

EliA™ JOURNAL



The 5th International Congress on Autoimmunity
November 29 – December 3, 2006
Sorrento, Italy

Editorial

The International Congress on Autoimmunity has become established as one of the major meetings in the field of immunology, taking place every two years. The lectures and discussions during this congress cover every aspect of autoimmunity from basic research attempting to clarify etiology or pathogenesis up to the latest innovations in treatment or diagnostics and the congress brings together some of the top autoimmunologists in the world.

The 5th International Congress on Autoimmunity took place in Sorrento, Italy, from November 29 to December 3, 2006. As for the previous Congresses on Autoimmunity, the 5th congress was sponsored by Phadia and we had a presentation area with an ImmunoCAP 250 instrument and the possibility to have a detailed introduction to its features and the tests which can be run on this highly automated instrument for immunoglobulin detection.

Similarly to the 4th International Congress on Autoimmunity in Budapest in November 2004, many posters and oral presentations were about the new rheumatoid arthritis marker anti-CCP and of course often about EliA CCP (see page 3 to 9). The session on anti-CCP (Chairmen: W.J. Van Venrooij and P.von Landenberg) was one of the best attended ones and showed the still growing interest in that diagnostic marker.

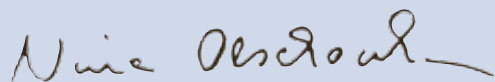
Also very well attended was the session on the European Autoimmunity Standardization Initiative (EASI) (Chairmen: M. Haass and A. Wiik). EASI was initiated and is still organised by Phadia. M. Haass gives an introduction on this topic on page 18 of this EliA Journal.

Several posters and oral presentations at the congress were about Phadia products such as EliA CCP, EliA Symphony or on Varelisa products such as Varelisa CTD Screen or Varelisa PR3 Capture and even some research assays, for example a Varelisa for antibodies crossreacting with a 28 kDa Drosophila antigen for the diagnosis of ankylosing spondylitis.

To make these developments available to our customers we summarize all posters which involve Phadia products in this special issue of the EliA Journal. In most cases, the posters come from independent labs and do not always reflect our opinion.

The high number of oral presentations and posters on EliA products shows us the high level of interest of labs in our fully automated autoimmunity testing and confirms our strategy of producing specificity-focused, high-quality products.

Enjoy reading,




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A comparative evaluation of 11 second and third generation ELISA methods for the detection of antibodies to citrullinated proteins

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Objective: To analyze the diagnostic accuracy of 11 second and third generation kits which use synthetic citrullinated peptides derived from filaggrin, synthetic citrullinated IgG, EBV-derived citrullinated peptides, human mutated citrullinated vimentin or recombinant citrullinated rat filaggrin.

Patients and Methods: 100 samples from patients with RA (diagnosed according to the ACR criteria), 128 disease controls (39 with connective tissue diseases, 12 with other rheumatic diseases, 39 with various viral infections, 6 with Lyme disease, 15 with autoimmune thyroid diseases and 17 with different kinds of cancer), and 74 blood donors were measured with kits from the following manufacturers: Aesku (Germany), Astra (Italy), Axis-Shield (UK), Eurodiagnostica (Netherlands), Euroimmun (Germany), Genesis (UK), Inova (USA, 2nd and 3rd generation, IgG and IgA conjugate), Orgentec (Germany), Phadia (Germany). Rheumatoid factor (RF) was measured with laser nephelometry.

Results: At the cut-off levels provided by the manufacturers, sensitivity of the 11 kits ranged from 61 to 89%, and specificity from 59 to 98.5%. Sensitivities at predefined specificity levels of 97%, 98% and 99% calculated by means of the ROC curves, are indicated in table I. Sensitivity and specificity of the RF were 54 and 86%. The majority of false positive results were observed among patients with viral infections (Table II).

The 3rd generation kit (Inova CCP3) performed slightly better than the 2nd generation kit from the same manufacturer; however, the use of combined anti-IgG and anti-IgA conjugate antibodies (kit Inova CCP3.1) didn't improve sensitivity in comparison to the assay that uses only an anti-IgG conjugate.

Kit	Cut-off	Sens. (%)	Spec. (%)	AUC	Sens. at 99% spec. (%)	Sens. at 98% spec. (%)	Sens. at 97% spec. (%)
Aesku	15	82	53	0.805	33	44	48
Astra	11	61	92.6	0.839	41	47	51
Axis-Shield	5	70	97	0.862	67	70	71
Eurodiagnostica	25	75	97	0.912	70	73	75
Euroimmun	5	72	98.5	0.919	64	73	73
Genesis	6.2	61	94.6	0.787	36	41	45
Inova CCP 2	20	65	97	0.865	64	64	66
Inova CCP 3	20	73	96	0.891	66	67	69
Inova CCP3.1	20	89	64.9	0.889	65	67	68
Orgentec	20	72	93.1	0.822	60	62	64
Phadia	10	73	98.5	0.896	69	74	74
RF	20	54	86.1	0.631	13	17	17

Table 1: Sensitivity and specificity of 11 kits and of RF at the cut-off levels provided by the manufacturers and sensitivity at 97, 98, and 99 % specificity as determined by ROC curves (AUC, area under the curve)

Kit	Cut-off	CTD	ORD	Virus	Thyroid	Lyme	Cancer	Healthy	Total
n		39	12	39	15	6	17	74	202
Aesku	15	22	8	25	5	2	6	27	95
Astra	11	2	0	5	2	0	2	4	15
Axis-Shield	5	1	0	1	0	1	1	2	6
Eurodiagnostica	25	4	0	0	1	1	0	0	6
Euroimmun	5	1	0	0	0	1	1	0	3
Genesis	6.2	1	0	6	0	1	0	3	11
Inova CCP 2	20	1	1	2	0	0	0	2	6
Inova CCP 3	20	5	1	1	0	0	0	1	8
Inova CCP 3.1	20	7	7	22	5	0	15	15	71
Orgentec	20	4	0	10	0	0	0	0	14
Phadia	10	0	0	2	0	0	1	0	3
RF	20	16	1	8	0	0	2	1	28

Table 2: False positives at the cut-off values provided by the manufacturers, subdivided by disease group (CTD = connective tissue diseases, ORD = other rheumatic diseases)

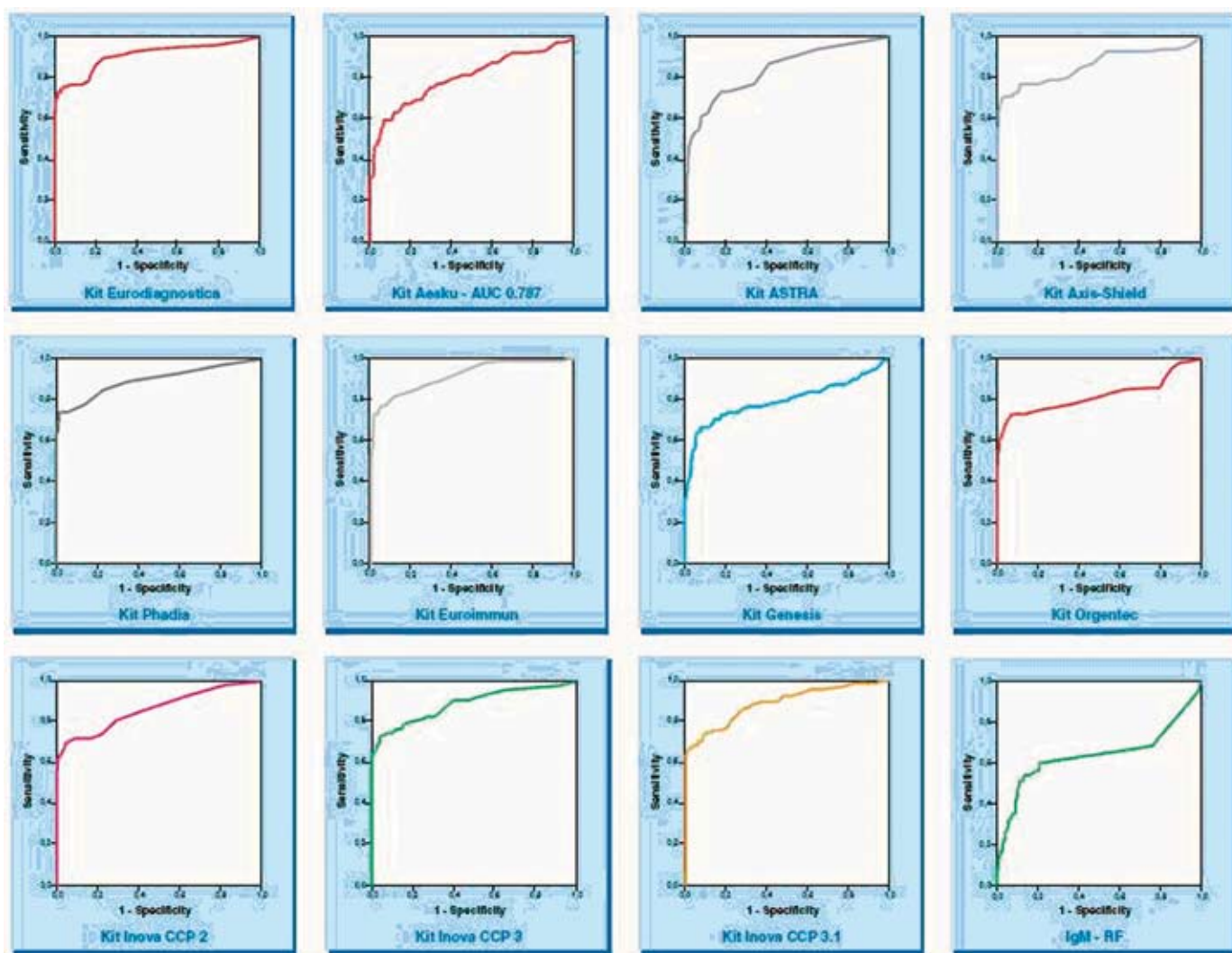


Figure 1: Receiver operator curves (ROC) for the 11 kits plus IgM RF

Conclusions: The results of this study show that commercial kits for the detection of anti-CP antibodies exhibit various degrees of diagnostic accuracy and that careful kit selection is needed to obtain reliable results.

Evaluation of anti-CCP antibodies by two different methods

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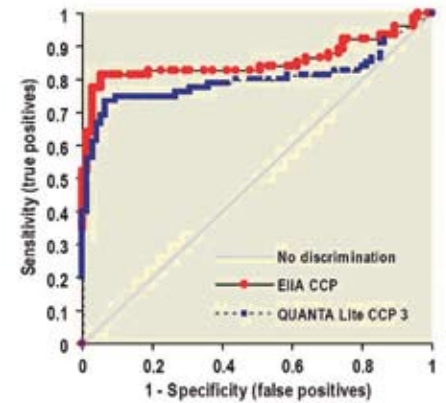
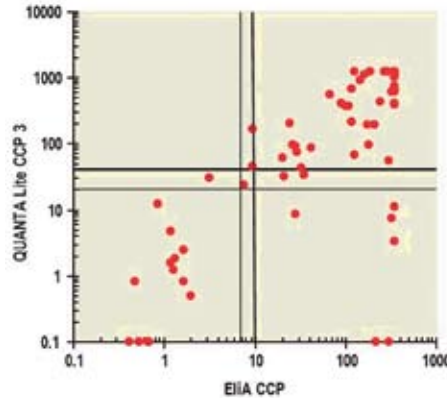
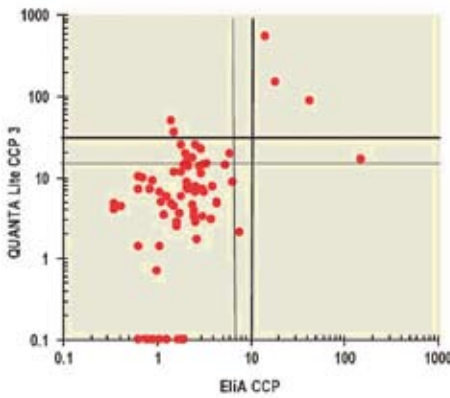
Objective: To compare the performance of two commercially available kits for the detection of anti-CCP Abs.

Patients and Methods: 75 RA, 75 disease controls (Non-RA) and 50 blood donors were analysed with EIA Quanta Lite CCP 3 (Inova, San Diego, USA) and EliA CCP on the ImmunoCAP 250 (Phadia, Freiburg, Germany).

Results:

75 RA 75 NonRA 50 BD	Inova CCP 3 (pos > 20 U/ml)	Inova CCP 3 (pos > 40 U/ml)	EliA CCP (pos > 7 U/ml)	EliA CCP (pos > 10 U/ml)
Sensitivity (%)	74.7	69.3	81.3	77.3
Specificity (%)	90.4	95.2	96.0	96.8
PPV (%)	82.4	89.7	92.4	93.5
NPV (%)	85.6	83.8	89.6	87.7
Efficiency (%)	84.5	85.5	90.5	89.5

Table 1: Sensitivity, specificity predictive values and efficiency of Quanta Lite CCP3 and EliA CCP



The agreement of both methods was 90%. The ROC curve showed an area under the curve (AUC) of 0.813 for the Inova assay and 0.842 for the EliA assay (see figure 3)

Figure 1 and 2: NonRA and RA patients in Quanta Lite CCP 3 and EliA CCP

Figure 3: ROC curve Quanta Lite CCP 3 vs EliA CCP (Blood Donors excluded)

Conclusions: Both tests showed high specificity, good sensitivity as well as good agreement. EliA CCP showed a higher clinical sensitivity and specificity. The lower specificity in Quanta Lite CCP 3 can be due to 4 presumptive false positives detected in blood donors (one had high antibody concentration, and three around the cut-off).

Rheumatoid factor and different CCP tests in rheumatoid arthritis and control patients

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Objective: To compare the clinical performance of two 2nd generation CCP tests (conventional ELISA and automated test, EliA), a CCP test with a different antigen composition (CCP 3) and the IgM class rheumatoid factor (RF) in rheumatoid arthritis (RA) patients and controls from routine.

Patients and Methods: 86 RA and 90 disease controls (various infections such as hepatitis, further connective tissue diseases, arthritic diseases) were quantitatively measured with the following methods: Quanta Lite CCP (Inova Diagnostics, San Diego, USA), EliA CCP (Phadia, Freiburg, Germany), Quanta Lite CCP 3 (Inova Diagnostics, San Diego, USA), and immuno-nephelometry for RF IgM (BN ProSpec System Dade Behring).

Results:

Total n = 176 86 RA, 90 NonRA	RF Pos > 20	CCP 2 ELISA		CCP 3 ELISA		EliA CCP	
		Pos > 20	Pos > 40	Pos > 20	Pos > 40	Pos > 7	Pos > 10
Sensitivity (%)	66.3	73.3	62.8	66.3	65.1	68.6	67.4
Specificity (%)	84.4	94.4	95.6	94.4	96.7	96.7	97.8
PPV	80.3	92.6	93.1	91.9	94.9	95.2	96.7
NPV	72.4	78.7	72.9	74.6	74.4	76.3	75.9
Efficiency	75.6	84.1	79.5	80.7	81.3	83.0	83.0

Interestingly, the CP ELISA using the 2nd generation CCP antigen has the most discrepancies compared to the other tests. In total, 5 sera are detected falsely positive by this test. Out of these only one is also positive in the EliA CCP test using the same antigen. This sample was also positive in the CCP 3 test. Overall, there is no improvement in clinical performance found by the CCP3 test. False positive anti-CCP results may create some problems in interpretation having in mind that CCP is regarded as highly specific test.

Conclusions: All different CCP tests showed a performance superior to RF.

It is important to have a close look on the performance of the different CCP tests available on the market. Recently newly developed CCP tests do not necessarily have a superior performance.

Value of low positive anti-CCP results in a diagnostic routine setting

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Objective: Specificity of anti-CCP tests especially in low positive samples is a diagnostic issue. Therefore, low positive CCP results in our diagnostic routine setting were retrospectively studied by confirmatory testing for anti-CCP and compared to rheumatoid factor (RF) status and clinical diagnosis of RA.

Patients and Methods: Between 2003 and 2006, sera were analyzed for CCP antibodies using the Quanta Lite CCP2 assay (Inova). Results above the cut-off of 20 U/ml up to 50 U/ml were re-measured with EliA CCP on the ImmunoCAP instrument (Phadia, Germany) and with the Quanta Lite CCP3 assay (Inova, USA). Rheumatoid factors were determined using Waaler-Rose and latex fixation test.

Results: 3% of tested samples (48/1690) showed low CCP positivity using routine testing. 19 of 48 samples tested CCP positive with all three assays used (Figure 1, red points). In this group a high correlation with RA and seropositivity for RF was observed.

28 of 48 samples resulted negative with both confirmatory CCP tests (Figure 1, yellow points). In this group, RF was negative and clinicians dismissed diagnosis of RA. Non-specific binding may explain discrepant results, since confirmation employed CCP2 and CCP3 epitopes.

Observation of false positives (29/1690) in this study depended on an arbitrary and narrow definition of "low positive" and may underestimate the actual rate of false CCP positivity.

Conclusion: In a routine clinical setting, low-level false positive CCP results were seen in 2% of samples and were unrelated to either binding to the CCP2 epitope or the diagnosis of clinical RA. Low-level CCP results obtained with MTP-based assays should therefore be interpreted with caution, in particular in rheumatoid factor negative cases.

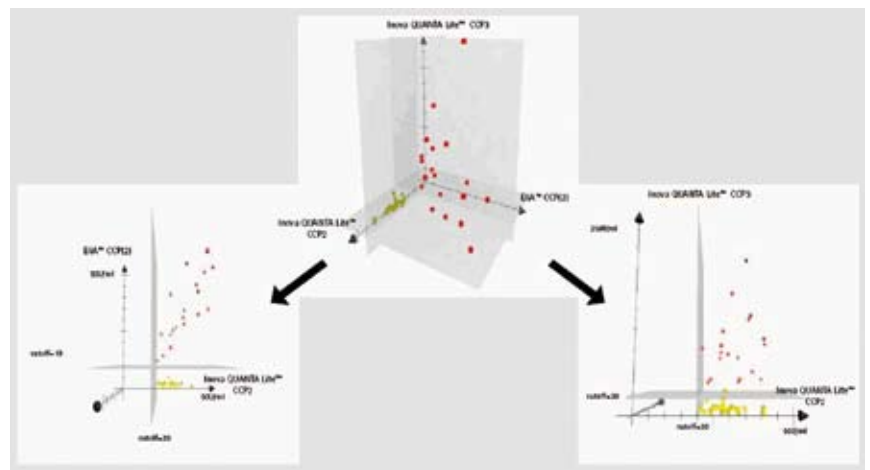


Figure 1: Results of the routinely used Quanta Lite CCP compared with 2 confirmatory CCP tests

Association of anti-cyclic citrullinated protein antibody concentrations and markers of disease activity in rheumatoid arthritis

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Objective: To assess possible associations of the anti-citrullinated protein (anti-CP) antibody titre and clinical and serological markers of disease activity including disease activity score 28 (DAS-28), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) in patients with rheumatoid arthritis (RA).

Patients and Methods: 102 stored sera of RA patients were tested for the presence of anti-CP antibodies using an automated second generation anti-cyclic citrullinated protein (anti-CCP2) enzyme immunoassay (EliA CCP from Phadia, Austria) with a recommended cut-off of 10 U/ml. Data on DAS-28, ESR and CRP were retrieved by chart review. Statistical analysis was performed using the Spearman-Rho rank correlation test and the Mann-Whitney U test as appropriate.

Results: A weak correlation was found between anti-CCP2 levels and DAS-28 scores (corr.coeff. 0.290; $p < 0.01$), ESR (corr.coeff. 0.228; $p < 0.05$) and CRP levels (corr.coeff. 0.355; $p < 0.01$). However, no significant differences were found in median DAS-scores (4.2, range 2.0-7.5; 3.5, 1.0-7.0 and 5.1, 1.6-8.1), ESR (18 mm/1st hour, 6-86; 28, 2-66 and 30, 10-100) and CRP levels (5.4 mg/L, 0.6-92.0; 9.4, 1-92.4 and 15.1, 1-129.0) between patients with

a low (as defined by titres between 10 and 100 U/ml), intermediate (100-200 U/ml) and high positive (>200 U/ml) anti-CCP2 titres, respectively.

Conclusion: Although the anti-CP antibodies are among the most specific serological markers to diagnose RA, anti-CP levels do not give additional information on disease activity.

Changes in anti-cyclic citrullinated peptide (CCP) antibody values in either relatives or not relatives of rheumatoid arthritis patients

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Objective: Some doubts still remain on the clinical significance of borderline anti-CCP values. We aimed at assessing anti-CCP profile in subjects with a first result included in the grey zone.

Patients and Methods: 32 relatives of RA patients and 24 cases without a family history were selected by having had at least 3 anti-CCP determinations over the last 15 months with a first value ranging from the cut-off threshold (5 U/ml with Diastat and 7 with EliA) to 30 U/ml. The sera were tested with 2 anti-CCP tests: Diastat Anti-CCP (Axis-Shield Diagnostics, UK) and EliA CCP (Phadia, Germany).

Results: Among family members, one patient was affected by rheumatoid arthritis (RA). Three subjects presented borderline values. None of these three subjects has been found to have rheumatoid arthritis up to now (Figure 1). Anti-CCP profile in the second group (no family history) showed two patterns of titre fluctuations with both assays: 1) subjects with stable values in the low-to-moderate range of borderline concentrations (n= 9), including 3 patients with a definite diagnosis of Sjögren’s syndrome and one with psoriatic arthritis (Figure 2); 2) subjects in whom anti-CCP values increased over time up to concentrations > 100 U/ml (n=15). The median doubling time of anti-CCP titres was 5.3 months (Figure 3). RA was subsequently diagnosed in 13 out of these 15 subjects.

Conclusion: Changes in anti-CCP antibody values over time may be used as a reliable diagnostic support in the monitoring of subjects presenting with borderline anti-CCP titres. Anti-CCP testing should be repeated at 6-month intervals in the laboratory follow-up of these cases.

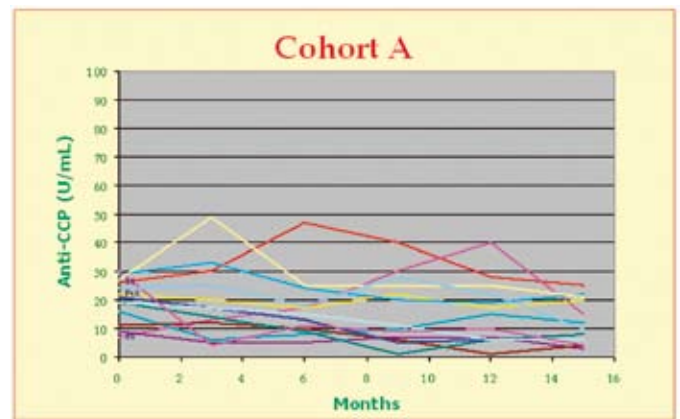


Figure 2: CCP levels in patients with no family history with stable values in the low-to-moderate range of borderline concentrations (Group A)



Figure 1: CCP levels in family members

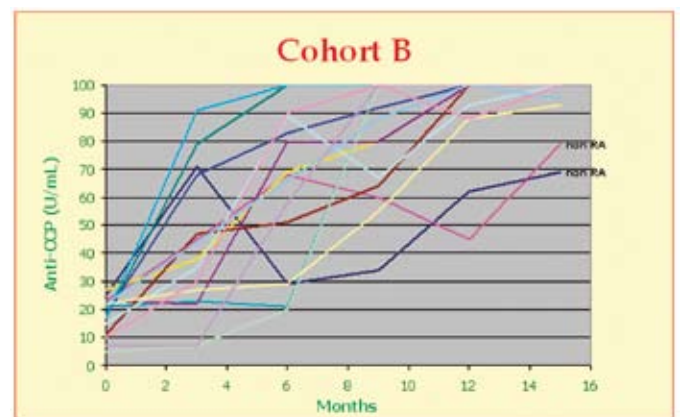


Figure 3: CCP levels in patients with no family history in whom anti-CCP values increased over time (Group B)

Significance of anti-CCP antibodies and rheumatoid factor in a rheumatologic outpatient setting

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Objective: To evaluate the significance of anti-CCP antibodies and rheumatoid factor in a rheumatologic outpatient setting.

Patients and Methods: 240 patients with suspected rheumatoid arthritis (RA) were classified according to ACR criteria. 138 were classified as RA, 24 had osteoarthritis, 16 had spondylarthritis, 7 fibromyalgia and 8 others. In 47 patients no rheumatologic disease could be diagnosed.

All patients were tested for rheumatoid factor and anti-CCP using EliA CCP (Phadia, Germany).

	RF	Anti-CCP	RF and / or anti-CCP
Sensitivity (%)	65.2	49.2	71.0
Specificity (%)	61.2	96.1	59.2
PPV (%)	68.2	94.4	70.0

Table 2: Results in RA patients

n total = 240	n	RF+ CCP+	RF+ CCP-	RF- CCP+	RF- CCP-
RA	138	60	30	8	40
Still's Disease	4	0	0	0	4
PMR	2	0	1	0	1
SCTD	2	0	1	0	1
OA	24	2	13	0	9
Fibromyalgia	7	0	4	0	3
Spondylarthritis	16	0	2	0	14
rheumatic disease not diagnosed	47	2	16	0	29

Table 1: Prevalence of CCP and RF in different diseases

Conclusion: Anti-CCP showed a high specificity for RA and was superior to RF with respect to specificity and PPV. The sensitivity of anti-CCP antibodies, however, was quite low, possibly due to a rheumatoid factor biased patient selection. This analysis confirms the value of anti-CCP testing for establishing the diagnosis in patients suspected of suffering from RA.

Diagnostic performance of anti-citrulline antibodies and IgM rheumatoid factor in rheumatoid arthritis

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Objective: To compare the sensitivity and specificity of two diagnostic markers, the 2nd generation CCP assay (anti-CCP2) and the IgM class rheumatic factor (RF) in Portuguese patients with established rheumatoid arthritis (RA) recruited from our outpatient rheumatology clinic.

Patients and Methods: 56 patients with RA, 43 with spondylarthropathies and 50 blood donors were tested for anti-CCP with EliA CCP (Phadia, Germany) on the ImmunoCAP 100 instrument and for IgM RF with an indirect enzyme immunoassay (DiaMeDix, IVAX Diagnostics Inc., Florida, USA).

Results: 52% of RA patients (29/56) were found positive with both tests. 11 patients were only positive for anti-CCP and 5 were only positive for RF. None of the controls were anti-CCP positive but 4 of 43 disease controls had RF. EliA CCP is superior in both aspects performing about 10 % better than the RF test.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Efficiency (%)	AUC
EliA CCP Cut-off >10 EliA U/ml	71.4	100	100	72.9	83.8	0.866
RF Cut-off >20 IU/ml	60.7	90.7	89.5	63.9	73.7	0.787

Table 1: Sensitivity, specificity, predictive values, efficiency and area under the curve for EliA CCP and rheumatoid factor

Conclusions: The anti-CCP2 showed an overall superior performance compared to the rheumatoid factor. The specificity of the anti-CCP2 is the most relevant aspect of this test.

The clinical utility of the anti-CCP test. Practicability of the EliA method for determination

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Objective: To evaluate EliA CCP and the practicability of the instrument ImmunoCAP 250.

Patients and Methods: 86 consecutive samples from patients with suspected rheumatoid arthritis (RA), sent in within 5 months in 2006, were tested for anti-CCP and rheumatoid factor (RF). 46 of the patients were diagnosed with RA.

The practicability of the ImmunoCAP 250 instrument was evaluated for environment, organisation of work, versatility and flexibility, security controls, staff training and maintenance through a questionnaire with a score ranging from 1 (very good) to 4 (very bad).

Results: 30/86 samples were positive for anti-CCP (35%) and 4 of these 30 were RF negative. 25 anti-CCP positive patients were diagnosed for RA, 5 could not be classified as having RA.

The practicability of the instrument was scored at 1.2 (see table).

Conclusion: The results confirm the diagnostic use of anti-CCP. The practicability study showed that the system is very useful because of its flexibility, simplicity etc.

With the same instrument IgG and IgA against other autoantigens as well as IgE against allergens can be measured and so in both the autoimmunity and the allergy lab time-saving is possible.

Environment (need of electricity, temperature...)	1.0
Work organisation	1.53
Start operations	2.66
Sample processing	1.0
Sample display and type of samples	1.83
Sample identification	1.0
Sample aspiration	1.66
Reagents	1.3
Speed	1.0
Methods	2.25
Information processing	1.0
Versatility and Flexibility: it is a closed analytical system	1.1
Security controls:	1.25
Easy access to analytical modules	1.15
Calibration control	1.0
Quality control	1.22
Staff training	1.22
Maintenance	1.0
Operational costs	1.5
Total	1.2

Table 1: Criteria for evaluating the practicability

Optimization project for an autoimmunity laboratory

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Objective: To establish a new organization, new instruments, methods and software in our lab and to evaluate the impact of this new organization.

Strategy: The project includes several actions with the test requesters in addition to all the typical activities looked after by the laboratory, in order to optimize the requests, the test setting and the reports.

Pre-analytical phase: It is very important to receive appropriate requests. This was obtained by meetings with clinicians, setting up of diagnostic algorithms, keeping clinicians informed using a company “on line” magazine and by supporting GPs as they generate their requests with a new software update.

Analytical phase: To improve the effectiveness, a powerful software and a new and fully automated instrument for test processing were used. Additionally, other instruments for specific purposes were used.

Post-analytical phase: To prepare valid reports, the authors inserted the analytical CV for every quantitative test, comment all results, store the samples for at least 30 days and examine the effectiveness of the reports.

Conclusion: Our experience is that the efficiency of our laboratory is significantly higher when the cooperation with the test requesters is active and effective.

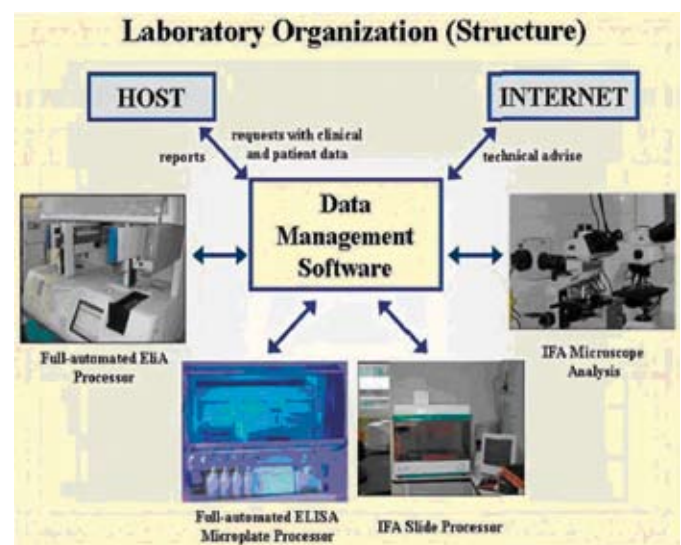


Figure 1: laboratory organization

Diagnostic accuracy of IgG anti-tissue transglutaminase antibody assays in celiac disease

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Objective: To evaluate 9 commercially available kits for IgG anti-tissue transglutaminase (tTG) antibodies for detecting Selective IgA Deficiency (SIgAD) which is the most common primitive immunodeficiency and is associated with an increased prevalence of celiac disease (CD) and to compare the results with those obtained with a test for IgG anti-gliadin (AGA) and a test for IgG to deaminated gliadin peptides (DGP).

Patients and Methods: 20 consecutive CD patients with SIgAD diagnosed according to the revised ESPGHAN criteria, 63 disease controls (9 patients with SIgAD without CD but affected by other gastrointestinal disorders; 54 patients with chronic liver disease) and 49 healthy subjects were tested with 9 different IgG anti-tTG assays (Euroimmun; Orgentec; Phadia; Immco; D-tek; Aesku; Generic Assay; Inova; Radim). IgG AGA were measured by a commercial ELISA assay (Radim, Italy) and IgG anti-DGP were measured using the Quanta Lite Gliadin IgG II ELISA assay (Inova Diagnostics, USA).

Results: Using the cut-off proposed by each manufacturer, the sensitivity ranged from 75 to 95% and the specificity from 88 to 100%. On the other hand, choosing the best cut-off by ROC curves, the sensitivity ranged from 90 (1 kit) to 95% (8 kits) and the specificity from 95 (1 kit) to 100% (8 kits); the area under the ROC curve was high (range 0.968 – 0.994) and not statistically different among the 9 assays evaluated. However, as Bland-Altman analysis demonstrated, absolute antibody values are not interchangeable and standardization is needed.

Manufacturer	Manufacturers' cut-off			ROC plot analysis-derived cut-off		
	Cut-off	Sens (%)	Spec (%)	Cut-off	Sens (%)	Spec (%)
Euroimmun	1 (ratio)	80	100	0.7 (ratio)	95	100
Orgentec	10 U/ml	95	94	31.5	95	100
Immco	20 U/ml	95	91	56.1	95	100
Phadia	7 U/ml	95	100	4.0	95	100
D-tek	25 U/ml	75	97	31.4	75	98
Aesku	15 U/ml	85	95	11.5 U/ml	95	94
Generic Assay	20 U/ml	95	91	33.8 U/ml	90	95
Inova	20 U/ml	75	99	11.6 U/ml	95	99
Radim	7 U/ml	95	88	11.0 U/ml	95	100
IgG AGA (Radim)				15 RU/ml	40	87
IgG anti-DGP (Inova)				7 U/ml	80	98

Table 1: Sensitivity and specificity of different tests using the manufacturers' cut-offs and the ROC plot analysis-derived cut-offs.

Conclusion: When choosing the best cut-off by ROC curve, all the IgG anti-tTG assays showed a good diagnostic accuracy for CD in SIgAD patients, even though they perform differently.

Because of its low efficiency the detection of IgG AGA should not be routinely used for diagnosing CD in SIgAD.

The new assay using deaminated gliadin peptides as antigen for AGA detection was more accurate than the conventional assay, and therefore it can be used if there is a high suspicion of CD and IgG anti-tTG antibodies are not detected.

Automated ANA/ENA Screening (EliA) as an alternative to IIF (HEp-2) in daily routine

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Objective: To compare the clinical performance of two different ANA/ENA screening tests.

Patients and Methods: 236 consecutive patients were analysed (40 connective tissue disease (CTD), 39 disease controls (Non-CTD), 155 where no diagnosis was available (NoD)). IIF on HEp-2 (BIO-RAD Laboratories, USA) was carried out and values above 1:80 were regarded as positive. The

fully automated tests EliA Symphony with a defined number of antigens and EliA dsDNA were performed on the ImmunoCAP 100 instrument (Phadia, Germany).

Results: HEp-2 IIF detected 36/40 CTD patients and 19/39 Non-CTD patients. EliA Symphony plus EliA dsDNA found 18/40 CTD and 2/39 Non-CTD patients. The clinically false positive HEp-2 results were found in patients with diseases such as Crohn's disease, thyroid disorders, cryoglobulinemia, liver disorders and infections, where unspecific ANAs are described.

The data resulted in a positive likelihood ratio of 1.85 for HEp-2 and 10.78 for EliA.

n = 79 (40 CTD, 39 Non-CTD)	Sensitivity (%)	Specificity (%)	Positive Likelihood Ratio	Negative Likelihood Ratio
HEp-2	90	51.3	1.85	0.19
EliA Symphony & EliA dsDNA (combined)	55	94.9	10.78	0.47

Table 1: Sensitivity, specificity and likelihood ratios for the detection of ANAs with HEp-2 versus EliA Symphony and EliA dsDNA.

Conclusions: Screening by HEp-2 IIF is most sensitive, but displays a low specificity. This has consequences for the calculated positive likelihood ratio and makes the value of a positive HEp-2 result questionable, at least by applying the indicated cut-off. The sensitivity of screening using defined antigens is lower, but specificity is excellent. The majority of patients missed suffer from SLE, maybe at an inactive disease state as indicated by the negative dsDNA results.

Evaluation of EliA dsDNA and EliA Symphony vs. CLIFT and routine ENA antibodies in the diagnostics of systemic autoimmune diseases

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Objective: To evaluate the diagnostic efficacy of an automated detection system for antibodies against dsDNA and extractable nuclear antigens (ENA) – comparison with immunofluorescence (CLIFT), immunodiffusion and/or immunoblotting (ID/IB).

Patients and Methods: 223 sera from 178 patients were tested for dsDNA and ENA antibodies between 2001 and 2002. Clinical evaluation was done retrospectively: 69 patients had a connective tissue disease and 109 had non-CTD.

ENA Screen, anti-dsDNA, and single ANA specificities were measured with the EliA method on the ImmunoCAP 100 instrument (Phadia, Germany). Routine ID/IB (except for Ro) was performed using rabbit thymus extract (Pel-Freeze Biologicals). For Ro human spleen extract was used. Weak or borderline positive ID/IB results were confirmed using specific ELISAs using purified antigens (Axis-Shield Diagnostics). Routine immunofluorescence for anti-dsDNA (CLIFT) was performed using a kit from Immuno Concepts.

Results: EliA dsDNA was more sensitive than CLIFT in detecting SLE with nephritis (11/18 vs. 8/18) or without nephritis (8/16 vs. 4/16). Both showed high disease specificity (2/35 and 1/35 positive in other CTDs).

EliA Symphony was more sensitive than ID/IB (20/34 vs. 16/34 SLE patients and 36/69 vs. 31/69 CTD patients) with equal specificity (15/109 vs. 14/109 non-CTD patients).

Anti-RNP antibodies were found in SLE (7/16 without nephritis vs. 5/18 with nephritis) and anti-Sm were found in active SLE (3/9 with active SLE vs. 2/25 with inactive SLE).

16 patients classified as suffering from non-CTD/non-autoimmune diseases were positive by EliA dsDNA or EliA Symphony with specificity

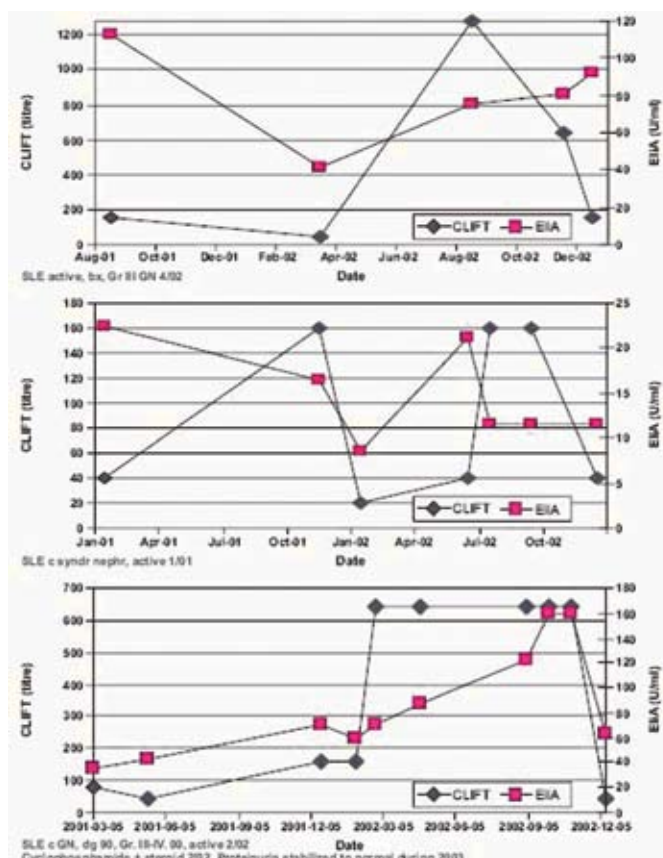


Fig. 1: Follow-up of three SLE patients with active nephritis showed good correlation between flare and dsDNA antibody titre, measured by EliA dsDNA or CLIFT.

for Ro, La, RNP, Sm or CENP: 8/105 non-CTD patients had anti-Ro antibodies, 3 had anti-Ro and anti-La, one patient with chronic glomerulonephritis had anti-Ro and anti-dsDNA (in both CLIFT and EliA positive) and 1/105 had centromere antibodies. 3/105 were misclassified due to missing patient history files.

Conclusion: EliA dsDNA was more sensitive than CLIFT in detecting SLE and a good correlation was found between SLE flare and the level of antibodies in follow-up samples. Both tests showed high diseases specificity. The EliA system was more sensitive than routinely used ID/IB.

Clinical evaluation of the new Varelisa ANA CTD Screen

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Objective: To evaluate a new automated ANA screening method as a routine technique to aid in the diagnosis of rheumatic autoimmune diseases.

Materials and Methods: 62 samples with established clinical diagnosis were measured with two ANA screening ELISAs, both measured on an automated ELISA processor:

The Varelisa ANA CTD Screen (Phadia, Germany) and the Quanta Lite ANA Screen (Inova Diagnostics, USA). The Quanta Lite assay was used as reference to define the diagnostic value of the new Varelisa ANA CTD Screen.

Both ELISAs include dsDNA, histone, Jo-1, Sm/RNP, SS-A, SS-B, Scl-70, centromere and ribosomal P protein. The Inova assay additionally has PCNA while the Phadia assay additionally includes Pm-Scl.

Antigenic specificities were determined using Quanta Lite ENA (Inova) and EliA dsDNA (Phadia).

Results: The results were obtained using the Inova ANA Screen as a reference (table 1)

Conclusion: ANA CTD Screen has good sensitivity and specificity for connective tissue diseases, as well as PPV and NPV. The efficiency of the new assay is similar to that of our routine method for the diagnosis of CTD.

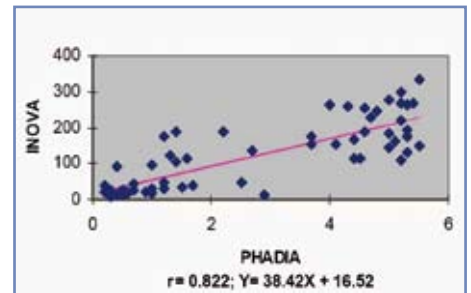


Figure 1: Quanta Lite ENA and EliA dsDNA in a regression curve

Sensitivity	93 %
Specificity	88 %
Positive predictive value	95 %
Negative predictive value	84 %
Positive likelihood ratio	7.75
Negative likelihood ratio	0.07

Table 1: Sensitivity, specificity, predictive values and likelihood ratios for Varelisa ANA CTD Screen with Quanta Lite ENA as reference

Evaluation of a new enzyme immunoassay for detection of antinuclear antibodies

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Objective: To evaluate the Varelisa ANA CTD Screen for the screening of connective tissue diseases (CTD) and to compare it with another immunoassay routinely used in the lab.

Patients and Methods: 223 consecutive patient samples with a request for ANA screening were tested with Varelisa ANA CTD Screen (Phadia) and with ANA Screen ELISA (Meridian). Positive results were confirmed by IIF on HEp-2 cells. Discrepant results were measured for dsDNA and specific ENA (Ro, La, RNP, Sm, Scl-70, CENP-B, Jo-1) with EliA (Phadia).

Results:

		Varelisa ANA CTD Screen		
		Negative	Positive	Total
ANA Screen ELISA	Negative	174	14	188
	Positive	12	23	35
	Total	186	37	223

Table 1: Varelisa ANA CTD Screen versa ANA Screen ELISA from Meridian

All samples positive in both ELISAs were also positive in IIF. Discrepant samples: Almost all sera which were positive by ANA Screen ELISA and negative by Varelisa react with uncommon antigens not related to antigens present in the Varelisa Screen. 8 of 14 samples which were negative by ANA Screen ELISA but positive by Varelisa were negative in IIF but many of them were EliA Ro positive.

Conclusion: Varelisa ANA CTD Screen is a good screening assay to aid in the diagnosis of systemic rheumatic diseases. It detects anti-Ro 52 antibodies that could be missed by IIF on HEP-2 cells and by other ELISA.

Performance of CTD Screen as screening for connective tissue diseases

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Objective: To assess the clinical performance of the new Varelisa ANA CTD Screen in the diagnosis of connective tissue diseases.

Patients and Methods: 287 patients with connective tissue diseases (220 SLE, 41 Sjögren’s syndrome, 26 Scleroderma with CREST symptoms), 217 disease controls and 105 blood donors were tested with Varelisa ANA CTD Screen, which includes the following antigens: dsDNA, RNP, Sm, Ro, La, Scl-70, centromere, Jo-1, histones, Pm-Scl and ribosomal P protein.

Results:

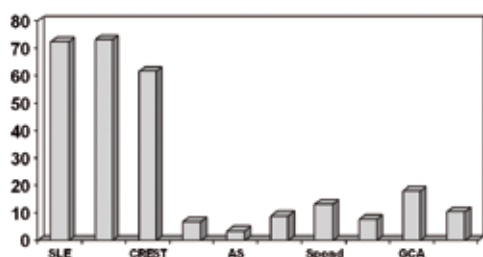


Figure 1: Frequency of samples positive in the Varelisa ANA CTD Screen

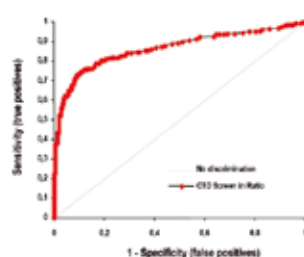


Figure 2: ROC analysis for Varelisa ANA CTD Screen (AUC: 0.866)

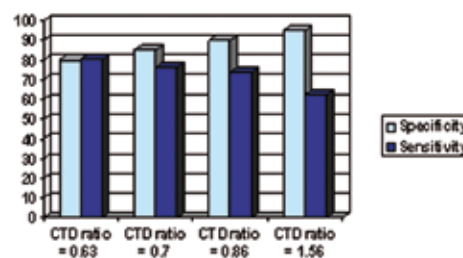


Figure 3: Specificity (light blue) and sensitivity (dark blue) with different cut-offs

Conclusion: A test employing a subset of the most prevalent specificities reveals a good compromise as indicated by a high positive likelihood ratio. However, the presence of ANA in diseases other than connective tissue diseases may be of clinical significance as well. In such cases an IIF test is still mandatory, especially in autoimmune laboratories.

	Cut-off ratio > 1.0	Cut-off ratio > 1.4
Sensitivity (%)	71.4	64.8
Specificity (%)	91.2	94.5
Positive likelihood ratio	8.2	11.7
Negative likelihood ratio	0.31	0.37

Table 1: Sensitivity, specificity and likelihood ratio with different cut-offs. With the cut-off at 1.0, 10 blood donors (10.48 %) were positive but none with the cut-off at ratio 1.4.

Comparison of different methods for the detection of antinuclear antibodies

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Objective: To compare the Varelisa CTD Screen (Phadia) with AtheNA Multi-Lyte ANA Test System (Zeus Scientific) used routinely in the lab.

Patients and Methods: 156 consecutive patient samples with a request for ANA screen were tested with Varelisa CTD Screen and AtheNA Multi-Lyte ANA Test System. Positive results were confirmed by indirect immunofluorescence (IIF) on Hep-2 cells. The Varelisa screens for antibodies against dsDNA, RNP (RNP70, A, C), Sm (B, B’, D), SS-A/Ro (52 kDa, 60 kDa), SS-B/La, Scl-70, CENP-B, Histone, Ribosomal P Protein and Jo-1 in a single well. The AtheNA Multi-Lyte system allows performing multiple individual assays in 1 well (dsDNA, SSA, SSB, Sm, RNP, Scl-70, Jo-1, Centromere-B and Histones).

Results: We found an excellent agreement between both tests.

2 sera were negative in both assays but positive in IIF. 4 were positive in Athena but negative in Varelisa, 2 of these 4 were positive in IIF. 2 were positive in Varelisa but negative in Athena, 1 of these 2 were IIF positive.

Conclusion: Varelisa CTD Screen assay gives comparable results to Athena Multi-Lyte ANA Test System.

Critical evaluation of positivity for anti-dsDNA by the Farr assay

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Objective: To investigate the significance of persistent positive anti-dsDNA antibodies by the Farr assay in patients suspected of having SLE, but with no organ involvement and scarce clinical evidence.

Patients and Methods: 28 SLE patients, 21 patients with a silent clinical setting (ECLAM<4, group A) and 7 with active SLE (ECLAM>6, group B), all positive by Farr, were tested for IgG-IgM anti-dsDNA by CLIFT (Immuno Concept) and by ELISA (Varelisa dsDNA Antibodies, Phadia). Additionally, they were tested for IgG-IgM anti-nucleosome (anti-NCS) in ELISA (Euroimmun, Inova).

Results: A Rheumatologist, blinded to anti-dsDNA values, made a revision of clinical and serological records of group A (low activity). 10 of 21 patients were classified as SLE in a remission phase, 7 as non-SLE connective tissue diseases (3 MCTD, 3 UCTD and 1 RA) and 4 as non systemic autoimmune diseases (1 chronic active hepatitis; 1 former thymoma; 1 monoclonal gammopathy of uncertain significance; 1 Kikuchi disease). One of the SLE patients in inactive stage was positive only for IgM; the patient with Kikuchi was negative for both isotypes of anti-dsDNA and anti-NCS (Fig 1). Farr positivity could be explained either as IgA anti-dsDNA positivity or as nucleosome/anti-nucleosome complexes. However, this was not confirmed by anti-NCS ELISA.

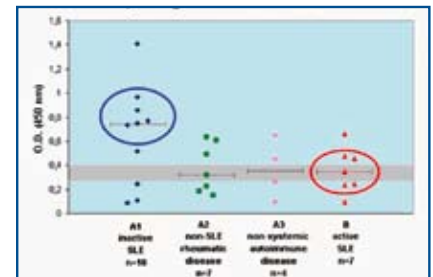
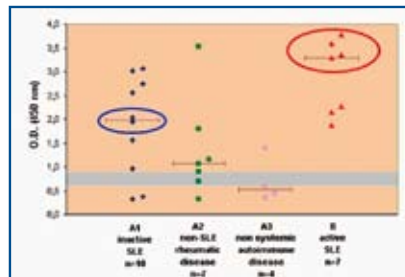
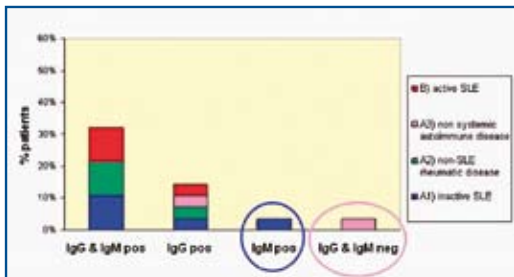


Figure 1: Anti-dsDNA isotypes (CLIFT and ELISA)

Figure 2 and 3: IgG anti-dsDNA ELISA and IgM anti-dsDNA ELISA

IgG anti-dsDNA titres were significantly higher during SLE flares, while IgM were mostly present during remission (Figure 2 and 3).

The concordance between 2 different IgG anti-nucleosome tests was low (Cohen’s Kappa Test K=0.32), for IgM anti-nucleosome tests it was high (K=0.81). In contrast to anti-dsDNA, IgG and IgM anti-nucleosome failed to be significantly different between active and remitting SLE.

Conclusion: This study shows that a positive Farr assay is not limited to an SLE diagnosis. 11 out of 21 Farr positive patients with a silent setting were diagnosed later as having other diseases.

Isotype-specific methods can be useful to identify subsets of patients. One SLE patient with only IgM antibodies was identified.

IgG titres were significantly higher during SLE flares, while IgM were mostly present during remission of disease. This finding seems to be concordant with the recent demonstration that IgM anti-dsDNA could have a protective role against organ damage in SLE, especially nephritis.

Evaluation of novel assay systems for the determination of antibodies to double-stranded DNA in patients with SLE

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Objective: To evaluate 2 novel assay systems for determination of anti-dsDNA antibodies, the EliA dsDNA assay (Phadia) and the microsphere based Fidis immunoassay (Biomedical Diagnostics).

Patients and Methods: 99 SLE patients and 270 disease controls (139 patients with other connective tissue diseases, 75 with inflammatory arthritis, 43 patients with non-inflammatory rheumatic diseases and 13 with non-rheumatic diseases) were evaluated.

The samples were measured with the following methods: CLIFT (substrate from Bios, Munich, Germany), Farr assay (Ortho-Clinical Diagnostics, Amersham, UK), EliA (automated dsDNA test on the ImmunoCAP instrument, Phadia, Freiburg, Germany), Fidis (quantitative homogeneous microparticle based immunoassay using Luminex technology from Biomedical Diagnostics SA, Marne La Valle, France) and Chromatin ELISA (histone H1 depleted calf thymus chromatin, Quanta Lite, INOVA, San Diego, USA).

Disease activity was determined with ECLAM score.

Results:

	CLIFT	Farr	EliA	Fidis	Anti-Chromatin
n	493	265	493	232	352
Sensitivity (%)	23	64	54	65	61
Specificity (%)	97	88	91	83	85

Table 1: Sensitivity and specificity for different test systems.

EliA and Fidis equal the “gold standard” Farr assay with respect to sensitivity and specificity for SLE. Values of both assays correlated well with the Farr assay. Fidis showed the highest correlation with Farr and EliA showed the highest correlation with disease activity (ECLAM). EliA and Farr assay showed similar associations with various SLE organ manifestations which, however, did not reach statistical significance. Determination of anti-chromatin antibodies turned out to be of no additional diagnostic value.

Conclusion: Sensitivities and specificities of the two novel anti-dsDNA assay systems (EliA and Fidis) were comparable to the Farr assay and sensitivity was considerably superior to the Chritidia assay. Therefore, both assays are reasonable alternatives to the Farr assay for determination of anti-dsDNA antibodies.

Evaluation of the EliA technique for detection of anti-bodies to proteinase 3 and myeloperoxidase in patients with systemic vasculitis

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Objective: To evaluate the diagnostic performance of EliA tests for anti-PR3 and anti-MPO combined with the indirect immunofluorescence (IIF) ANCA test in a larger cohort of patients with clinically defined AASV (ANCA-associated systemic vasculitides).

Patients and Methods: 126 patients with AASV and 100 disease controls (89 with chronic inflammatory diseases and 11 with acute infectious diseases) were collected. Additionally, 15 blood donors were tested and were negative in all of the tests.

ANCA were detected by IIF (Inova Diagnostics), always controlled by an ANA test on HEp-2 cells (Inova Diagnostics). Anti-PR3 and anti-MPO were measured with EliA on ImmunoCAP 100 (Phadia).

Results:

n = 226 (126 AASV, 100 Non-AASV)	Sensitivity (%)	Specificity (%)
ANCA IIF	77.0	55.0
EliA PR3 or EliA MPO	67.5	89.0

Table 1: Sensitivity and specificity for ANCA IIF and EliA PR3 or EliA MPO

n = 226 (126 AASV, 100 Non-AASV)	Positive Predictive Value (%)
ANCA IIF	68.3
ANCA IIF and EliA PR3 or EliA MPO	88.3

Table 2: Positive predictive value for IIF alone and IIF combined with EliA PR3 or MPO

Evaluating the clinical significance of the results for all the patients, the combination of EliA PR3 and EliA MPO showed a lower sensitivity (67,5%) but a better specificity (89,0%) than ANCA-IIF test alone (77,0% and 55,0%, respectively) for AASV.

The combination of ANCA-IIF positive tests with the positive results of EliA PR3 or MPO gave a PPV of 88,3%; the fact that some of the AASV patients were in remission of the disease supports our belief that the true PPV of the test is actually higher.

Conclusion: The automated EliA system, apart from being fast, flexible and easy to operate, demonstrates a good sensitivity and excellent specificity for detection of antibodies to PR3 and MPO in patients with ANCA-associated systemic vasculitides.

Performance of a new solid-phase assay to detect ANCA, specific for proteinase 3

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Objective: To evaluate the diagnostic sensitivity of Varelisa PR3 Capture for Wegener's granulomatosis (WG), in comparison with the direct PR3 ELISA routinely used.

Patients and Methods: 35 WG, 72 disease controls and 13 blood donors were tested on IIF for C-ANCA, on the home-made direct PR3 ELISA which is routinely used in this lab and on the new Varelisa PR3 Capture. In addition, 485 consecutive serum samples from routine were tested in the three devices.

Results:

n = 35 WG, 31 MPA, 43 disease controls	C-ANCA (IIF)	Direct PR3 ELISA	Varelisa PR3 Capture
Sensitivity (%)	82.9	51.4	82.9
Specificity (%)	100	95.9	98.6

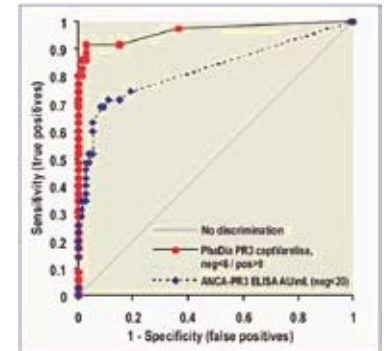


Figure 1: ROC curve of direct PR3 ELISA and Varelisa PR3 Capture.

Table 1: Sensitivity and Specificity for C ANCA IIF, direct ELISA and Varelisa PR3 Capture

n = 481 (4 equiv. excluded)		Varelisa PR3 Capture		
		Negative	Positive	Total
C-ANCA	Negative	427	10	437
	Positive	3	41	44
	Total	430	51	481

Table 2: Positive / Negative agreement of C-ANCA (IIF) and Varelisa PR3 Capture results in 481 consecutive routine samples. The 10 Varelisa PR3 Capture positive/c-ANCA negative samples were all from WG patients.

Conclusion: Both in the retrospective and in the prospective phase of the study, Varelisa PR3 Capture had a higher sensitivity than the direct PR3 ELISA maintaining a very good specificity. Moreover, its diagnostic performance is very close to IIF.

Increased sensitivity in anti-PR3 detection with a new capture assay

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Objective: To evaluate the clinical performance of a new PR3 Capture ELISA.

Patients and Methods: The presence of anti-PR3 was evaluated with a new PR3 Capture ELISA (Varelisa PR3 Capture, Phadia) in 89 patients with ANCA-associated vasculitis (AAV; 55 WG, 31 MPA, 3 CSS) and 66 disease controls (54 with SLE and 12 with other inflammatory rheumatic disorders). The activity status of 19 of 55 WG patients was scored as active, 36 inactive. Results for AAV samples were compared against IIF and our routinely used direct anti-PR3 ELISA.

Results:

	Direct ELISA for PR3	Varelisa PR3 Capture
Sensitivity (%) for WG	64	80
Sensitivity (%) for AAV	55	64
Specificity (%) for AAV	97	97

Table 1: Sensitivity for Wegener's granulomatosis and for AAV and specificity for AAV

17 of 19 clinically active WG (89%) and 28 of 36 remission sera (78%) were highly positive. 8 WG samples and 1 MPA sample were positive in the capture assay but negative in IIF (2 of them in active stage). 3 WG and 2 MPA samples were positive for c-ANCA in IIF but negative or equivocal in the capture assay.

Conclusions: With this serum panel, the new PR3 capture assay had a clearly higher sensitivity than the direct PR3 ELISA and even a higher sensitivity than IIF with a still excellent specificity.

High positive likelihood ratios of autoantibodies cross-reacting with a 28 kDa Drosophila antigen for diagnosis of ankylosing spondylitis

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Objective: To test the diagnostic value of autoantibodies cross-reacting with a 28 kDa Drosophila antigen for the diagnosis of ankylosing spondylitis (AS).

Patients and Methods: 371 AS patients, 165 disease controls and 37 blood donors were tested using a Research ELISA from Phadia. Western blotting was performed to exclude detection of E-coli antigens by patients' sera and a 13.5% separation SDS gel was used to confirm the purity of the antigen used for the ELISA-plates.

Results: Increased serum concentrations of antibodies cross-reacting with a 28 kDa Drosophila antigen were found in AS patients compared to healthy controls (39.5 U/ml vs. 22.6 U/ml; $P=0.004$, Fig. 1). The positive likelihood ratios of this ELISA test for AS were between 1.9 [95% confidence intervals 1.2–3.8] for a cut-off level of 50 U/ml and 3.8 [1.6–15.4] for a cut-off level of 75 U/ml. The sensitivities were between 42.1% [37.0–47.3%] for a cut-off level of 50 U/ml and 30.7% [26.1–35.7%] for a cut-off level of 75 U/ml (Table 1).

Antibody concentrations were independent from age of the patients, HLA-B27 predisposition, BASMI, BASFI and S-HAQ scores. Although not significant, women tended to have higher antibody concentrations than men ($P = 0.091$).

	Cut-off > 50 U/ml	Cut-off > 60 U/ml	Cut-off > 75 U/ml
Sensitivity (%)	42.1	36.1	30.7
Specificity (%)	78.4	83.8	91.9
Positive likelihood ratio	1.9	2.2	3.8

Table 1: Sensitivity, specificity and positive likelihood ratio at different cut-offs

Conclusion: Serum ELISA tests for autoantibodies cross-reacting with the 28 kDa Drosophila antigen have low sensitivities, but high positive likelihood ratios for AS depending on the cut-off levels used.

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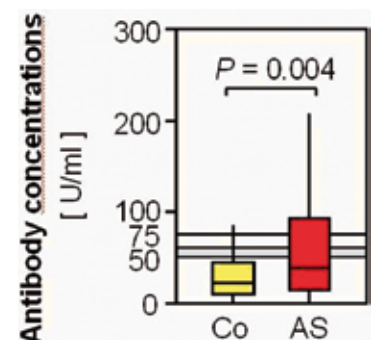


Figure 1: Increased concentrations of antibodies cross-reacting with a 28kDa antigen in AS patients compared to healthy controls (Co).

A novel anti-IgA antibody and IgA deficiency assay based on the EliA platform on ImmunoCAP 250 and ImmunoCAP 100 system

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Objective: To evaluate a human IgA coated well for the detection of IgG antibodies against IgA based on the commercial EliA IgG platform.

Patients and Methods: 42 total IgA deficient patients, 9 patients with suspected low total IgA levels, 119 blood donors and 12 suspected celiac disease patients, whereof 5 were total IgA deficient were tested.

Special human IgA coated EliA wells (Phadia, Germany) were used for the detection of IgG anti-IgA antibodies. All other reagents were standard commercial EliA reagents (Phadia AB, Sweden). Measuring range for the EliA IgG method was 0-600 µg/l and for EliA IgA 0-80 µg/l (WHO IRP 67/86).

Inhibition study was performed in 2 sera using purified (1.9 mg/ml) human IgA (Europe bioproducts Ltd, UK), and also performed by using human donor plasma.

Results: EliA IgG anti-IgA could discriminate normal from abnormal samples with a suggested cut-off at 3 µg/l and an equivocal range of 3-7 µg/l to indicate the dynamic of the antibody level over time. Inhibition study shows inhibition of > 50-90 % of IgG anti-IgA concentration by pre-incubation of samples with purified human IgA, and to a lesser extent (>30-60%) with pre-incubation of normal donor serum.

EliA Low level IgA application can be used to select Total IgA deficient patients from patients with normal or low Total IgA concentration with a preliminary cut-off at 10 µg/l IgA, which also makes it suitable for the differential diagnosis of celiac disease. Very good agreement was seen between ImmunoCAP 250 and ImmunoCAP 100 results. CV % for the EliA IgG anti-IgA assay was found to be < 10%.

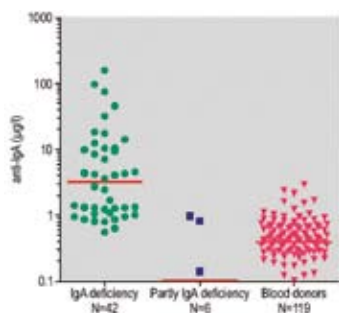


Figure 1: EliA IgG anti-IgA antibodies.

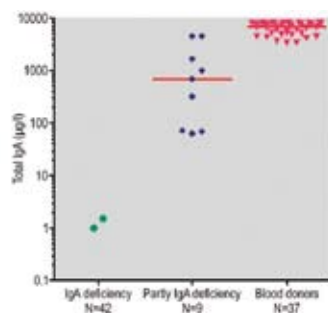


Figure 2: EliA low Total IgA application.

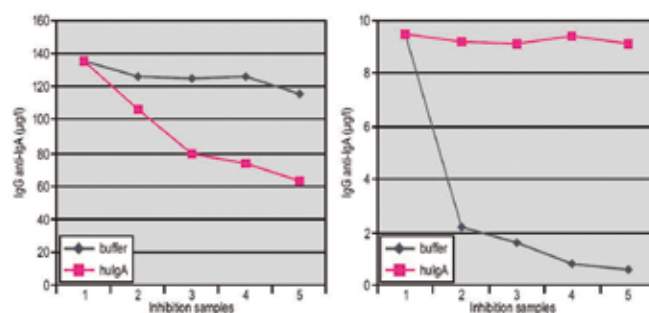


Figure 3: IgG anti-IgA inhibition with human IgA dilutions 1:16, 1:8, 1:4, 1:2

Conclusions: The described assay provides an easy method for accurate quantification of IgG antibodies against human IgA, on both the ImmunoCAP 250 and ImmunoCAP 100 instruments. By using the recommended cut-off at 3 µg/l the assay is capable of discriminating between absence or elevated levels of IgG anti-IgA with high sensitivity. The assay is also capable of discriminating quantitatively between patients with total IgA deficiency, low total IgA and probably normal IgA levels, using the recommended cut-off at 10 µg/l.

An IgE anti-IgA antibody assay should be developed to cover some anaphylactic transfusion reactions not detected by the described IgG anti-IgA antibody assay.

Article

EASI - the European Autoimmunity Standardization Initiative

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Introduction

Autoimmune diseases (AID) are a very large and complex group of disorders, which can be organ-specific (affecting one organ only) or systemic (affecting different organ systems of the whole body) [1, 2]. They are characterised by a broad variety of clinical symptoms which, in the initial phases of the diseases, may often be weak and uncharacteristic, and here the finding of characteristic autoantibodies in the patients' serum can lead to recognition of early disease [3, 4].

A thorough medical examination of a patient must precede analysis and interpretation of combined clinical and serological data [5]. Decisions

on technologies and reporting routines should be based on a close collaboration between clinicians and laboratory specialists. Experiences in the different European countries, however, demonstrate that, due to changes in the health care systems and the organisation of laboratories, clinicians and laboratory specialists are working more and more in isolated units.

EASI was founded six years ago with the initial intention of improving diagnostics in chronic rheumatic disorders by strengthening the collaboration between clinical and laboratory scientists responsible for rheumatology diagnostics at any given level of the health care systems in Europe.

Situation of the diagnostics of autoimmune rheumatic diseases

An analysis of how such diseases are diagnosed in several European countries resulted in the identification of three areas where changes in the actual work-up could lead to an earlier, better and faster diagnosis of patients and an overall reduction of health care costs.

1. Communication between clinicians and laboratory needs to be improved

Through the introduction of new techniques and methodologies in clinical and laboratory practice and the efforts made by most of the European health care systems to increase efficiency and reduce costs, clinical and labora-

tory units are organized to work more and more independently, sometimes even isolated from each other. Initially this leads to a less frequent communication between the parties and finally can result in an exchange of information solely by electronic media. Through the loss of direct contacts between the laboratory, the clinician and the patient the importance of interactive diagnostics becomes weaker.

A stronger communication and cooperation network between the different groups would lead to an earlier, more precise and faster diagnosis of patients, would allow a rational follow-up and sometimes an earlier treatment all of which would reduce the overall health care costs.

2. Testing algorithms are not standardised

Thorough clinical work-up is frequently not the basis for the usage of laboratory tests. Old established test methods are often exchanged by new methods without recognition of the differences such exchange may confer to the clinical use of test results. Often, the optimal way of reporting laboratory results to the clinic has not been agreed upon between clinicians and laboratory scientists.

There are no standardised algorithms used by the different laboratories for ensuring the rational use of laboratory tests. Such algorithms should be locally customised taking into account the various methods available and the results that can be derived by using these tests should be studied.

A standardisation/harmonisation of testing algorithms across Europe could lead to a more efficient use of the laboratory tests.

3. International standards for test kits, methodology and use of results are needed

Only a few assay systems used in the determination of autoantibodies are based on international standards (like WHO Wo/80 for anti-dsDNA [6] or MRC 65/93 and 66/387 for anti-Tg and anti-TPO, respectively). Most of the test

systems refer to arbitrary units resulting from internal calibrations by the manufacturers or the laboratory itself.

Comparability of results, however, is not given even if international reference preparations are used due to the very different reaction conditions in different assay systems. FARR assays, Crithidia luciliae immunofluorescence test (CLIFT) or regular ELISA systems, which are all calibrated against the WHO Wo/80 standard, can give very different results in the various detection systems for antibodies against dsDNA, depending on their ability to determine antibodies of high or low avidity [7, 8, 9]. For the determination of Tg/TPO antibodies, the situation is similar [10, 11].

Currently, there are no international guidelines available on how to standardise autoimmunity test kits. A common concept to standardise assay systems internationally could increase the clinical value of the test systems, improve the accuracy of the diagnosis and could guide the manufacturers of assay systems.

The practical approach

An EASI international team consisting of expert rheumatologists / internists / laboratory scientists from Denmark, France, Germany, Israel, Italy, The Netherlands, Spain, and the United Kingdom was formed to discuss how interactions between laboratories and clinics could be improved in practice, how algorithms in autoantibody testing could be harmonized, and what an international concept of standardisation of diagnostic strategies in this area could look like.

In national EASI teams, which were founded in many European countries, clinical and laboratory experts from different clinical areas now also meet regularly to discuss practical improvements in their country-specific approaches.

All EASI groups build a close EASI network, which allows a frequent exchange of information and experiences and enables a close cooperation across borders on particular EASI projects (Fig.1). Newsletters and an internet platform as well as individual e-mail conversation are used to make communication as easy and fast as possible. To review the common activities regularly and to plan new European projects, the coordinators of all EASI teams meet annually in the EASI forum.

Beside the communication within the different teams, EASI also presents and discusses the results of their activities with other experts in the field. To this end, EASI conferences are organised biannually during the International Congress on Autoimmunity.

Actual projects of the EASI network

1. Publication of guidelines for family doctors

A paper describing diagnostic screening algorithms for systemic rheumatic autoimmune diseases and intended for use by family practitioners is being prepared by the EASI international team.

2. Booklet(s) for family doctors

Authors from the different EASI teams will write, in collaboration, the chapters of a booklet, covering each of the rheumatic / autoimmune diseases. Beside the EASI team members, external collaborators will also contribute to the booklet.

Future booklets should cover all areas in autoimmunity. Each of the chapters will focus on one disease and discuss the symptoms of the different disease phenotypes, recommend markers which can help to diagnose or exclude the disease and describe diagnostic algorithms that are recommended for use.

3. Multicentre Study

A multicentre study on the performance of the different methods used for autoantibody testing should be initiated. All assay systems which are commercially available should be included.

Books and papers published by the EASI network

1. **Wiik A, Cervera R, Haass M, Kallenberg C, Khamashta M, Meroni PL, Piette JC, Schmitt R, Shoenfeld Y (2006)** European attempts to set guidelines for improving diagnostics of autoimmune rheumatic disorders *Lupus* 15, 391 - 396
2. **Cervera R, Plaza A (2006)** Guías de práctica clínica y de laboratorio: Autoanticuerpos y enfermedades autoinmunes, 210 pages (currently in Spanish only), ISBN: 84-8473-333-5
3. **Chyderiotis G, Claudel E, Fabien N, Musset L, Olsson NO, Pham BN (2006)** Autoanticorps utiles au diagnostic et au suivi des maladies auto-immunes systémiques 43 pages (currently in French only)

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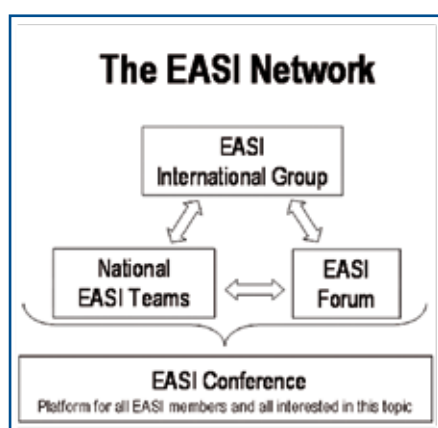


Figure 1: The EASI network

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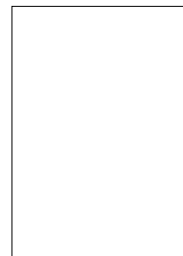
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