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# The 4th International Congress on Autoimmunity Budapest, Hungary, November 3 - 7, 2004

Phadia

4th INTERNATIONAL CONGRESS ON AUTOIMMUNITY
Budapest, Hungary, November 3 – 7, 2004

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Following the successful 3rd International Congress on Autoimmunity in Geneva, Switzerland in 2002, the 4th Congress was held in Budapest, the capital city of Hungary.

The Congress on Autoimmunity, which takes place every two years, covers both novel scientific (basic immunlogy) and clinical aspects of autoimmunity and autoimmune diseases, and brings together some of the top autoimmunologists in the world.

As for the three previous Congresses on Autoimmunity, the 4th Congress was sponsored by Pharmacia Diagnostics and, naturally, we had a presentation area where interested people could be informed about and could discuss our products with our autoimmunity specialists. Several posters and oral presentations at the congress were about our new EliA products.

In this special edition of the EliA Journal we would like to give you some impressions of the Congress this year. Most importantly, however, we summarize those posters and presentations, which present studies on new Pharmacia products, especially on EliA PR3, EliA MPO and on EliA CCP. It is worthwhile mentioning that the new EliA CCP assay showed an excellent performance in all independent studies (see page 3 to 5).

The high number of oral presentations and posters on EliA products shows that many laboratories are very interested in our high-quality fully automated autoimmunity testing.

Enjoy reading,
Your EliA Journal editorial team.

# Summary of Oral Presentations and Posters

## at the congress, involving Pharmacia Diagnostics products

### Clinical utility of the anti-CCP (cyclic citrullinated peptide) assay: diagnostic value in a mixed population of patients with rheumatic disorders and first experiences with an automated test system

M.Gaubitz

Universitätsklinikum Münster

**Objective:** To assess the clinical utility of anti-CCP assays in patients with rheumatic disorders and to compare an ELISA with the new automated EliA.

**Patients and Methods:** 500 consecutive patients of an outpatient-clinic were included in this study (376 women and 124 men). 185 of these were RA patients, 315 were disease controls. Rheumatoid factor was measured by agglutination in the Waaler-Rose-Test and by nephelometry in the Latex-Test. CCP antibodies were measured by ELISA (Euroimmun) and EliA (Pharmacia Diagnostics).

**Results:** 115 of 185 RA patients (62%) were positive for rheumatoid factor, 125 (68%) were positive for anti-CCP. In the disease controls, 63 (20%) were positive for rheumatoid factor and only 12 (4%) were positive for anti-CCP (details see table 1).

All 500 sera were tested with the CCP ELISA and EliA CCP. Controversial results were found in only 8 sera. In 6 of 8 the EliA-CCP correlated with the diagnosis according to accepted criteria, in 2 of 8 the ELISA did.

| | RF positive | Anti-CCP positive |
|---|---|---|
| Rheumatoid Arthritis (n=185) | 115 (62%) | 125 (68%) |
| Disease Controls | | |
| SLE (n=58) | 11 (19%) | 3 (5%) |
| Sjögren`s syndrome (n=20) | 12 (60%) | 1 (5%) |
| Other connective tissue diseases (n=29) | 2 (7%) | 0 (0%) |
| Vasculitis (n=32) | 7 (22%) | 1 (3%) |
| Psoriasis associated arthritis (n=18) | 1 (6%) | 2 (11%) |
| Osteoarthritis (n=30) | 5 (17%) | 0 (0%) |
| Noninflammatory myalgia (n=26) | 5 (19%) | 4 (15%) |
| Other rheumatic entities (n=43) | 9 (21%) | 0 (0%) |
| Chronic hepatitis (n=59) | 11 (19%) | 1 (2%) |

*Table 1: Rheumatoid factor versus anti-CCP in rheumatoid arthritis and disease controls*

| | RF | anti-CCP |
|---|---|---|
| Sensitivity (%) | 62.2 | 67.6 |
| Specificity (%) | 80.0 | 96.2 |

*Table 2: Sensitivity and specificity of RF and anti-CCP in 185 RA sera and 315 disease controls, respectively*

**Conclusions:**

- Anti-CCP-antibodies are specific markers for RA.
- Anti-CCP-antibodies are a useful tool to discriminate RA patients from those with other RF-positive diseases.
- While displaying higher specificity, they still show similar sensitivity when compared to the classical rheumatoid factor.
- The automated EliA-CCP-system provides a reliable, cost-effective possibility to test for anti-CCP-antibodies.

### Clinical diagnostic value of EliA fluorenzyme-immunoassay for anti-cyclic citrullinated peptide (CCP) antibodies in patients with rheumatoid arthritis

B. Gilburd, S. Praprotnik, O. Shovman, Y. Shoenfeld

Center for Autoimmune Diseases, Department of Medicine B, Chaim Sheba Medical Center Tel-Hashomer, Sackler Faculty of Medicine, Incumbent of the Laura Schwarz-Kipp Chair for Research of Autoimmune Diseases, Tel-Aviv University, Israel, University Clinical Center, Ljubljana, Slovenia.

**Objective:** To evaluate the diagnostic and clinical value of EliA anti-cyclic citrullinated peptide antibodies (CCP) test (Pharmacia Diagnostics, Germany).

**Patients and Methods:** The study was performed on sera obtained from 305 subjects: rheumatoid arthritis (n=107), other inflammatory joint disease (n=30), vasculitis (n=30), Sjogren (n=38), and healthy control (n=100). All sera were tested for the anti-CCP antibodies by EliA- test using UniCAP machine (Pharmacia Diagnostics, Germany).

**Results:** Receiver operating characteristic (ROC) analysis of EliA anti-CCP antibody assay yielded very high sensitivity (88.8%) and specificity (98%) for patients with rheumatoid arthritis (RA). In other studied groups, the frequency of anti-CCP antibodies ranged from 0% (Vasculitis) to 7.9% (Sjogren's syndrome). Additionally, anti-CCP antibodies were found in 57.1 % of rheumatoid factor (RF) negative RA patients. In early RA, the diagnostic sensitivity of RF and anti-CCP antibodies was 73.9 and 91.3% respectively.

**Conclusions:**

The EliA anti-cyclic citrullinated peptide antibodies (CCP) test is highly sensitive and specific for rheumatoid arthritis. Positive EliA anti-CCP test supports the diagnosis of rheumatoid arthritis. EliA anti-CCP test may be an additional diagnostic tool in RF negative patients with rheumatoid arthritis.

### Anti-CCP: usefulness in routine Data based on evaluation of routine samples collected over some months

M. Herold

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**Objective:** To compare different methods for the detection of anti-CCP antibodies.

**Patients and Methods:** Sera from 543 patients were evaluated in the routine laboratory. 66 of these patients have rheumatoid arthritis (RA), 42 possibly have RA, 328 have definitely no RA and the clinical background of 107 patients is unknown.
The fully automated EliA CCP was compared to the ELISA of Inova (QUANTA Lite™ CCP).

**Results:** 442 sera were negative in both assays, 68 were positive in both assays. 13 sera were positive in EliA but negative in the ELISA.

8 of these 13 sera were from RA patients, but 2 of the 13 patients definitely do not have RA (3 other cases are not defined or not known). 3 were positive in the ELISA but negative in EliA. One patient of these 3 definitely has no RA. The clinical background of the two other sera is not known.

Thus, the sensitivity of the ELISA was lower than that of EliA (68.2% vs 80.3%) and the specificity was comparable (96.9 and 97.0, respectively).

**Conclusions:**

The new EliA CCP assay gives comparable results to the ELISA tested. This was expected, as both assays use the same antigen. However, EliA CCP is fully automated, easy to use and the analysis of single samples is possible.

## Testing the true clinical specificity of anti-CCP antibody assays in the laboratory management of rheumatoid arthritis

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Laboratory of Clinical Chemistry, San Giovanni Battista Hospital, Torino, [2)] Andrea Hospital, Vercelli, and [3)] Cairo Montenotte Hospital, Savona; ITALY
[1)] on behalf of G.A.L. (Gruppo Autoimmunità di Laboratorio del Piemonte e Valle d'Aosta)

**Objective:** To evaluate the clinical usefulness of three anti-CCP2 assays with a cohort of subjects selected on the basis of possible interferences or confounding factors and to compare results with those from two other assays in terms of diagnostic accuracy.

**Patients and Methods:** 155 patients with an established diagnosis of rheumatoid arthritis, and 297 controls, 146 patients with various other diseases (rheumatic diseases, arthritis of various nature, gammopathies, HCV-related disorders, inflammatory bowel disease, autoimmune thyroiditis) and 151 (apparently) healthy controls were measured.

The following assays were performed: N Latex RF (Rheumatoid Factor) from Dade Behring, Aeskulisa RA/CCP*-Detect (with IgG anti-human synthetic IgG) from Aesku.Diagnostics, Diastat Anti-CCP (2nd generation CCP) from Axis-Shield Diagnostics, EliA CCP (2nd generation) from Pharmacia Diagnostics, and Anti-CCP (2nd generation) from Euro-Diagnostica AB.

**Results:** While the two tested assays for rheumatoid factor detection showed a wide distribution of results among the three study groups, a good discrimination could be observed with all three CCP2 tests. However some positive results appeared both in diseased and in healthy control populations. This was more evident for Diastat assay, also considering its application on a larger series of samples (n=434) than the two other methods (n=230). ROC curve analysis on our protocol population for CCP2 assays confirmed their high diagnostic performances (see figure 1a-d). AUC values were 0.91, 0.97 and 0.97 for Diastat, EliA and Euro-Diagnostica, respectively, as compared to 0.80 and 0.74 for Aeskulisa and Latex RF. All three anti-CCP kits revealed a higher than expected sensitivity level, when considering cut-offs suggested by manufacturers, whereas an increase in the threshold limits might be recommended in order to obtain a specificity > 97-98% in our study groups (see table 1).

| | Diastat | | Euro-Diagnostica | | EliA CCP | |
|---|---|---|---|---|---|---|
| Cut-Off (U/ml) | < 5 | < 10 | < 45 | < 35 | < 7 | < 20 |
| Sensitivity (%) | 83 | 83 | 92 | 92 | 94 | 94 |
| Specificity (%) | 94 | 98 | 93 | 99 | 95 | 100 |

*Table 1: Sensitivity, specificity of different anti-CCP assays*

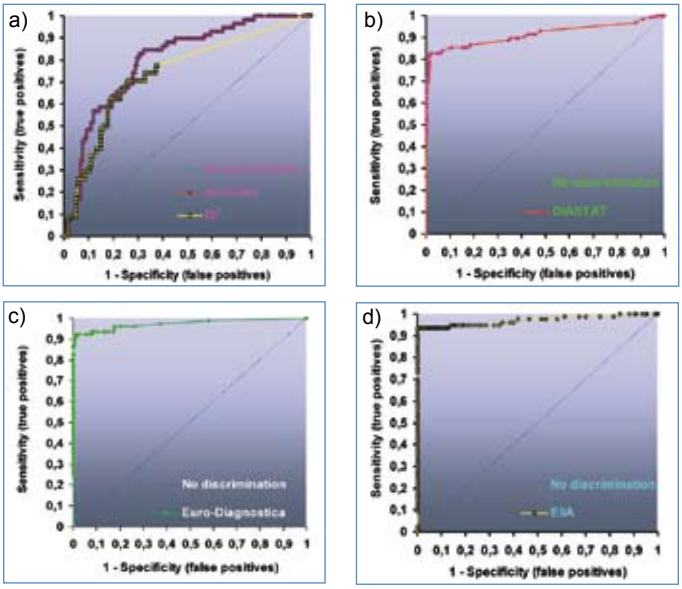


*Fig.1: ROC plot analysis of a) N Latex RF and Aeskulisa RA/CCP*-Detect, b) Diastat Anti-CCP, c) Anti-CCP from Euro-Diagnostica AB, and d) EliA CCP.*

**Conclusions:**

When applied to a "difficult" clinical setting, all three tested CCP assays yielded comparable diagnostic accuracies. The hypothesis that supposedly false positive results may predict an early phase of rheumatoid arthritis development is intriguing and could account for the need for increasing threshold limits to include those subjects with anti-CCP antibody concentrations in the lower range of positivity. Clinical significance of these low anti-CCP titres should be addressed in a prospective study. Moreover, possible implications of positive tests, even at moderate-to-high titres, such as those found in this study in a cryoglobulinemic HCV-patient or in a son of an affected patient, should be verified with all new assays.

## Correlation of a new fluoroenzyme immunoassay for measurement of anti-cyclic citrullinated peptide (anti-CCP) antibodies with an anti-CCP enzyme linked immunassay and disease activity in patients with rheumatoid arthritis (RA)

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**Objective:** To evaluate the diagnostic value of a new anti-CCP fluoroenzyme immunoassay in rheumatoid arthritis in comparison with an established anti-CCP ELISA and to evaluate the correlation of anti-CCP serum concentrations with markers of disease activity.

**Patients and Methods:** Anti-CCP serum concentrations were measured in a cohort of 101 RA patients with a mean disease duration of 62 months. Anti-CCP antibodies levels were measured by ELISA (Euroimmun) and EliA CCP (Pharmacia Diagnostics). Both tests use 2nd generation CCP Antigen (Axis Shield). Quality of life was measured by health assessment questionnaire (HAQ), disease activity by swollen joint count (SJC), tender joint count (TJC) and disease activity score (DAS). Degree of inflammation was indicated by measurement of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).

**Results:** 80 of 101 sera were found positive for anti-CCP in either one of the two methods. The two evaluated anti-CCP tests showed a high correlation (r=0,976, P<0,0001). No correlation was found between anti-CCP levels and any markers of disease activity, such as patients' HAQ (mean value 0,98), SJC (mean 3,19), TJC (mean 5,94), DAS (mean 3,66), ESR (mean 15,6 mm) or CRP levels (mean 0,98 mg/dl).

| | | EliA CCP | | |
|---|---|---|---|---|
| | | positive | negative | Total |
| **CCP ELISA** | positive | 75 | 3 | 78 |
| | negative | 2 | 21 | 23 |
| | Total | 77 | 24 | 101 |

*Table 1: Correlation of CCP ELISA and EliA CCP in 101 RA sera*

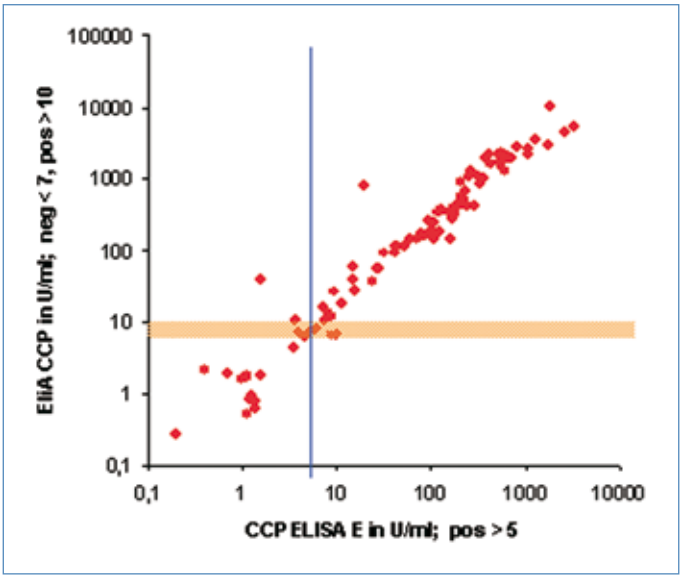


*Figure 1: Correlation of CCP ELISA and EliA CCP in 101 RA sera*

**Conclusions:**

The new fluoroenzyme immunoassay for anti-CCP (EliA CCP, Pharmacia Diagnostics) will be a useful detector of anti-CCP antibodies in routine evaluation of patients with suspected rheumatoid arthritis. So far no further correlation was detected with any activity marker, SJC, TJC, DAS, ESR, CRP nor patients' general health as measured by HAQ.

## Infliximab treatment and autoantibodies in patients with rheumatoid arthritis: Comparison with untreated patients and follow-up study

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**Objective:** To investigate the long-term effect of anti-TNF treatment on induction and fluctuation of various autoantibodies associated with rheumatoid arthritis (RA).

**Patients and Methods:** 53 patients were investigated, 20 with undetermined arthritis, 33 with RA. 20 of the latter group were treated with infliximab for 2 years, 13 were treated with other treatment protocols (in the following referred to as "untreated").

The following markers were measured: anti-CCP antibodies and anti-dsDNA antibodies on EliA (Pharmacia Diagnostics) and IgM rheumatoid factor (RF) measured by agglutination assays.

**Results:** 2 of 20 patients with undetermined arthritis were positive for anti-CCP and 2 were positive for RF. 23 of 33 patients with RA were positive for anti-CCP and 20 were positive for RF. Comparing the untreated and treated RA patients, for anti-CCP there was no significant difference (9 of 13 = 69% vs. 14 of 20 = 70%), for anti-RF the percentage of positives was slightly higher in untreated patients (9 of 13 = 69% vs. 11 of 20 = 55%). Indeed, in the follow-up of 16 patients with RA during 2 years of infliximab therapy, this tendency was confirmed: while the level of anti-CCP did not change in the 2 years of treatment, the RF titre decreased. IgG anti-dsDNA were detected in 20% of patients with RA treated with infliximab, but in no untreated patients (see figure 1).

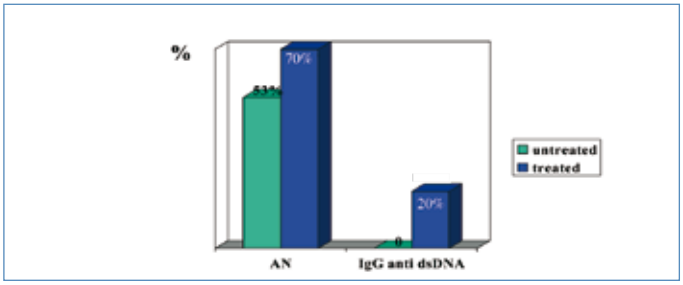


*Fig. 1: Antinuclear antibody profile in RA patients untreated and treated with infliximab.*

**Conclusions:**

Long-term infliximab therapy leads to reduction in RF titres, induction of anti-DNA IgG, and does not modulate anti-CCP levels.

## Evaluation of a new fluorescent-enzyme immunoassay for diagnosis and follow-up of ANCA-associated vasculitis

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**Objective:** To assess

- the diagnostic performance of the new fluorescent-enzyme immunoassay (EliA, Pharmacia Diagnostics) in detection of PR3- and MPO-ANCA.
- whether quantification of the ANCA titre by the new fluorescent immunoassay (EliA) is able to predict relapses in patients with ANCA-associated vasculitis (AAV).

**Patients and Methods:** 87 patients with biopsy-proven, pauci-immune necrotizing and crescentic glomerulonephritis (NCGN) were included for evaluation of PR3- and MPO-ANCA detection by EliA. Sera contained PR3- (n=39), MPO-ANCA (n=38), or no ANCA (n=10) as determined by a combination of IIF, direct- and capture-ELISA. 40 healthy and 32 disease controls (SLE, IgM-RF, polyclonal hyper-IgG) were included.

The diagnostic performance of PR3-ANCA detection by EliA was examined in a cohort of 60 Wegener's Granulomatosis (WG) patients, as compared to 30 healthy and 30 rheumatoid arthritis controls used in a previous multicenter study for comparison of direct and capture ELISAs.

For prediction of relapses PR3-ANCA were measured in 5 consecutive samples of 23 PR3-AAV patients prior to relapse, and of 23 matched patients with inactive PR3-AAV. An increase of 100% was used as cut-off based on a ROC-curve.

**Results:** EliA MPO and EliA PR3 showed good sensitivity and specificity (see table 1 and 2, respectively) compared to the results of a

combination of IIF, direct- and capture-ELISA. The predictive value of EliA PR3 for relapses in Wegener's granulomatosis was comparable to ELISA methods.

| PR3 | Multistep ANCA Procedure | EliA Cut-off 10 | EliA Cut-off 3 |
|---|---|---|---|
| Relative Sensitivity (n=39) | 100% | 92.3% | 97.4% |
| Overall Sensitivity (n=87) | 44.8% | 43.7% | 47.1% |
| Specificity (n=72) | 100% | 95.8% | 95.8% |

*Table 1: PR3 detection for diagnosis AAV*

| MPO | Multistep ANCA Procedure | EliA Cut-off 10 | EliA Cut-off 3 |
|---|---|---|---|
| Relative Sensitivity (n=38) | 100% | 94.7% | 97.4% |
| Overall Sensitivity (n=87) | 43.7% | 41.4% | 43.7% |
| Specificity (n=72) | 96-100% | 100% | 100% |

*Table 2: MPO detection for diagnosis AAV*

| PR3 | EliA Cut-off 10 | EliA Cut-off 4 | Direct ELISA | Capture ELISA |
|---|---|---|---|---|
| Sensitivity | 60.0% | 71.7% | 64.0% | 74.3% |
| Specificity | 100% | 96.7% | 99.0% | 100% |

*Table 3: PR3 detection for diagnosis WG*

| PR3 | Rise in ANCA | Relapse/ Rise | No Relapse/ No Rise |
|---|---|---|---|
| IIF | 24 | 15/24 | 14/22 |
| Direct ELISA | 24 | 17/24 | 16/22 |
| Capture ELISA | 21 | 16/21 | 18/25 |
| EliA | 26 | 18/26 | 15/20 |

*Table 4: Prediction of relapses in patients with WG by rise in PR3-ANCA*

**Conclusions:**

- Detection of PR3- and MPO-ANCA by EliA has excellent performance in terms of diagnosis of AAV patients.
- The sensitivity of PR3-ANCA detection by EliA in patients with WG is more comparable to direct ELISAs than to capture ELISAs.
- Detection of rises in PR3-ANCA by EliA for prediction of relapses reveals similar results to those by ELISA techniques.
- Introduction of the capture technology in the EliA system may further improve the sensitivity.

## Multicentre evaluation of a new automated method for determination of antibodies against PR3 and MPO

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[4] Servicio De Inmunología, Hospital Universitario La Paz, Madrid;
[5] Servicio De Inmunología, Hospital Universitario Gregorio Marañón, Madrid

**Objective:** To compare the diagnostic performance of fully-automated ANCA tests EliA™ PR3/MPO (Pharmacia Diagnostics, Freiburg, Germany) to a commercially-available reference ELISA using a panel of patients with ANCA-associated vasculitis (AASV) and disease controls.

**Patients:** 94 patients with AASV, (25 with WG and 69 with various other vasculitides) were tested. The control group consisted of 98 patients with different diseases (see table 1).

**Results:** Agreement of methods: A kappa agreement of over 80% was found between the EliA and ELISA method, for MPO as well as for PR3. As expected, the agreement of IIF to ELISA as well as EliA was lower, but still on a moderate level. ANCA IIF showed highest sensitivity but worst specificity. ELISA and EliA displayed similar sensitivity and specificity (see table 2).

Patients with active WG showed significantly higher PR3 titres than those with inactive WG. Patients with active AASV showed significantly higher MPO titres than those with inactive AASV. Fisher Exact Test and F-Test differentiated clearly between WG patients and disease controls (in category 'pos./neg.' as well as titre). Significant differences were also found in titres of active vs inactive WG. In this respect EliA showed (low) significance in differentiating WG with and without nephritis.

| Vasculitides | n |
|---|---|
| ANCA associated vasculitides (AASV) | 94 |
| Wegener's Granulomatosis (WG) | 25 |
| Microscopic Polyangiitis | 34 |
| Churg-Strauss Syndrome | 5 |
| Rapidly Progressive Glomerulonephritis (RPGN) | 14 |
| Other AASV | 16 |
| Disease Controls | 98 |
| Non AASV | 17 |
| SLE | 17 |
| RA | 24 |
| HIV | 21 |
| Other Diseases | 19 |

*Table 1: Details of patients*

| | n | cANCA or pANCA pos. | ELISA PR3 or MPO pos. | EliA PR3 or MPO pos. |
|---|---|---|---|---|
| ANCA associated Vasculitides (AASV) | 94 | 78,8 | 57,4 | 54,3 |
| Wegener's Granulomatosis (WG) | 25 | 76,5 | 68 | 60 |
| Disease Controls | 98 | 9,2 | 4,1 | 4,1 |

*Table 2: Sensitivity of different methods*

**Conclusions:**

- The new automated EliA™ PR3 and MPO tests demonstrate a comparable performance to the reference assay.
- Low, but significantly better association to disease status with EliA™ is found.
- The automation of the ANCA tests allows fast and cost-efficient quantification of PR3/MPO antibodies.

## Evaluation of a new automated fluorescence immunoassay (EliA) for PR3 and MPO-ANCA detection

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**Objective:** To evaluate the diagnostic performance of a new fluorescence immunoassay (EliA) for PR3 and MPO-ANCA detection and to compare the results with traditional ("home made") ELISAs and an indirect immunofluorescence test.

**Patients:** 100 serum samples from 71 patients with ANCA associated vasculitides (AASV, 27 with WG, 32 with microscopic polyangiitis and 12 with Churg-Strauss syndrome) were tested. The control group comprised 58 patients with different diseases and 35 blood donors. Disease activity was judged by clinicians using BVAS criteria.

**Results:** Sensitivity, specificity and predictive value of the ELISA and EliA for PR3 and MPO were calculated. Sensitivity for both ELISA PR3 and EliA PR3 was 51.9% for WG. Specificity was 98.9 and 97.8%, respectively.

Sensitivity for MPO was 63.6% (ELISA) and 70.5% (EliA) for microscopic polyangiitis and Churg-Strauss syndrome. Specificity was 98.9% for both assays.

The kappa Agreement of ELISA and EliA was very good (0.88). As expected, the agreement was not as high with immunofluorescence and ELISA and EliA, respectively, but it was still acceptable (0.80-0.86 for c-ANCA/PR3 and 0.52-0.55 for p-ANCA/MPO).

ELISA and EliA performed comparably in terms of correlation with disease activity (see fig. 1 and 2).

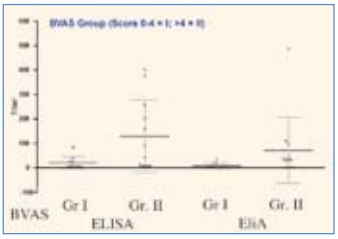


*Fig.1: Results of ELISA and EliA PR3 in active and inactive WG*

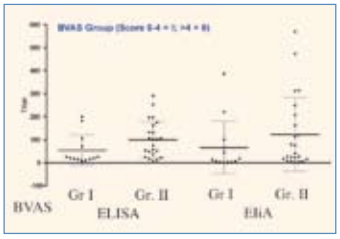


*Fig.2: Results of ELISA and EliA MPO in active and inactive AASV (WG excluded).*

**Conclusions:**

- The automated EliA system for PR3 and MPO-ANCA detection has high sensitivity and very good specificity for ANCA-associated systemic vasculitis (AASV).
- The fluoro-immunoassay system performs as well as a classical "reference" ELISA, which has been used for more than 10 years in our laboratory.

## Anti-glomerular basement membrane (GBM) antibodies in the diagnosis of Goodpasture syndrome: comparison of different methods

A. Radice, C. Corace, B. Bollini, R.A. Sinico

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**Objective:** To compare different methods for the detection of circulating anti-GBM antibodies.

**Patients and Methods:** 32 sera of 17 patients with GBM disease were measured, 12 of the patients had lung involvement. 70 sera of 42 patients with ANCA associated vasculitis (n=32), SLE (n=9) and pulmonary fibrosis (n=1) and 20 blood donors were used as controls.

Three ELISAs and EliA GBM were used, two ELISAs with human antigen and two test systems with recombinant human antigen, expressed in insect cells (Varelisa and EliA). Cut-off limits, sensitivity, specificity, PPV, and NPV were calculated for each assay using the Receiver Operating Characteristic (ROC) curves.

**Results:** Sensitivity for GBM autoantibodies was found to be very similar for the different assays tested (see table 1 and figure 1). EliA GBM showed the highest specificity. For two patients a follow-up was done: both patients had high positive titres at onset and had negative or low titres after treatment (see figure 2).

| | ELISA 1 | ELISA 2 | ELISA 3 (Varelisa) | EliA GBM |
|---|---|---|---|---|
| Sensitivity (%) | 100 | 94.4 | 94.4 | 94.4 |
| Specificity (%) | 90.6 | 96.9 | 93.8 | 100 |
| PPV (%) | 94.4 | 85.7 | 89.5 | 100 |
| NPV (%) | 96.9 | 100 | 96.8 | 97.0 |

*Table 1: Sensitivity, Specificity and predictive value of different anti-GBM assays*

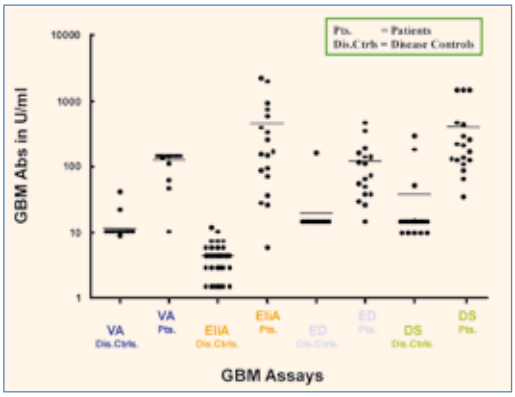


*Figure 1: Performance of four different anti-GBM assays*

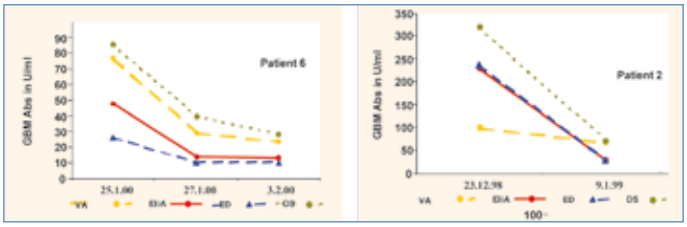


*Figure 2: Monitoring of anti-GBM in two patients with four different anti-GBM assays*

**Conclusions:**

- All assays employed in our study showed a comparable good sensitivity (>94%), whereas specificity varied considerably.
- The best performance, in terms of sensitivity/specificity, was reached by the fluoro-enzymatic-immunoassay EliA.
- The data confirms the importance of testing pathological, rather than normal, controls when evaluating diagnostic performance of different assays.
- Detection of circulating anti-GBM is useful in follow-up of patients treated for anti-GBM antibody-mediated disease, both in the acute phase as well as in the late phase, because occasional relapses and late recurrence have been reported.

## A novel method for detection of anti-human tissue transglutaminase IgA antibodies

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**Objective:** To compare the performance of Celikey, the anti-tTG IgA assay in use for a few years in our laboratory, with EliA Celikey IgA, a new fully automated assay recently introduced in the market.

**Patients and Methods:** We tested 161 serum samples obtained from four groups of subjects: 96 CD patients, blood drawn at the time of diagnosis; diagnosis confirmed by EMA and biopsy, 15 CD patients during gluten-free diet follow-up, and 50 controls, 28 blood donors and 22 disease controls with inflammatory bowel disease (IBD).

In this study we used Celikey (Pharmacia Diagnostics, Freiburg, Germany), a quantitative anti-tTG IgA ELISA assay using human recombinant transglutaminase expressed in baculovirus/insect cell system. The reference values used for interpretation of results are: negative < 5 U/ml, equivocal between 5-8 U/ml and positive > 8 U/ml.

The results were compared to the EliA Celikey IgA (Pharmacia Diagnostics, Freiburg, Germany), a new Fluoro Enzyme Immuno Assay (FEIA) for quantitative detection of anti-tTG IgA antibodies. This is a fully automated test. It uses monowells as solid phase, coated with the same human recombinant antigen used in Celikey. The reference values are: negative < 7 U/ml, intermediate between 7-10 U/ml and positive > 10 U/ml.

**Results:** Each serum was tested quantitatively with both methods detecting the levels of anti-tTG IgA autoantibodies. In each run a positive control was assayed in order to define the inter-assay CVs (table 1). We calculated sensitivity, specificity, positive and negative predictive values for each method (table 2). Both tests show good clinical performance with EliA Celikey being slightly more sensitive in CD patients. This is also confirmed by the ROC-plots. In addition we evaluated the degree of concordance between Celikey on ELISA and EliA Celikey. tTG IgA antibody levels seem to decrease slower using EliA Celikey compared to Celikey on ELISA in the group of CD patients following a gluten-free diet (see figure 1).

| | **Inter-assay CV** |
|---|---|
| **EliA Celikey IgA** | 8.34 % (8 runs) |
| **Celikey ELISA** | 4.47 % (4 runs) |

*Table 1: Inter-assay coefficients of variation (CVs)*

| | **EliA Celikey IgA** | **Celikey ELISA** |
|---|---|---|
| Sensitivity (%) | 97.9 | 91.7 |
| Specificity (%) | 100 | 100 |
| PPV (%) | 100 | 100 |
| NPV (%) | 93.3 | 77.8 |

*Table 2: Sensitivity, Specificity and predictive value of Celikey on EliA and on ELISA.*

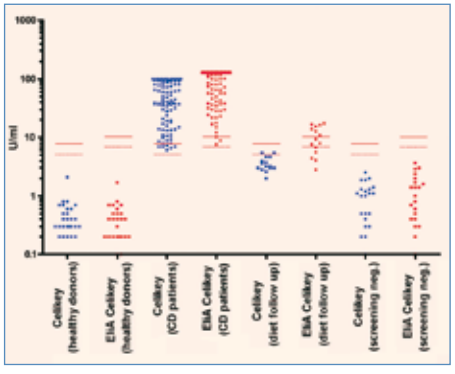


*Figure 1: Comparison of EliA™ Celikey® IgA and Celikey® on ELISA. Full line red bars represent the cut-offs. Dotted red line bars represent the lower limit of the equivocal range.*

**Conclusions:**

The analysis of results confirmed the high quality of both methods for reliably detecting CD patients with high specificity. The main difference between Celikey on ELISA and EliA Celikey is an apparent slower decrease in antibody titre in patients on gluten-free diet using EliA Celikey. The EliA Celikey monowell technology can improve the workflow in CD laboratory testing.

## Relationship between titres of anti-dsDNA antibodies measured by EliA dsDNA and SLE disease activity

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**Objective:** To assess the relationship between titres of anti-dsDNA Abs measured by the EliA dsDNA test and disease activity in a large cohort of SLE patients and to compare these results with those obtained with CLIFT.

**Patients and Methods:** 1017 sera were collected from 181 SLE patients attending the Rheumatology Clinic between July 1997 and March 2004. All the patients fulfilled the 1982 ACR criteria.

Disease activity was defined with modified SLEDAI (M-SLEDAI). IgG anti-dsDNA Abs were measured with the EliA dsDNA assay (Pharmacia Diagnostics, Freiburg, Germany) and with the classical CLIFT (Mardx, Carlsbad, CA). EliA dsDNA was considered positive at titres above 15 IU/ml whereas CLIFT was positive at a serum dilution of 1/10 or higher. Serum concentration of C3 and C4 was quantified by standard nephelometry (Dade Behring, Marburg, Germany).

**Results:** Measuring anti-dsDNA antibodies by the EliA dsDNA assay and CLIFT in SLE patients in lupus flares and renal relapses showed that both tests performed similarly for lupus nephritis but the EliA dsDNA assay could detect higher percentages of positive sera in active SLE than CLIFT. There were significant differences in titres of anti-dsDNA antibodies in patients with active SLE vs non-active SLE when measured by EliA dsDNA, but not when measured by CLIFT.

Sensitivity, specificity and predictive values of EliA dsDNA and CLIFT for diagnosing lupus and nephritis flares are shown in figure 1, table 1 and table 2.

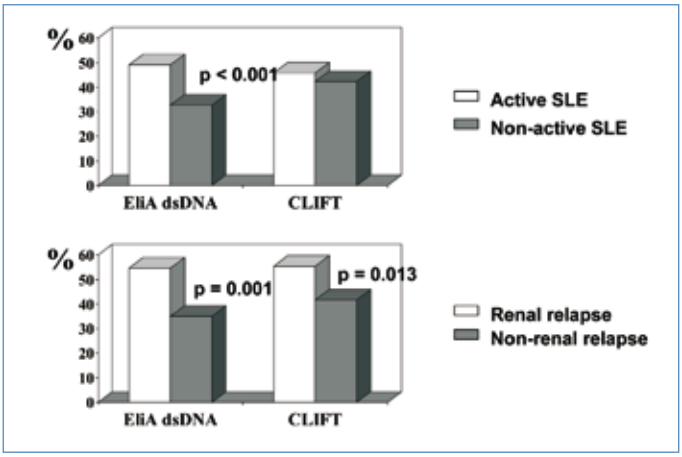


*Figure 1: Frequencies of sera positive for anti-dsDNA antibodies by the EliA dsDNA and the CLIFT in SLE patients in lupus flares and renal relapses.*

| | EliA dsDNA | CLIFT |
|---|---|---|
| Sensitivity (%) | 49.1 | 45.8 |
| Specificity (%) | 67.2 | 57.8 |
| PPV (%) | 33.5 | 27.8 |
| NPV (%) | 79.7 | 75 |

*Table 1: Sensitivity, specificity and predictive values of EliA dsDNA and CLIFT for diagnosing lupus flares.*

| | EliA dsDNA | CLIFT |
|---|---|---|
| Sensitivity (%) | 54.7 | 55.4 |
| Specificity (%) | 64.9 | 58.3 |
| PPV (%) | 18.4 | 15.6 |
| NPV (%) | 90.8 | 90.4 |

*Table 2: Sensitivity, specificity and predictive values of EliA dsDNA and CLIFT for diagnosing nephritis flares.*

**Conclusions:**

This retrospective study of a large cohort of patients demonstrated that the new EliA dsDNA assay is of slightly more diagnostic value for detecting lupus and nephritis flares than CLIFT. These data, together with the advantages of being automated, fast and quantitative, makes the EliA dsDNA assay a suitable tool for the monitoring of SLE patients.

## Investigation of an automated ANA/ENA screening system (EliA) as an alternative to IIF (HEp2) for routine use

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**Objective:** To investigate an automated dsDNA/ENA analysis technique (EliA from Pharmacia Diagnostics) as an alternative to HEp2 IIF with the goals of allowing us to perform the screening in-house and so reduce costs while maintaining or improving the service to patients.

**Patients and Methods:** 110 consecutive patient samples received by our laboratory with a request for ANA screen were sent to a local reference lab for testing by HEp2 IIF (Inova) according to the manufacturer's instructions and utilising a cut-off titre of 1:160. An aliquot of every sample was tested in our laboratory using EliA Symphony and EliA dsDNA. EliA Symphony incorporates a solid-phase well which is coated with a mixture of 8 autoantigens selected by the manufacturer for their clinical relevance to CTD.

The patients' medical notes were obtained and assessed by an independent pathologist according to ACR criteria for CTD (excluding any ANA results) and the patients were characterised as being CTD positive, CTD negative or CTD status unable to be determined. This third group comprised 14 patients who were consequently excluded from the study. Disease activity at the time of the study was not evaluated.

**Results:** Of the 96 patients included, 15 had a connective tissue disease and the remaining 81 were negative for this diagnosis. HEp2 was positive in 41 of 96 patients (42.7%). 10 of these 41 patients had a connective tissue disease, 31 did not. EliA was positive in only 10 sera (10.4%), of which 6 were from patients with a connective tissue disease. Sensitivity, specificity and predictive value are shown in table 1.

Sending our ANA screen samples to an external reference laboratory, cost our institution an all-inclusive price (defined here as X) for each HEp2 screen performed. A follow-up test at the reference lab cost 1.57X for each analyte tested. Reagents for EliA Symphony/dsDNA screening cost a total of 0.27X and follow-up tests cost 0.15X for each analyte.

In the reference laboratory, a positive HEp2 result generated an average of 4.11 follow-up tests. For the 41 samples requiring follow-up after a positive HEp2 result, the cost of the follow-up tests totalled 265X. Taking the totals of screening and follow-up tests, the average cost of each sample screened was 3.76X.

A positive Symphony Screen was followed up with tests for autoantibodies to each of the 8 antigens present in the Symphony wells (but not to dsDNA as these antibodies were fully identified and quantified in the screening process when present). The 10 samples positive in the EliA screening system cost a total of 12X in follow-up tests. The costs of screening and follow-up tests using the EliA system averaged out to 0.40X per sample screened (costs and savings see figure 1).

Labour costs were not calculated and could be expected to add to this cost, however, the EliA system is fully automated and required minimal hands-on time. The instrument cost was included in the reagent cost assessment.

| | HEp2 | EliA |
|---|---|---|
| Sensitivity (%) | 66.7 | 40.0 |
| Specificity (%) | 61.7 | 95.1 |
| pos. predictive value (%) | 24.3 | 60.0 |
| neg. predictive value (%) | 90.9 | 89.5 |

*Table 1: Sensitivity, specificity and predictive value for connective tissue diseases of HEp2 and EliA*

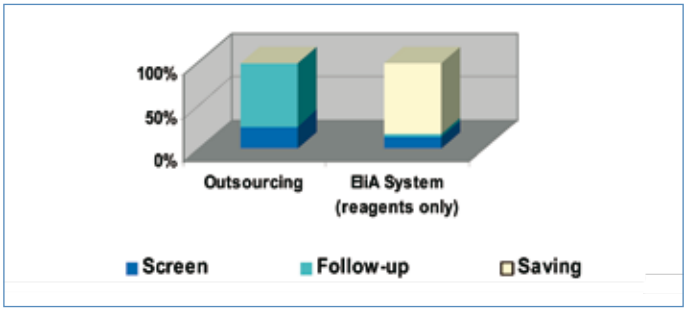


*Figure 1: Cost Comparison of HEp2 and EliA System*

**Conclusions:**

Compared to EliA, HEp2 IIF was the more sensitive method, but the high rate of false positives reduced the significance of a positive result to the clinician and generated very high costs in unnecessary follow-up testing. The EliA system failed to detect several CTD patients detected by HEp2 but the high specificity gave a more helpful result to the clinicians when a positive was found. The low sensitivity of EliA in this study may be a reflection of the limited range of antigens present in the assay or alternatively, may have been affected by the disease activity status of the patients involved as EliA dsDNA is said to better detect disease-active patients. The possible cost savings to our institution represented a major benefit to us in allowing us to better utilise a limited budget. Nevertheless, we wished to maintain our access to HEp2 testing for the few patients where the clinician might disagree with a negative EliA result. The compromise of retaining the option to send samples to the reference centre for HEp2 testing and follow-up for these controversial patients while transferring the majority of our ANA screen workload to the EliA format was agreed on by all laboratory and clinical staff involved and this protocol has been successfully implemented.

## Screening of anti-ENA antibodies by EliA™ Symphony vs. routine immunodiffusion and/or immunoblotting – a two-year patient material study from Turku University Hospital

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**Objective:** Evaluation of an automated detection system for antibodies against extractable nuclear antigens (ENA).

Comparison with classical immunodiffusion and/or immunoblotting (ID/IB).

**Patients and Methods:** 653 patient sera sent for routine ENA testing during 2001-2002 were retested with EliA™ dsDNA and EliA™ Symphony, followed by testing using specific ENA antigens. Clinical evaluation of the patients was done retrospectively by medical history. 99 patients had a systemic autoimmune disease (connective tissue disease, CTD). 12 of these had SLE (2 with nephritis), 14 MCTD, 30 Sjögren's Syndrome, 3 scleroderma, 2 DM/PM, and 38 had undefined connective tissue disease. Furthermore, there were 29 patients with rheumatoid arthritis, and 6 with juvenile rheumatoid arthritis or Morbus Still patients.

Automated fluorescence immunoassay, EliA™ Symphony containing 9 recombinant antigens (RNP [70, A, C], Ro [52, 60], La [48], Scl-70, CENP, Jo-1) and purified Sm, in a single well, was carried out using UniCAP® 100 instrument. Quantitative in vitro measurement of IgG antibodies to specific antigens was performed using the UniCAP EliA system. Routine ID/IB (except for Ro) were performed using rabbit thymus extract (Pel-Freeze Biologicals). For Ro (SS-A) human spleen extract (Scimedx E-102 ENA Poly II Antigen Extract) was used. Weak or borderline positive ID/IB results were confirmed using specific ELISAs utilizing purified antigens (Axis-Shield Diagnostics).

**Results:** EliA™ Symphony was more sensitive than ID/IB (54 vs. 40 positive out of 99 CTD samples; Figure 1), although 10 samples were only CENP-positive (CENP was not included in ID/IP testing). EliA™ Symphony was less specific than ID/IB (53 vs. 27 positive out of 554 nonCTD samples).

Moderately higher sensitivity for EliA™ Symphony than ID/IB was observed in clinical subgroups (Table 1) from patients with SLE (10/12 vs. 9/12), MCTD (14/14 vs. 12/14), SS (18/30 vs. 16/30), and clearly higher sensitivity in undefined systemic autoimmune diseases i.e. collagenosis (10/38 vs. 4/38) and in scleroderma (3/3 vs. 0/3). The latter is explained by 2 CENP-positive sera not included in ID/IB testing. However, one serum positive for Scl-70 by EliA™ was negative by routine ID/IB.

There were 44 patients classified as suffering from "other", i.e. no clinical systemic autoimmune disease, which screened positive by EliA™ Symphony and were further characterised as specific for Ro, La, RNP, Sm or CENP. Among them there were 20 patients with symptoms suggestive of a developing systemic autoimmune disease or with a diagnosis of discoid or subacute lupus, and 4 patients with symptoms suggesting Sjögren's syndrome. Of the 24 subjects positive with Elia™ Ro only, 14 had symptoms or signs suggestive of an autoimmune disease, and of those with La antibodies the figure was 11 out of 12. Altogether 15 out of 44 had no signs or symptoms of an autoimmune disease (13 of them Ro positive and one dsDNA positive).

| | ENA ID/IB | EliA Symphony |
|---|---|---|
| Sensitivity (%) | 40.4 | 54.5 |
| Specificity (%) | 95.1 | 90.4 |
| PPV (%) | 59.7 | 50.5 |
| NPV (%) | 89.9 | 91.8 |

*Table 1: Sensitivity, Specificity and predictive value. After a detailed analysis of the patients classified as "other", it would be justified to interpret some of the "false positive" results as clinically correct, hence the specificity and PPV of the tests would improve significantly.*

**Conclusions:**

The EliA™ System was a more sensitive detector of ENA antibodies than routinely used ID/IB. The presence of ENA and dsDNA antibodies in nonCTD patients suspected of CTD may indicate a false positive result, but could also be a marker of a developing autoimmune disease.

## Anti-ribosomal P proteins antibodies detected by ELISA in patients with rheumatic diseases

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**Objective:** to assess the prevalence, the specificity and the association with IgG aCL of anti-Rib-P Abs, detected by an indirect noncompetitive EIA kit, in patients with SLE and other systemic autoimmune diseases.

**Patients and Methods:** 80 patients were enrolled for the study: 20 with SLE without neuropsychiatric involvement, 15 with primary anti-phospholipid syndrome (pAPS), 15 with primary Sjogren syndrome (pSS), 15 with systemic sclerosis (SSC), and 15 with rheumatoid arthritis (RA). Additionally, 20 healthy subjects were included in the study.

Sera were analyzed by Varelisa Rib PO, P1, P2 Abs and by Varelisa Rib P2 Abs (Pharmacia Diagnostics, Freiburg, Germany, for research only). These are indirect noncompetitive EIAs in which full-length recombinant human P0, P1, P2 hexa His tagged and full-length recombinant human P2 hexa His tagged, expression system Baculovirus /Sf9cell, were used as antigens. All sera were tested by established methods for IgG/IgM aCL Abs and for other common autoantibodies (ANA, anti-ENA, anti-dsDNA) frequently found in autoimmune diseases.

**Results:** Anti-Rib-P Abs were positive (>10 U/ml) in 2/20 SLE patients: samples no. 3 and no. 19. Three sera gave equivocal results (range 5-10 U/ml), confirming the prevalence previously reported by other authors in SLE. All the remaining sera tested negative for Anti-Rib-P Abs, suggesting a very high specificity of this test for SLE. We did not find any correlations between anti-Rib-P Abs and aCL, or with the other autoantibodies tested for.

**Conclusions:**

On the basis of our results, we can observe that anti-Rib-P Abs, found in about 10% of patients with SLE, are fairly specific for the disease. It also seems that full-length recombinant human antigens, as used in the Varelisa assays, are specific. Moreover, the assay for Rib PO, P1, P2 Abs is more sensitive than the assay for Rib P2 Abs although the specificity was the same. Because of the low prevalence of positive sera in our cohort of patients, these preliminary data do not allow us to confirm the association of anti-Rib-P Abs with aCL, as previously reported by other authors, but further studies, performed on a larger set of samples, are in progress to investigate this aspect.

# The Pharmacia Diagnostics Social Event

In the evening of November the 5th, Pharmacia Diagnostics had the pleasure of welcoming customers and scientists from all over the world to a social event. We were happy that over 90 people joined us to enjoy a very special and enjoyable evening. After picking up all participants at the hotel by bus, we drove to Budafok, south of Budapest. This is a famous Hungarian traditional wine-growing district. The evening started with a sightseeing tour of a champagne cellar followed by a wine tasting (quite a matter of taste!). In the restaurant "Borkatakomba", located in an ancient vault cellar we were welcomed with a Hungarian drink and Gipsy music. While we ate a typical Hungarian 4-course dinner, we were entertained by folk dancing and other traditional displays. The chance to mix and talk with colleagues from around the world made for a very interesting and enjoyable evening.

# Pharmacia Diagnostics' Competition

At our stand stood a metre-high glass tube filled with EliA™ Wells. The participants of the congress were asked to estimate how many EliA™ Wells it contained.

110 congress participants took part. The estimates ranged from 550 to 500,000!

**However, the exact number of EliA™ Wells in the glass tube was 9,278.**

With her surprisingly close estimation of 9,370 EliA™ wells, Dr F. Paciello from Torino, Italy, won the 1st prize, a portable CD-player. With their estimation of 9,000 wells, Dr. A.S. Bansal from Carshalton, UK, and Dr. M. Bukilica from Belgrade, Serbia, won a 128 MB pendrive and a laser-pointer, respectively. These two prizes were allocated by a draw.


Congratulation to the winners and thank you to all participants!

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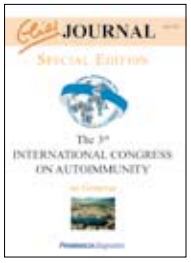

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