombinant fibrillarin, produced with baculovirus/ insect cell system.
Ninety-five sera were examined: 15 SSc (10 Cutaneous Diffuse, 5 Limited form, anti-centromere or anti-Scl70 antibodies negative), 10 Systemic Lupus Erythematosus, 10 Sjogren Syndrome, 20 Rheumatoid Arthritis, 10 patients with circulating immunocomplexes, 10 HCV and 20 healthy subjects. All sera were tested with ANA IIF, starting dilution 1:160 (HEp2000, ImmunoConcepts), EliA Symphony screening (Thermo Fisher Scientific) and further EliA tests for single ANA specificities (new anti-fibrillarin included) on Phadia250 instrument.

Results: Correspondence was demonstrated between anti-fibrillarin positivity and ANA IIF clumpy nucleolar pattern in 2 SSc patients: one patient showed diffuse cutaneous SSc, ANA 1:640, fibrillarin 129 ug/l; the other one showed limited cutaneous SSc, ANA 1:5120, fibrillarin 301 ug/l; both patients were affected by pulmonary hypertension.

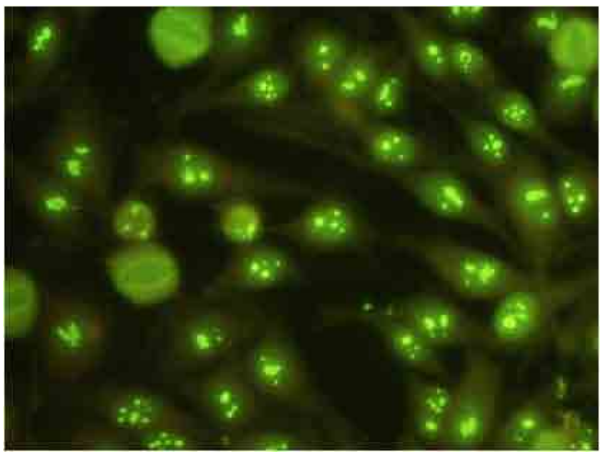

Figure 1: clumpy nucleolar pattern in one SSc patients

No positivity was found in disease controls and healthy subjects, corresponding to a positive predictive value (PPV) of 100% and infinite positive likelihood ratio (LR+).

Conclusions: The new EliA anti-fibrillarin test shows very high specificity while identifying SSc patients with clumpy nucleolar pattern with good sensitivity. Due to high PPV and LR+ the new test is useful in clinical routine.

Could anti-Ribosomal P protein IgG autoantibodies be important for Systemic Lupus Erythematosus diagnosis?

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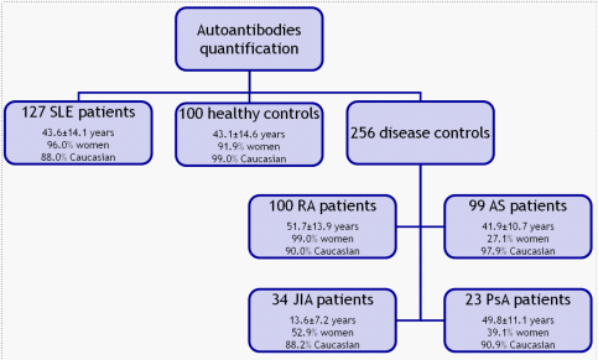
Objective: To evaluate the diagnostic performance of anti-Rib-P in comparison to anti-Sm and anti-dsDNA antibodies in SLE patients and to identify anti-Rib-P association with clinical manifestations in SLE patients.

Patients and Methods: Autoantibody titers were determined using EliA Rib-P, EliA Sm, and EliA dsDNA, from Thermo Fisher Scientific. Receiver operating characteristic (ROC) curves were performed and the cut-off values of



positivity were determined. The relationship between demographic parameters, clinical features, and autoantibody titers was assessed by univariable followed by multivariable linear regression analyses.

Results: anti-Rib-P test performance: sensitivity = 14.2%; specificity = 99.4%; PPV = 90.0%; NPV = 76.4%. manufacturer cut-off = 10 U/ml; New cut-off established = 4.45 U/ml
For anti-Sm determination the cut-off was established as 3.4 U/ml (test performance: sensitivity = 9.4%; specificity = 100%; PPV = 100%; NPV = 75.6%).
For anti-dsDNA determination the cut-off was kept according to manufacturer's indication on 15 U/ml (test performance: sensitivity = 49.6%; specificity = 96.9%; PPV = 85.1%; NPV = 84.4%).

	SLE patients	Healthy controls	Rheumatic disease controls
Anti-Rib-P (U/ml) n (%)	4.9±20.2 18 (14.2%)	0.07±0.21 0 (0%)	0.6±1.8 2 (0.8%)
Anti-Sm (U/ml) n (%)	2.7±13.8 12 (9.4%)	0.02±0.11 0 (0%)	0.1±0.3 0 (0%)
Anti-dsDNA (U/ml) n (%)	44.6±73.8 63 (49.6%)	3.5±8.1 6 (6.0%)	2.6±4.2 5 (2.0%)

Table 1: Autoantibody titers and positivity for three clinical groups.

Conclusions: Antibodies against Rib-P proteins are very specific for SLE and the EliA test ensured accurate results. Anti-Rib-P autoantibody determination should be considered for inclusion in SLE classification criteria.

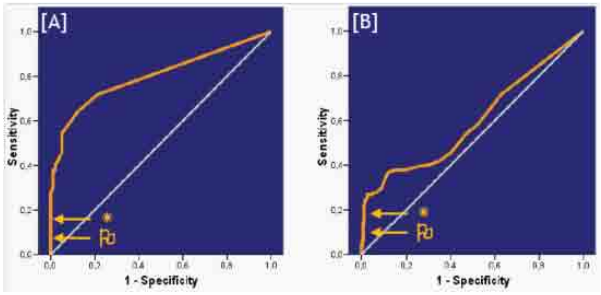


Figure 1: ROC curves for anti-Rib-P autoantibodies quantification for [A] discriminations between SLE and healthy controls and for [B] discriminations between SLE and control diseases.

Evaluation of a new automated fluoroimmunoenzymatic assay for the detection of anti-ribosomal P antibodies in SLE patients

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Objective: This study was designed to evaluate a new immunoassay for the detection of anti-Rib P antibodies (EliA Rib-P, Thermo Fisher Scientific) and its association with the presence of anti-dsDNA antibodies.

Patients and Methods: Serum samples from unselected patients with SLE (n=231) and a control group (n=222) were tested with the EliA Rib-P and EliA dsDNA assays (Thermo Fisher Scientific). The control group included patients with infectious diseases (n=48), non SLE connective tissue diseases (n=123), organ specific autoimmune disorders (n=22), vasculitis (n=9) and healthy individuals (n=20).

Results: The results revealed that 11.3% of SLE patients had a positive result using EliA Rib-P assay, confirming the reported prevalence of 10 to 40% for anti-Rib P antibodies in SLE patients.

The diagnostic performance of the EliA Rib-P assay revealed a similar positive predictive value (92.9%) as the EliA dsDNA assay (92.7%) for SLE, but a higher specificity (99% versus 95.5%), although a lower sensitivity (11.3% versus 49.4%).

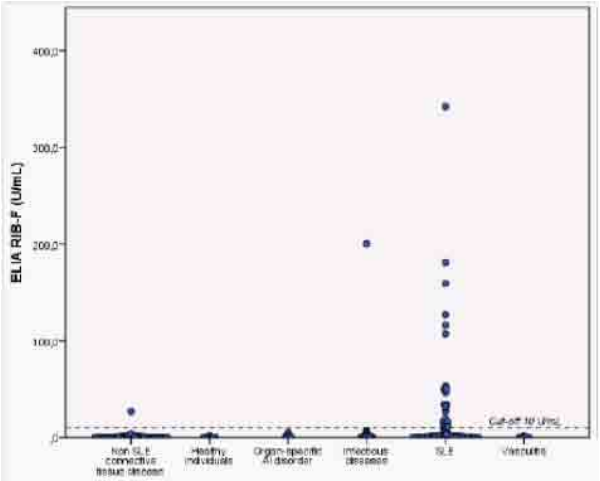

Figure 1: Anti-Rib P results in SLE patients and control group.

Most SLE patients were not in flare, which can explain the low sensitivity found, and, similar to other recent studies, almost all of the anti-Rib P positive SLE patients were also anti-dsDNA positive (23/26).

	Rib-P	DNA
Sensitivity	11.30%	49.40%
Specificity	99.00%	95.50%
PPV	92.90%	92.70%
NPV	49.40%	62.30%

Table 1: Sensitivity, Specificity, PPV and NPV of the EliA Rib-P assay.

Additionally, it was observed that most of the anti-Rib-P positive samples did not show the typical IIF pattern on HEp-2 cells.

Conclusions: The excellent diagnostic value of the anti-Rib P antibodies for SLE, using the EliA Rib-P assay was confirmed. Due to the high specificity and positive predictive value for SLE, patients with anti-Rib P antibodies should be carefully monitored, even if negative for anti-dsDNA antibodies.

Evaluation of a new test for anti-RNA polymerase III antibodies determination in French patients

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[1]Autoimmunity and Hypersensitivity Unit, [2]Rheumatology department, [3]Dermatology Department, [4]Internal Medicine Department, [5]Pneumology Department, APHP, Hospital Bichat-Claude Bernard, Paris, France and [6]Immunology Lab, [7]Pneumology Department, APHP, Hospital Cochin, Paris, France

Objective: To evaluate the performance of a new EliA technique for detection of anti-RNA polymerase III auto-antibodies.

Patients and Methods: 67 serum samples were collected according to their RNA polymerase III auto-antibody status (34 positive and 33 negative) as determined by a classical ELISA method (Quanta Lite® RNA Pol III, Inova) performed manually. All samples were then tested for anti-RNA polymerase III antibodies by the new EliA well (Thermo Fisher Scientific) on a Phadia250 analyzer (Thermo Fisher Scientific). Nine sera which were positive and 10 which were negative in both techniques as well as the 13 discordant sera were further analyzed by an immunodot technique performed manually (Euroline Systemic Scleroris Profile, EuroImmun).

Results: The specificity of anti-RNA polymerase III for both ELISA and EliA for SSc was 100%. The sensitivity of EliA compared to ELISA was 62%. The global agreement between ELISA and EliA was 81% with a Kappa coefficient of 0.61. Anti-RNA polymerase III research was performed by immunodot on the 13 discordant sera (A) and also on 9 positive (B)

and 10 negative (C) sera as controls. The Immunodot was positive for RNA polymerase III for all positive samples and negative in all negative samples. Interestingly it could weakly detect anti-RNA polymerase III in only 2/13 (15%) discordant sera, while another anti-SSc auto-antibody could be found in 9/11 (82%) remaining discordant sera. It was previously demonstrated in a large cohort that anti-RNA polymerase III do not often associate with another SSc antibody (17% of association, Meyer et al. J Rheumatol 2010), so it seems doubtful that all these patients are truly positive for anti-RNA polymerase III. Rather than using the gold standard technique (radiolabeled immunoprecipitation) that is usually not available in a routine lab, it is recommended to re-evaluate the clinical relevance of anti-RNA polymerase III auto-antibodies using this new technique.

Conclusions: The new EliA showed 81% agreement with the ELISA technique, with 62% sensitivity (in relation to the ELISA) and 100% specificity. However, ELISA-positive patients that were negative for EliA were also mostly found negative by a third test. Thus, it is unclear if those patients should be considered positive for anti-RNA polymerase III antibodies. A study on a large scleroderma cohort is needed to assess the clinical relevance of such antibody profile, particularly in regard to severe complication such as renal crisis.

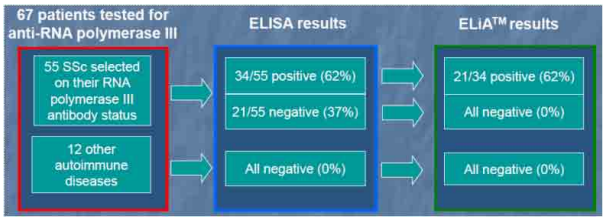

Figure 1: RNA polymerase III antibodies positivity by ELISA and EliA.

A.

	ELISA (ratio)	EliA™ (ratio)	Immunodot result
Discordant1	0,1	0,1	anti-RNA Pol III (weak)
Discordant2	0,1	0,0	anti-RNA Pol III (weak)
Discordant3	0,1	0,0	anti-centromere B
Discordant4	0,4	0,0	anti-Scl70
Discordant5	0,2	0,0	anti-NOR90
Discordant6	0,2	0,0	anti-centromere B
Discordant7	0,1	0,0	anti-Pm-Scl75
Discordant8	0,1	0,0	anti-Scl70
Discordant9	0,1	0,0	anti-centromere B
Discordant10	0,1	0,0	anti-Scl70
Discordant11	0,1	0,0	-
Discordant12	0,1	0,0	anti-centromere B
Discordant13	0,1	0,0	-

B.

	ELISA (ratio)	EliA™ (ratio)	Immunodot result
Positive1	5,0	7,8	anti-RNA Pol III
Positive2	7,3	2,3	anti-RNA Pol III
Positive3	7,2	8,6	anti-RNA Pol III
Positive4	6,1	7,9	anti-RNA Pol III
Positive5	5,9	10,7	anti-RNA Pol III
Positive6	2,7	1,0	anti-RNA Pol III
Positive7	2,3	2,5	anti-RNA Pol III
Positive8	1,9	2,4	anti-RNA Pol III
Positive9	1,5	3,1	anti-RNA Pol III

C.

	ELISA (ratio)	EliA™ (ratio)	Immunodot result
Negative1	0,8	0,3	anti-fibrillarin
Negative2	0,6	0,3	-
Negative3	0,5	0,0	-
Negative4	0,4	0,2	anti-centromere B
Negative5	0,3	0,5	anti-centromere B
Negative6	0,3	0,9	-
Negative7	0,3	0,6	anti-Scl70
Negative8	0,3	0,9	anti-Ro52
Negative9	0,2	0,5	-
Negative10	0,1	0,2	-

Figure 2: Immunodot results

Prevalence of IgA rheumatoid factor in HCV positive patients

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Objective: This study evaluated the distribution of RF-IgA isotype in HCV positive patients.

Patients and Methods: 126 serum samples were collected from HCV positive patients (54 male and 72 female). All samples were tested with RF-IgM and total IgA

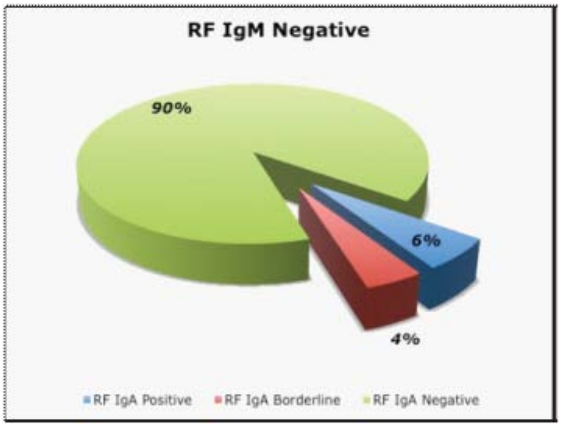
nephelometric (View, Siemens), EliA RF IgA (fluoroimmunoenzymatic, Thermo Fisher Scientific) and EliA CCP (ACPA IgG, fluoroimmunoenzymatic, Thermo Fisher Scientific).

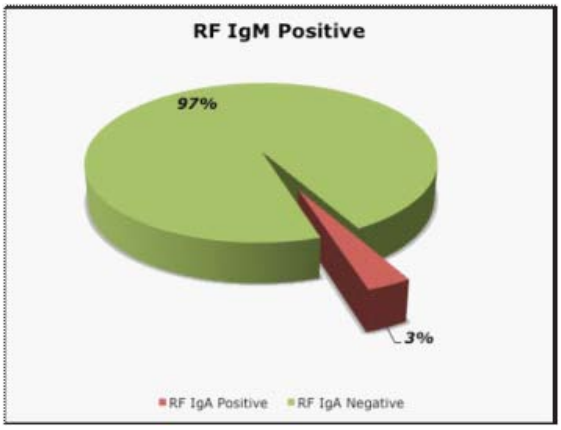
Results: Of the 126 patients sera tested for RF-IgM 67 (53,2%) were found positive and 59 (46,8%) negative.
Among the RF IgM positive samples:
- 4 were found positive for RF IgA
- and 3 of these 4 were also found positive for ACPA IgG
- 3 were found borderline for RF IgA

Among the RF IgM negative samples:
- 2 were found positive for RF IgA
- 2 samples other than the above were found positive for ACPA IgG

4 of the 5 patients positive for ACPA IgG were additionally affected by rheumatic pathologies.

Conclusions: Results show a low prevalence (7,1%) of RF-IgA in HCV positive samples when compared to RF-IgM (53,2%). This could indicate that the IgA isotype is a better marker for RA than IgM, given that ACPA still remain the best marker for this pathology.





Thermo Fisher Scientific EliA System is a valuable method for RF and ACPA detection in early RA

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Objective: The main goal of this study was to assess the performance of a recent available high throughput and automated detection kit for RF (IgA and IgM) and ACPA, namely anti-CCP2 antibodies, in early and established RA.

Patients and Methods: RF (IgM and IgA) and ACPA autoantibodies were determined in serum samples of 28 healthy controls, 10 untreated early RA (ERA) patients with less than 1 year of disease duration and 136 established RA patients under disease modifying anti-rheumatic drugs (DMARD) treatment by EliA CCP, EliA RF IgM and EliA RF IgA (Thermo Fisher Scientific) using a Phadia100 instrument.

Results: It was observed that in the ERA group 20% of patients were positive for RF IgA, 50% were RF IgM positive and 60% of them were ACPA positive. In the established phase of the disease it was found that 42% of patients were positive for RF IgA, 72% were RF IgM positive and 74% of them were ACPA positive. Of note, all healthy controls were seronegative for RF IgA and ACPA, while only 7% were positive for RF IgM. Interestingly, while RF IgM was significantly increased in the established phase of the disease, RF IgA and ACPA were augmented since the early phase of RA when compared to healthy controls.

Conclusions: EliA system showed a good performance, comparable to what has been described for other tests. In fact, as expected, ACPA measured by the EliA system has a better sensitivity and a better specificity in ERA and established RA as compared to RF. The new kits from EliA system (Thermo Fisher Scientific) for RF and ACPA measurements combine a high throughput and highly automated technology with standardized quality.

	Healthy controls (n=28)	ERA (n=10)	RA (n=136)
RF IgM (U/ml)	1.0 (0.4 – 12.3)	6.8 (0.6 – 92.7)	15.1 (0.3 – 263.0)
RF IgA (U/ml)	3.3 (1.1 – 11.4)	6.4 (3.1 – 89.9)	13.6 (1.3 – 214.0)
ACPA (U/ml)	1.4 (0.5 – 3.4)	146.9 (1.0 – 340.0)	93.65 (0.7 – 461.0)

Table 1: Autoantibody levels in healthy controls, ERA and established RA patients. Values are represented as median (range).

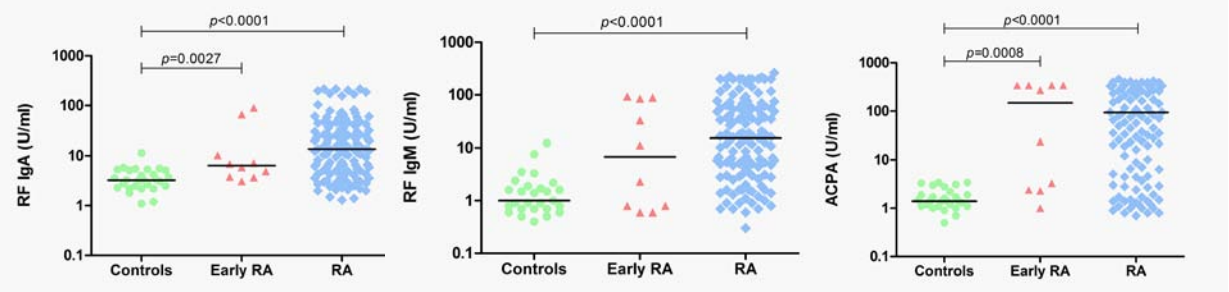

Figure 1: Quantification of RF (IgA, IgM) and ACPA in serum samples from healthy controls, early and established RA patients. *Differences were considered statistically significant for p<0.05 in comparison with healthy controls.

Determination of rheumatoid factor isotypes in a selected population: diagnostic performances of new analytic procedure

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Objective: Purpose of the study was to evaluate the diagnostic performance of a new method for the determination of RF and its isotypes (RF EliA) in comparison with a commercially available assay (RF ELISA).

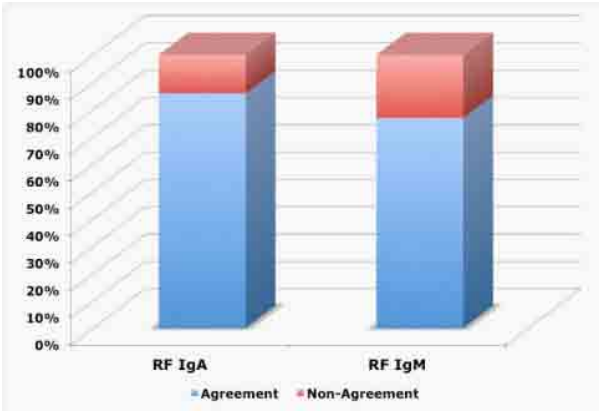
Patients and Methods: The study population consisted of 126 sera belonging to patients with rheumatoid arthritis not responding to treatment with DMARDs, before the treatment with biological drugs. On all patients sera CRP and RF-IgM nephelometric (View, Siemens), RF-IgA and RF-IgM EliA fluoroimmunoenzymatic (Thermo Fisher Scientific) and RF-IgA and RF-IgM ELISA (Orgentec) were performed.

Results: Comparison of EliA against ELISA methods shows the following results:

- RF-IgA: 82.5% of results were in agreement while 17.4% provided different results. In details, of the overlapping results 55.5% were positive and 27.0% were negative, while 3.9% were ELISA negative and EliA positive and 13.5% were ELISA positive and EliA negative.

• RF-IgM: 77.0% of results were in agreement while 23.0% were discordant. In details, of the overlapping results 69.1% were positive and 7.9% negative while 10.3% were ELISA negative and EliA positive and 12.7% were ELISA positive and EliA negative.
Moreover, comparison between EliA/ELISA IgM methods against nephelometric RF-IgM shows better correlation for EliA (overall agreement: 91.3%) with respect to ELISA (84.1%).

Conclusions: Statistical analysis of the discordant samples for the measurement of RF between the ELISA and EliA, with particular attention to the IgA isotype, did not show a significant difference when compared to clinical and inflammation parameters.



A comparison between IgG antibodies to cyclic citrullinated peptides and to modified citrullinated vimentin in rheumatoid arthritis

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Objective: It has been argued that antibodies to modified citrullinated vimentin (anti-MCV) are superior to antibodies to cyclic citrullinated peptides (anti-CCP), while others claim that anti-CCP is preferable because of higher diagnostic specificity for rheumatoid arthritis (RA).

Patients and Methods: 43 patients sera were used referring to the laboratory of autoimmunity by clinical suspicion of RA. Detection of anti-MCV antibody was performed with reactive (Palex Diagnostic) and anti-CCP with EliA (Thermo Fisher Scientific). Statistical analysis was performed with Medcalc statistic programs.

Results: 46.5% were positive for anti-CCP and 67.4% for anti-MCV. 10 discordant results were found. All discordant results showed anti-MCV positive values lower than 30 IU/ml (cut-off, 25 IU/ml) and negative results for anti-CCP. The diagnosis of RA was confirmed in 19 patients. None of the patients with low levels of anti-MVC and negative results for anti-CCP were diagnosed with RA. In the evaluation of both tests, the Kappa ratio was 0.553.

			anti-CCP		Total
			negative	positive	
anti-MCV	negative	amount	14	0	14
		% of anti-MCV	100.0%	0.0%	100.0%
		% of anti-CCP	58.3%	0.0%	32.5%
		% of total	32.6%	0.0%	32.6%
	positive	amount	10	19	29
		% of anti-MCV	34.5%	65.5%	100.0%
		% of anti-CCP	41.7%	100.0%	67.4%
		% of total	23.3%	44.2%	57.4%

Table 1: Association analysis of anti-CCP and anti-MVC

Conclusions: The results obtained by both techniques are moderately different. Analysis of the results reveals the existence of weak positive results for anti-MVC which are clearly negative for anti-CCP in patients without diagnosis of RA, that may be false positive results for anti-MVC tests.

Autoantibodies on celiac disease – laboratory protocol

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Objective: The aim of this study was to evaluate the diagnostic value of the autoantibodies IgA anti-endomysium (EMA-IgA), IgA anti-tissue transglutaminase (TTG-IgA), IgA and IgG anti-deaminated gliadin (DGA-IgA and DGA-IgG), as well as to review the existing protocols.

Patients and Methods: The cohort of 416 sera included: adults and children (over 3 years old) with celiac disease (CD), confirmed by intestinal biopsy (n=55), patients with signs and symptoms suggestive of CD (n=323) and blood donors (n=38) as healthy control group. These sera were used to evaluate the usefulness of these autoantibodies for screening and for monitoring of the gluten-free diet patients' sera for the monitoring.
EMA-IgA was performed by indirect immunofluorescence (IIF) on monkey oesophagus slides (BioSystems) TTG-IgA, DGA-IgA and DGA-IgG were performed by fluoroimmunoassay on the Phadia250 analyser with EliA Celikey IgA, EliA Gliadin^{DP} IgA, EliA Gliadin^{DP} IgG (Thermo Fisher Scientific).

	EMA-IgA	DGA-IgA	DGA-IgG	TTG-IgA
Sensitivity (%)				
Children (n=20)	100.0	78.9	84.2	100.0
Adults (n=9)	100.0	87.5	87.5	100.0
Specificity (%)				
Children	99.5	97.6	98.0	99.5
Adults	100.0	96.4	100.0	100.0
PPV (%)				
Children	95.4	75.6	80.0	95.2
Adults	100.0	63.6	87.5	100.0
NPV (%)				
Children	100.0	98.1	98.5	100.0
Adults	100.0	99.1	100.0	100.0

Table 1: Test performance in first study.

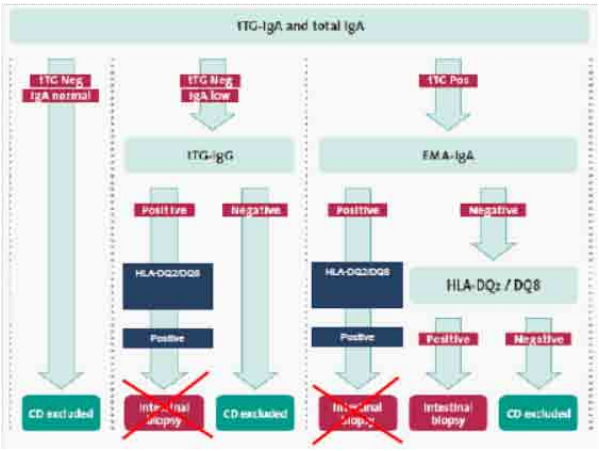

Figure 1: Algorithm for diagnostics of celiac disease. Adapted from Catassi 2010.

Results: The same sensitivity (100%) for EMA-IgA and TTG-IgA was found; DGA-IgA and DGA-IgG showed a lower sensitivity in children (78.9%, 84.2%) than in adults (87.5%, 87.5%). The specificity was very good and similar for all tests (96.4% to 100%). The high positive predictive value for EMA-IgA and TTG-IgA (95.4% to 100%) confirmed their utility for CD diagnosis.
In gluten-free diet patients, DGA-IgA showed a decreased positive predictive value (16.7% for children and 20% for adults) which confirmed the utility of DGA-IgA for monitoring the patients compliance to a gluten-free diet.

Conclusions: The results confirm the high diagnostic value of TTG-IgA. Since it is less time consuming than EMA-IgA, and without the subjectivity of IIF, it is preferable for the diagnosis of CD. EMA-IgA may be used as a confirmatory test.
Therefore, a scheme for CD laboratory study (Figure 1) is proposed, using TTG-IgA for the diagnosis of CD (in adults and children over 3 years), which is consistent with the new ESPGHAN criteria (2011), and DGA-IgA for the compliance to gluten-free diet.

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- EliA CTD Screen can be used as a sensible technique in order to detect the most relevant specificities in connective tissue diseases.
- The new EliA CTD Screen showed a good correlation with ANA IIF.
- EliA Fibrillarin, EliA PM-Scl 100 and EliA RNA Pol III may be considered a very accurate tool for the detection of these subsets of autoantibodies.
- The new EliA assays for RF and ACPA measurement (Thermo Fisher Scientific) combine a high throughput and highly automated technology with standardized quality.
- Anti-tTG-IgA is the premium test for the diagnosis of celiac disease (in adults and children over 3 years), and anti-DGP-IgA for the compliance to gluten-free diet.

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