

Journal

ImmunoDiagnostics

9th International Congress on Autoimmunity

Nice la Belle (Nice the Beautiful), located on the French Riviera, was named after Nike the Roman goddess of victory and it is said to be one of the oldest human settlements in Europe. From March 26th to 30th Nice la Belle was the venue of the 9th International Congress on Autoimmunity. More than 2000 participants attended this worldwide biggest congress on Autoimmunity. The congress boasted more than 100 sessions featuring over 500 speakers. Thermo Fisher Scientific is one of the main sponsors of this congress. Posters highlighting EIA products are summarized in this issue of the ImmunoDiagnostics Journal.



EiA CCP & EiA RF

EiA CTD Screen and EiA dsDNA

EiA Celikey, EiA Gliadin^{DP}

EliA in Nice



The biggest congress on Autoimmunity, the 9th International Congress on Autoimmunity, took place in Nice in March 2014.

For Thermo Fisher Scientific as one of the main sponsors, it was a very successful congress. Successful, because we made many

new contacts to potential customers, opinion leaders and collaborators; we had a beautiful booth which invited guests to have fruitful discussions while enjoying a good coffee; we started projects and discussed study protocols. We supported three scientific sessions: 1. Celiac and Related Disease, 2. Inflammatory Bowel Disease (IBD), Gastroenterology, and last but not least 3. The EASI Conference on Anti-CCP and rheumatoid arthritis.

We invited our guests to a dinner in the wonderful Hotel le Negresco where Yehuda Shoenfeld and the IDD president Joe Bernardo gave informative and entertaining presentations on Autoimmunity and the ImmunoDiagnostics Division at Thermo Fisher Scientific.

Additionally, eleven posters were presented which included our EliA products – for example, François et al report from their experience with their new Phadia 5000 instrument. The poster from Alcalá Peña et al. confirmed that neither anti-CCP nor RF correlate with disease activity in rheumatoid arthritis. Alpini et al. evaluated the prevalence of RF IgA in RF IgM-positive patients. Bizzaro et al. and Korsholm et al. both compared EliA CTD Screen to a competitor's screening test and IIF on HEp-2 cells, respectively, and found a very good correlation in the first and good complementation in the second case. Hémond et al. investigated the correlation of EliA dsDNA with disease activity.

In the second group of posters, gastrointestinal markers were investigated, e.g. in the study of Sousa et al, showing the typical request profile for celiac markers in France. Dufat et al. analysed the correlation of IgA deficiency and the background level of EliA Celikey IgA. Pham et al. asked more than 5500 physicians how diagnosis of celiac disease is done in France. And last but not least, Guis et al. investigated the performance of the new ASCA tests on EliA.

Please find all these posters summarized in this journal. The content of these posters came from independent studies, sometimes supported by Thermo Fisher Diagnostics but never influenced in a manipulative way.

Enjoy reading



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Phadia 5000

Evaluation of the analytical performance of the autoimmunity process lines of the new automated system Phadia 5000

François S, Raulin C, Jolivot A, Delanoë C.

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Objective: To evaluate the Phadia 5000 analytical performance for autoimmunity testing.

Patients and Methods: Repeatability and reproducibility are performed on 30 values for 21 autoantibodies (anti-SSA/Ro, SSB/La, Sm, U1RNP, Scl70, Jo1, centromere, ENA screening, dsDNA, CCP2, MPO, PR3, GBM, cardiolipin IgG and IgM, beta 2 glycoprotein 1 (B2GP1) IgG and IgM, deaminated gliadin peptide (DGP) IgG and IgA, transglutaminase (tTG) IgG and IgA). 30 samples selected on the entire measuring range are tested for the comparison study with Phadia 250 for 15 autoantibodies and 40 samples for the comparison study with ELISA for 6 autoantibodies.

Results:

Analytical performance of the Phadia 5000

Phadia 5000 versus Phadia 250:

EliA reagents are exactly the same between the Phadia 250 and the Phadia 5000 and there is no discrepancy for the interpretation of the 15 autoantibodies between the two systems.

Quality of results is maintained between Phadia 250 and Phadia 5000.

Phadia 5000 versus ELISA:

Analytical performance are in accordance with the expected requirements. The Phadia 5000's high capacity allowed the automation of 6 parameters which were still done by ELISA before.

Automation on Phadia 5000 system versus ELISA allows:

- Emergency management for anti-MPO and PR3 antibodies (daily occurrence)
- Faster results for APS investigation
- No need to make several samples for the same patients because all the antibodies are done on the same system.

It makes the pre-analysis faster and secures the results using only primary samples.



Productivity improvement for the Autoimmunity platform before and after the Phadia 5000

5 Phadia 250 were needed to permit all the Autoimmunity activity (4 dedicated to each antibodies family and 1 in mirror for all analysis) and ELISA techniques were still performed for 6 antibodies.

Qualitative advantages:

- More than 2 hours per day won for technicians so they can be focused on the results and technical validation.
- Only one machine to load and collect samples, at the same place, and not 5 different places = better ergonomics.
- The tube feeder (rack Input/Output module) is a great improvement for quality, rapidity and ergonomics.

Conclusions: The high productivity thanks to this unique system configuration allows valuing the technician work in his technical competency and the result validation. The high throughput permits the automation of new parameters and consequently to secure the pre-analytics. The Phadia 5000 uses the same reagents than the Phadia 250 so that allows continuity in the quality of returned results. The Phadia 5000 answers to all the quality and automation requirements of the laboratory.

EliA CCP & RF

Correlation between serological and clinical markers in rheumatoid arthritis

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Objective: To investigate the association between disease activity and levels of ACCP and rheumatoid factor (RF) in RA patients. As disease activity markers, Disease Activity Score (DAS)28 PCR, Simplified Disease Activity Index (SDAI) and a Spanish version of the Health Assessment Questionnaire (HAQ) are used.

Patients and Methods: DAS28 PCR, SDAI and HAQ were evaluated in 62 sera of 42 RA patients, diagnosed according to the American College of Rheumatology / European League Against Rheumatism 2010 classification criteria. In all of them, ACCP (Thermo Fisher Scientific) and RF were measured.

Results: When we analyze the different correlations between ACCP, RF and DAS28 PCR, SDAI and HAQ, we find no correlation between them (Figure 1).

Conclusions: We find no correlation between serological and disease activity markers in RA patients. Consequently, although ACCP and RF are essential for the RA diagnosis, they are not useful as disease activity indicators, and their continuous measurement in the monitoring of patients would not be indicated.

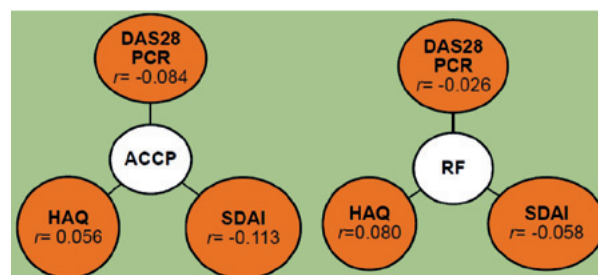


Figure 1: Pearson correlation (r) among serological data - anticyclic citrullinated peptide antibodies (ACCP) and rheumatoid factor (RF) - and clinical data - Disease Activity Score 28 (DAS28 PCR), Simplified Disease Activity Index (SDAI) and Health Assessment Questionnaire (HAQ) - in diagnosed RA patients.

EliA CCP

Clinical and serological response to Tocilizumab in patients with rheumatoid arthritis

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Objective: This study investigates the relationship between the presence of RF and anti-CCP and clinical response to Tocilizumab in patients with RA.

Patients and Methods: This was an observational longitudinal study in 27 patients with active, long-standing RA

despite previous treatment with >2 DMARDs and/or steroids. Patients were treated with Tocilizumab 8 mg/kg every 4 weeks. IgM-, IgA- and IgG-RFs and anti-CCP antibodies were assessed using ELISA and EliA, respectively, at baseline (T0), 3 months (T1), 6 months (T2), and 12 months (T3).

Results: All patients showed significant and sustained clinical response to Tocilizumab treatment. All clinical scales with the exception of HAQ significantly decreased. There was a significant correlation ($p=0.03$) between anti-CCP and SDAI changes from baseline at T1 and T2. Likewise there were no significant correlations between antibody count at T0 and changes in the DAS-28 VES at T1 and at T2. No significant relationship between clinical scales and antibody levels RF-IgG, IgA, IgM and anti-CCP levels were observed.

Conclusions: Tocilizumab is effective in treating the clinical symptoms of RA, and the efficacy of this molecule was neither correlated with RF nor anti-CCP levels. There is now a growing body of evidence suggesting that markers associated with clinical response may not be the same biomarkers that predict risk of further joint damage.

EliA RF IgA

Prevalence of IgA isotype rheumatoid factor in a population positive for IgM rheumatoid factor

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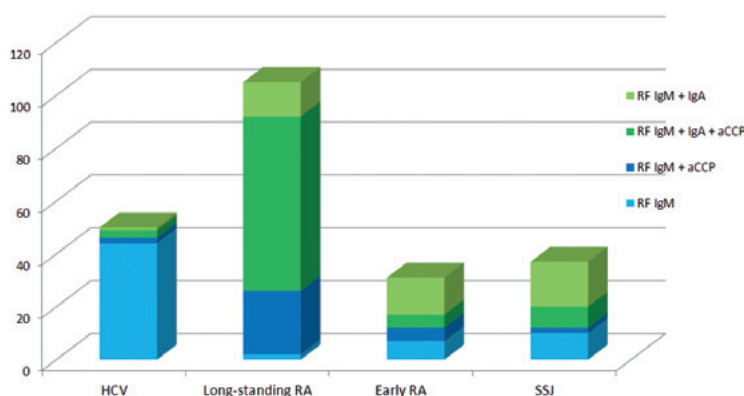
Objective: To investigate the prevalence of IgA isotype Rheumatoid factor (RF) in 223 RF IgM positive patients with systemic connective tissue disease and patients with HCV infection.

Patients and Methods: Anti IgM/IgA RF and anti-citrullinated protein / peptide antibodies were determined using the EliA™ Fluoroenzyme Immunoassay kits (Thermo Fisher Scientific), in 50 HCV infected patients (HCV RNA positive), 105 patients with long-standing RA and 31 early RA according to the 1987 ACR revised criteria and 37 patients with Sjogren's syndrome (SSJ) defined according to the European classification criteria.

Results: Prevalence of IgA RF was 56,5% in all patients; it was 8.0 % (4/50) in HCV patients, 3 also anti-CCP positive; 58.1% (18/31) in patients with early RA, 7 anti-CCP positive; 75.2% (79/105) in patients with long-standing RA, 66 anti-CCP positive and 67.5% (25/37) in patients with SSJ, 10 anti-CCP positive, respectively.

Conclusions: The presence of IgA RF is low in HCV infection. In both RAs groups IgA RF is well represented and possibly describes a subset of disease; it is also related to the response to drug treatment. In SSJ patients the high prevalence of IgA RF and anti-CCP, clinically confirmed by RA, suggests further monitoring of possible evolution towards RA of SSJ IgA RF positive patients.

Proportion of aCCP and RF IgA in RF IgM-positive patients



EliA CTD Screen

Clinical evaluation of QUANTA Flash CTD Screen Plus, a novel chemiluminescent immunoassay for the detection of autoantibodies in systemic autoimmune rheumatic diseases

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Objective: To compare a new chemiluminescent immunoassay (CIA) for the detection of anti-intracellular antibodies with EliA CTD Screen.

Patients and Methods: 325 patients with SARD and 142 controls were tested by QUANTA Flash CTD Screen Plus (CTD CIA, INOVA Diagnostics, US) and the results were compared with those obtained with EliA CTD Screen (Thermo Fisher Scientific). 40 sera with a nucleolar staining pattern were also tested, as the two assays differ in antigenic composition only in the Ku and Th/To (INOVA) and fibrillarin (Thermo Fisher Scientific) antigens. Reactivities to the individual antigens were detected using Dot Blot (D-tek, Belgium) and QUANTA Flash (QF) prototype tests (INOVA).

Results: 277/467 samples were positive and 145/467 were negative for both assays. In the SARD group, 14 samples were QF+/EliA- and 8 samples were QF-/EliA+ (agreement 93.2%). In the controls, 18 samples were QF+/EliA- and 5 were QF-/EliA+. Total percent agreement (TPA) between methods was 90.4%. Sensitivity and specificity were 86.8% and 81.0% for QF and 84.9% and 90.1% for EliA (Figure 1). When specificity

was set at 96.5% by ROC curve analysis, sensitivity was 68.3% for QF (AUC, 0.92) and 69.4% for EliA (AUC, 0.93).

Conclusions: The QUANTA Flash CTD Screen Plus showed good diagnostic accuracy, similar to EliA CTD Screen. Both tests can be reliably used as a screening test to select sera to be tested for specific antibodies.

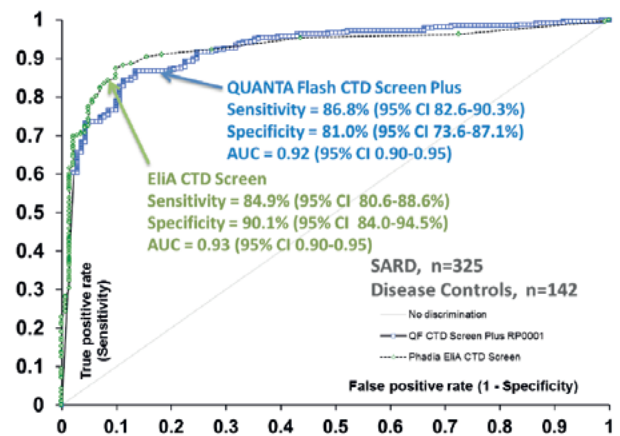


Figure 1: ROC analysis of QUANTA Flash CTD Screen Plus vs. EliA CTD Screen among SARD patients and disease controls. Summary of antigen formulation for QUANTA Flash CTD Screen Plus and EliA CTD Screen.

Disease Group	N=467	QF CTD Screen Plus No. Pos (%Pos)	EliA CTD Screen No. Pos (%Pos)
Systemic lupus erythematosus (SLE)	110	92 (83.6%)	92 (83.6%)
Sjogren's syndrome (SjS)	89	74 (83.1%)	75 (84.3%)
Systemic sclerosis (SSc)	80	74 (92.5%)	71 (88.8%)
Mixed connective tissue disease (MCTD)	38	35 (92.1%)	33 (86.8%)
Dermatomyositis (DM)	8	7 (87.5%)	5 (62.5%)
Rheumatoid arthritis (RA)	60	10 (16.7%)	3 (5.0%)
Infectious disease	39	10 (25.6%)	5 (12.8%)
Other	43	7 (16.3%)	6 (14.0%)

Table 1: Overview of sensitivities in each disease group for QUANTA Flash CTD Screen Plus and EliA CTD Screen.

EliA CTD Screen

Indirect immunofluorescence on HEp-2 cells versus EliA CTD Screen for the detection of anti-nuclear antibodies

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Objective: To estimate the diagnostic performance of indirect immunofluorescence on HEp-2 cells versus EliA CTD Screen for the detection of anti-nuclear antibodies (ANA). Furthermore, to evaluate the agreement of the two methods.

Patients and Methods: 211 samples from patients suspected of connective tissue disease (CTD) were analysed by EliA CTD Screen (Thermo Fisher Scientific) and IIF on

HEp-2 (ImmunoConcepts). The patients that were later diagnosed with a CTD were used in this project as incident patients (42). For additional comparison of the two methods (agreement) 124 patients already diagnosed with a CTD and fulfilling current consensus criteria were likewise analysed. The CTDs were systemic lupus erythematosus (SLE), primary Sjögren's Syndrome (SS), systemic sclerosis (SSc), mixed connective tissue disease (MCTD) and polymyositis/

n = 211	EliA CTD Screen		IIF on HEp-2	Combined
	≥0,7	>1,0	1:≥160	EliA+IIF
Sensitivity	88	80	85	90
Specificity	93	96	88	80
LR+	13	18	7	4,5
LR-	0,13	0,21	0,16	0,12

Table 1: Diagnostic performance, estimated from samples of incident CTD patients and diseased controls. LR: Likelihood ratio.

n = 211	n	EliA CTD Screen		IIF on HEp-2
		≥0,7	>1,0	1:≥160
SLE	11	91	82	91
SS	4	100	100	50
SSc	18	89	78	89
MCTD	5	100	100	100
PM/DM	1	0	0	0
SSc+SS	2	100	100	100
SSc+MCTD	1	100	100	100
DC	169	11	7	21

Table 2: ANA by IIF and by EliA in incident CTD patients and diseased controls. For each disease entity the likelihood of reactivity is shown. DC: Diseased controls.

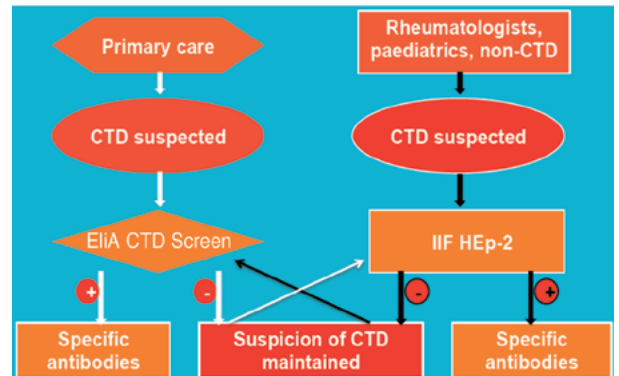
n = 124	n	EliA CTD Screen		IIF on HEp-2	at diagnosis
		≥0,7	>1,0	1:≥ 160	1:160
SLE	59	81	73	76	98
SS	18	83	83	67	78
SSc	29	72	69	72	86
MCTD	8	100	100	100	100
PM/DM	10	60	60	30	50

Table 3: Anti-nuclear antibodies by IIF on HEp-2 and by EliA CTD Screen in CTD patients. For each disease entity the likelihood of reactivity is shown. The IIF reactivity at time of diagnosis is shown to illustrate that the low reactivity in IIF and especially in the SLE group is due to quiescent / treatment induced remission of disease in some of the patients.

dermatomyositis (PM/DM). Furthermore, 100 blood donors were tested. All samples were analyzed simultaneously by IIF on HEp-2 and EliA CTD Screen. All reactive samples were further analyzed for antibody specificity.

Results:

The study indicates that the EliA CTD Screen with a cut off of 0.7 is superior in performance. However, agreement analysis reveals a substantial fraction of CTD patients, in which ANAs are detected by only one of the methods, substantiating that IIF on HEp-2 for some diseases is more sensitive, but for some specific antibodies (anti-SSA, -SSB, -Jo-1, RNA pol III, results not shown), the EliA CTD Screen demonstrates better recognition. IIF on HEp-2 is still recognised to be the reference method of choice for ANA screening, but its limitations should be kept in mind, being a laborious technique requiring highly qualified laboratory personnel, in addition to low specificity, making it unsuitable for the high-throughput screening of relatively unselected populations



with a low prevalence of CTD. We propose the algorithm on the right for optimal detection of ANA in various patient populations suspected of having CTD.

Conclusions: IIF on HEp-2 and EliA CTD Screen complement each other, and if either is used alone, negative samples should be analysed by the other method if clinically indicated.

EliA dsDNA

Determination of a new anti-dsDNA titer threshold measured by EliA assay to discriminate active and non-active SLE

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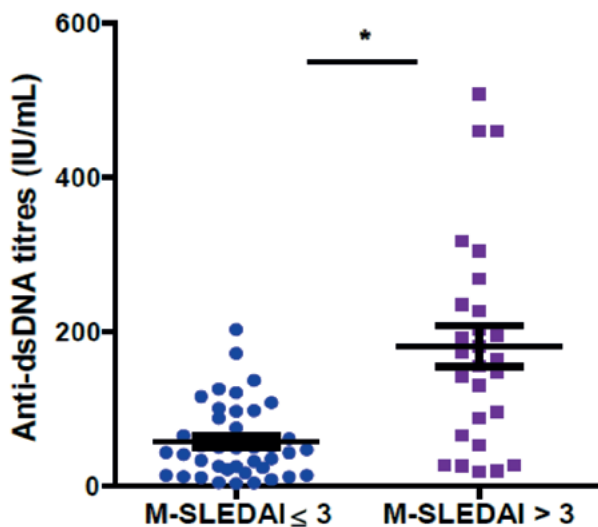


Figure 1: Comparison of mean anti-dsDNA titers in active and non-active SLE.

Objective: This study aimed at determining an appropriate anti-dsDNA threshold allowing better discrimination between active and non-active SLE.

Patients and Methods: 68 anti-nuclear antibody positive sera from 37 SLE patients were tested for anti-dsDNA by a commercial assay EliA dsDNA (Thermo Fisher Scientific). SLE disease activity was assessed at the time of each sample using a modified SLEDAI (M-SLEDAI) to remove anti-dsDNA parameter. SLE was considered to be active if the M-SLEDAI was more than 3. Determination of a new threshold allowing better discrimination between active and non-active SLE was performed using the ROC methodology.

	M-SLEDAI ≤ 3 (n=41)	M-SLEDAI > 3 (n=27)
% of positive anti-dsDNA titers (>10 IU/mL)	90 %	100 %
Mean titer of anti-dsDNA	57.3 IU/ml	181.1 IU/ml

	Manufacturer cut-off: 10 IU/ml	ROC cut-off: 128.5 IU/ml
Se (%)	100.0 (87.2-100.0)	66.7 (46.0-83.5)
Sp (%)	12.5 (4.2-26.8)	92.5 (79.6-98.4)
pLR	1.14	8.89
nLR	0.0	0.36

Results: Mean titer of anti-dsDNA was significantly higher in sera from active than from quiescent disease.

Anti-dsDNA titers were significantly correlated with M-SLEDAI ($p < 0.001$, $r = 0.533$). A significant correlation was also observed between anti-dsDNA titers and the C3 serum concentration ($r = -0.361$, $p = 0.007$) and the total hemolytic complement assay (CH50) ($r = -0.502$, $p = 0.001$).

With manufacturer's cut-off value, performance of the assay in discriminating quiescent and active SLE was poor. With a new higher threshold defined by ROC methodology (128.5 IU/mL) the accuracy was greatly improved.

Table 1: Optimisation of the assay in discriminating non-active and active SLE

We observed for several patients an association between the increase of anti-dsDNA titers and the changes in SLEDAI and C3 and C4 concentrations.

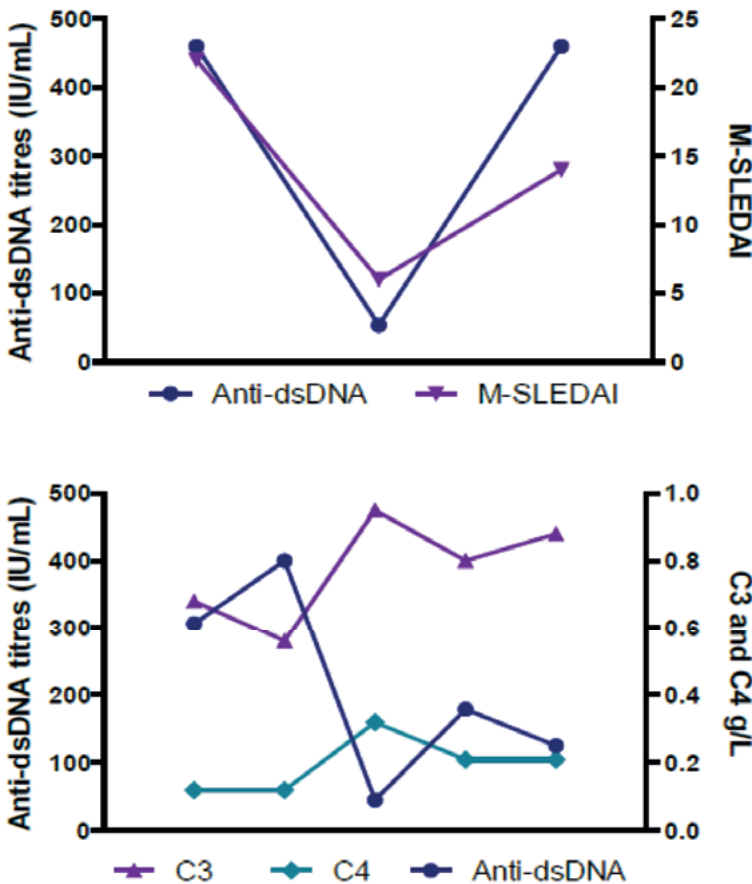


Figure 2: Changes in anti-dsDNA titers during follow-up of patients with SLE.

Conclusions: Anti-dsDNA titers measured by EliA dsDNA are correlated with SLE disease activity. Besides of the manufacturer's cut-off, the determination of a higher

threshold confers to this assay a supplementary tool to discriminate active and non-active SLE.

EliA Celikey and EliA Gliadin^{DP} Study of requests profile in celiac disease diagnosis

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Objective: After one year as Reference Laboratory (Protocol with the Portuguese Celiac Disease Association) in Celiac Disease, the authors want to show the preliminary results about the incidence of the disease in the patients with the specific requests.

Patients and Methods: We used a fluoroenzymeimmunoassay (FEIA, Thermo Fisher Scientific) for quantification of Anti-Tissue Transglutaminase Antibodies [IgA] (tTG) and Anti-Gliadin Antibodies [IgA and IgG] (AGA). We detect Anti-Endomysium Antibodies [IgA] (EMA) and Anti-Reticulin Antibodies [IgA and IgG] (ARA) with immunofluorescence methods for microscopy analysis (IF), with excitation filters to 495 nm and emission filters at 525 nm.

Results:

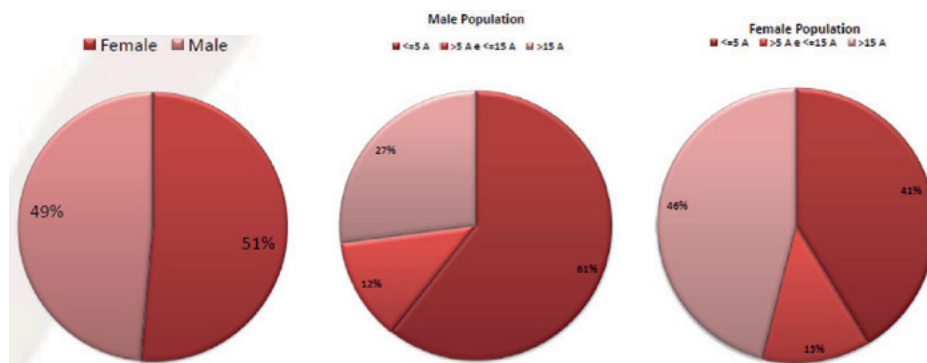


Figure 1: Age and sex distribution of the study population

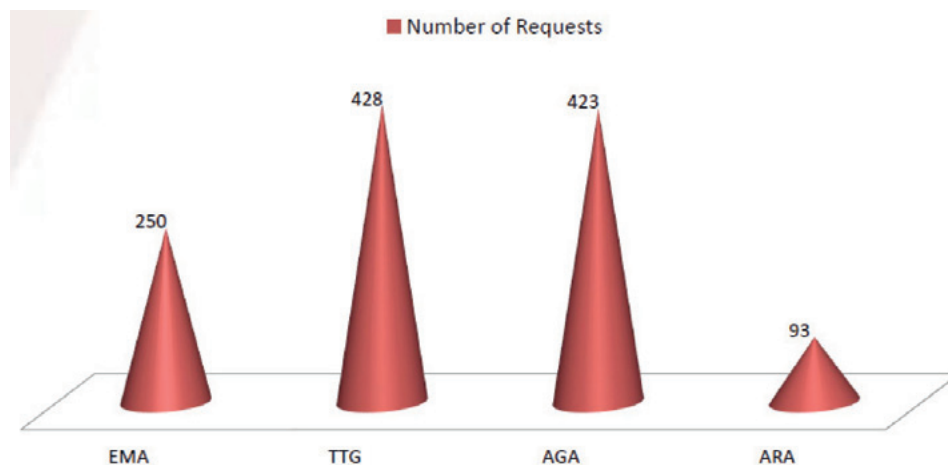


Figure 2: Amount of requests by parameters.

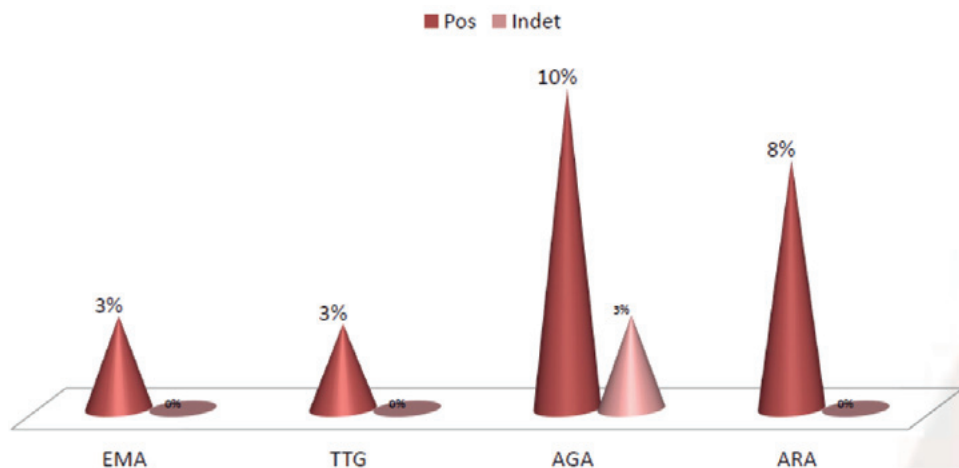


Figure 3: Evaluation of positive cases by parameter.

Conclusions: Antibodies against tTG are considered to be the main serological marker in celiac disease and they became essential in diagnosis and monitoring of the disease. Since the seroconversion from gliadin to tTG antibodies in children occurs often only at the age of 4 or 5 years, it is recommended to measure anti-gliadin antibodies in younger children. On the other hand it has to be considered, that anti-tTG and anti-gliadin antibodies may occur in children without the presence of clinical symptoms of celiac disease. In this preliminary study, 570 samples have been evaluated. The most frequent request (22%) was for antibodies against

tTG+EMA+AGA. Only one patient (age: 60 years) had all these antibodies and only one patient with 5 years of age was positive in anti-tTG and EMA. The second most frequent request was tTG+EMA+AGA+ARA (21%). Only one child (less than 5 years of age) was EMA and AGA positive, but anti-tTG negative.

All in all, 3% of the 570 cases were diagnosed with celiac disease and were positive for anti-tTG and EMA. 10% of the sera were AGA positive but could not be associated with the presence of celiac disease.

EliA Celikey

Background fluorescence levels of anti-tissue transglutaminase IgA EliA assays are correlated to the concentration of circulating IgA and enable the detection of selective IgA deficiencies

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Objective: To verify whether RU background levels of EliA Celikey IgA (anti-tTG IgA EliA) correlate with the concentration of circulating IgA, especially in sera with low concentration of circulating IgA.

Patients and Methods: Sera of 52 patients that were referred to the Pitié Salpêtrière hospital with blood routinely drawn for EliA Celikey IgA (Thermo Fisher Scientific) and measurement of circulating IgA were analyzed retrospectively.

In addition, 86 sera characterized by low concentration of circulating IgA (<1 g/L) measured by nephelometry (BNII, Siemens, Germany) were also included in the study and measured with EliA Celikey IgA. Among the latter sera, 25 were confirmed with specific IgA deficiency (slgAdef) as circulating IgA concentration through nephelometry was <0.06 g/L. All the sera were negative for anti-tTG IgA (i.e. <7 U/mL). Low RU were defined as undetectable RU while detectable RU were defined by RU>4.

Results:

Background RU levels of EliA Celikey IgA are highly correlated to circulating IgA concentrations (r=0.96 and p<0.0001 using Spearman non parametric correlation test). The correlation remained excellent when the comparison was made on the 86 sera with IgA levels <1 g/L (r=0.85 and p<0.0001).

We observed that all sera characterized by low RU (n=15) were indeed slgAdef. However, 10 other sera of slgAdef corresponded to detectable RU (table 1). Low RU had an excellent specificity for slgAdef (Spe=1) but a poor sensitivity (Se=0.6). The area under the curve of ROC analysis was of 0.97, indicating an excellent power for RU levels for the diagnosis of slgAdef. While the best likelihood ratio (94.9) was obtained with a cut-off of 9.5 (Spe: 0.99 and Se: 0.84), we reached a 100% sensitivity with a cut-off for RU of 22 with a 0.61 specificity. Therefore, all slgAdef could be detected when considering a cut-off of 22 in this study (figure 1).

Conclusions: Background levels of EliA Celikey IgA were highly correlated to circulating IgA concentrations. slgAdef could be overlooked when considering a cut-off of 22 RU. We further demonstrated that not all slgAdef could be detected by low RU as 40 % of slgAdef (10 out of 25) had detectable RU.

We therefore propose a decisional algorithm for the detection of slgAdef for sera with undetectable anti-tTG IgA (i.e. <7U/mL) in order to avoid measuring IgA concentrations in unnecessary situations and overlooking slgAdef diagnosis (figure 2):

RU / IgA	IgA >0.06 g/L	IgA <0.06 g/L	Total
Detectable RU	113	10	123
Low RU	0	15	15
Total	113	25	138

Table 1: Optimisation of the assay in discriminating non-active and active SLE

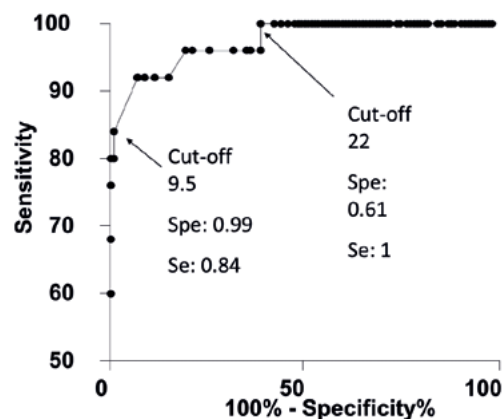


Figure 1: Receiver operating characteristics (ROC) analysis for RU levels for the diagnosis of slgAdef.

For all sera with RU >22, circulating IgA concentration should not be measured as slgAdef diagnosis is highly improbable. In such cases, RU levels under the positivity threshold for anti-transglutaminase IgA antibodies should lead to the conclusion of the absence of slgAdef and of anti-transglutaminase IgA antibodies.

For all sera with low RU, slgAdef is highly probable. Thus, with regards of the search for anti-transglutaminase antibodies, IgG isotype assays would be performed while measurement of circulating IgA would be optional.

For all sera with detectable RU below 22 RU, the sensitive measurement of IgA should be performed in order to confirm or rule out slgAdef. In case of confirmed slgAdef, IgG isotype assays would be performed while the conclusion of the absence of slgAdef and of anti-transglutaminase IgA antibodies would be drawn.

We conclude that the RU levels should be considered as an important parameter that enables the diagnosis of previously overlooked slgAdef.

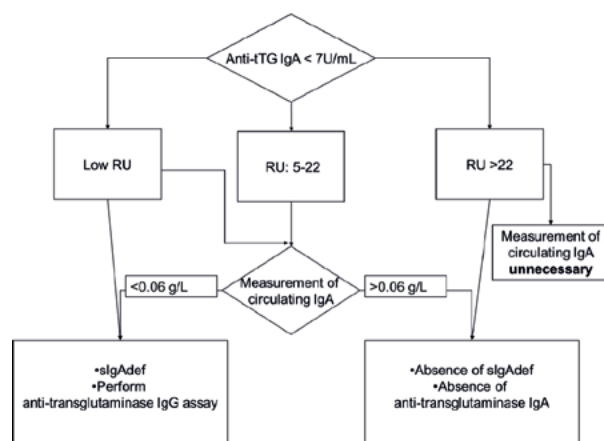


Figure 2: Proposition for an operational diagnosis algorithm for the diagnosis of slgAdef using RU levels for sera with undetectable anti-tTG IgA (<7 U/mL).

Celiac questionnaire

Coeliac disease diagnosis: Guideline impact on medical prescription in France

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Objective: Different guidelines for the diagnosis and management of coeliac disease have been recently published. The aim of that study was to determine whether recommendations issued from these guidelines have been adopted by physicians in France when coeliac disease is suspected.

Patients and Methods: A total of 5521 physicians were prospectively included in this study between January 2010 and February 2011. All 5521 physicians had a medical activity in the liberal sector. They were asked to fill in a detailed questionnaire about coeliac

QUESTIONNAIRE:

Five questions were asked as follows:

- 1 – How many patients with coeliac disease do you follow up?
- 2 – What type of symptoms lead you to evoke coeliac disease? (Open question)
- 3 – How many patients do you suspect with coeliac disease per year?
- 4 – When coeliac disease is suspected:
 - Do you request a duodenal biopsy to be performed?
 - Do you request serological tests to be performed?
 - Do you request a duodenal biopsy together with serological tests to be performed?
 - Do you request a duodenal biopsy regarding serological test results only?
 - Do you request serological tests regarding the duodenal biopsy feature only?
 - Do you refer your patient to a colleague experienced with coeliac disease?
- 5 – What type of serological tests do you prescribe to diagnose coeliac disease?
 - IgA anti-tissue transglutaminase: always, sometimes, never, marker not known?
 - IgA anti-endomysium: always, sometimes, never, marker not known?
 - IgA anti-gliadin: always, sometimes, never, marker not known?
 - IgA anti-reticulin: always, sometimes, never, marker not known?
 - Total IgA levels: always, sometimes, never, marker not known?

disease to evaluate their medical practice. Responses were obtained from 256 general practitioners, 221 gastroenterologists, and 227 pediatricians.

Prescription behavior when coeliac disease was suspected	General Practitioners (N = 256)	Gastroenterologists (N = 221)	Pediatricians (N = 227)
No answer or non-exploitable data	17	36	5
No answer from physicians directly referring the patient to specialized physicians	139 (54.3%)	0	30 (13.2%)
Duodenal biopsy only	1 (1%)	3 (1.6%)	0
Serologic tests only	26 (26%)	13 (7%)	82 (42.7%)
Both duodenal biopsy + serologic tests	21 (21%)	115 (62.2%)	5 (2.6%)
Duodenal biopsy after serologic tests	51 (51%)	45 (24.3%)	100 (52.1%)
Serologic tests after duodenal biopsy	1 (1%)	9 (4.9%)	5 (2.6%)

Anti-tissue transglutaminase antibodies (tTg Abs) prescribed by physicians when their patients were suspected with coeliac disease	General Practitioners (N=256)	Gastroenterologists (N=221)	Pediatricians (N=227)	p-value*
Prescription of tTg Abs N (%)				
tTg Abs marker not known	34 (21%)	0 (0%)	6 (3%)	<0.001
tTg Abs always prescribed	78 (48%)	196 (94%)	202 (91%)	
tTg Abs sometimes prescribed	33 (20%)	9 (4%)	12 (5%)	
tTg Abs never prescribed	19 (12%)	3 (1%)	3 (1%)	
Missing or non-exploitable data	92	13	4	

* Chi-squared test

Results: Response analysis showed that protean clinical presentations of coeliac disease may be better recognized by gastroenterologists and pediatricians than general practitioners.

Gastroenterologists requested duodenal biopsy much more often than general practitioners and pediatricians when coeliac disease was suspected.

Serologic testing behavior and knowledge of critical markers, as anti-tissue transglutaminase and total IgA dosage

prescribed to diagnose coeliac disease, were different when comparing general practitioners, gastroenterologists and pediatricians.

Conclusions: Analysis of medical prescriptions showed that the recommendations for coeliac disease diagnosis are not always followed by physicians, emphasizing the fact that the impact of the national or international guidelines on medical behavior should be evaluated.

EliA ASCA

Evaluation of the new EliA ASCA (anti-saccharomyces cerevisiae antibodies) on Phadia 2500

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Objective: To evaluate the new EliA ASCA assay on the Phadia 2500 (Thermo Fisher Scientific) by testing the analytical performances, the prevalence of ASCA IgG and/or IgA in IBD and non IBD populations and the correlation with ELISA ASCA assay performed on the Alegria system (Orgentec).

Patients and Methods:

Analytical performance

Intra-assay precision: coefficient of variation (CV) was calculated with the results of 28 determinations of an IgG and IgA positive serum in a single run.

Inter-assay precision: coefficient of variation (CV) was calculated with the results of 26 determinations of the IgG or IgA positive control in 26 different runs.

Prevalence of IgG and/or IgA ASCA in IBD and non IBD populations

58 patients with IBD serological markers prescription (ASCA and ANCA) were selected from our routine: 30 patients diagnosed CD with ASCA IgG and/or IgA positive (Alegria Orgentec) and negative ANCA (indirect immunofluorescence), 28 patients diagnosed UC with negative ASCA IgG and/or IgA (Alegria Orgentec) and atypical pANCA (indirect immunofluorescence). 51 patients were selected for the non IBD control group: 29 with celiac disease, 12 with active hepatitis (B,C and E), and 10 with lupus or Sjogren disease.

Correlation with ELISA assay (Alegria Orgentec)

A total of 133 sera were tested: 58 from the prevalence study with IBD serological markers prescription (ASCA and ANCA), 27 celiac disease and another 48 not selected, from our routine.

Results:

Analytical performance

Intra-assay precision CV was 8% for IgG and 7% for IgA. Inter-assay precision CV was 5.5% for IgG and 5.6% for IgA. In light of the absence of recommendation by scientific societies for autoimmunity analysis and from our experience, these CV's are satisfactory and in complete accordance with the requirement of our laboratory.

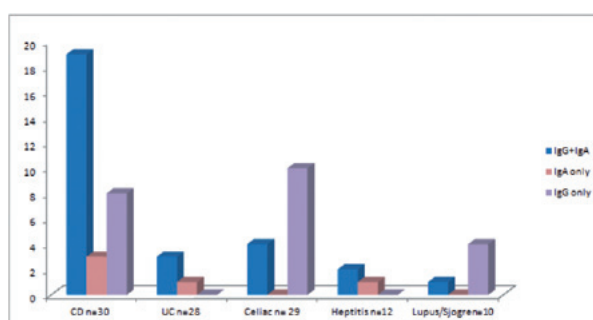


Figure 1: Numbers of patient with positive ASCA in the different groups

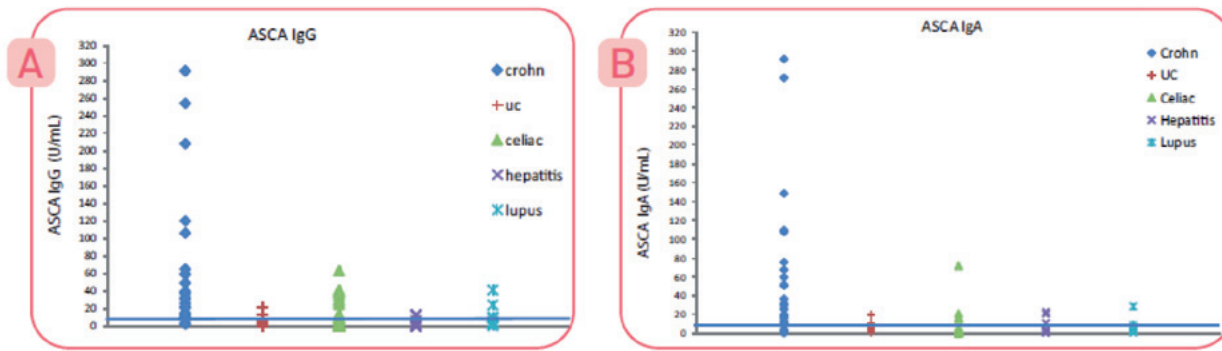


Figure 2: ASCA IgG (A) / IgA (B) values for CD patients range from low to very high titers. However most of UC and non IBD groups showed low or moderate titers.

		Phadia 2500		
		+	-	Total
Alegria	+	51	10	61
	-	8	64	72
	Total	59	74	133

Table 1: ASCA IgG. Agreements observed on 86.5% of the observations. Kappa=0.727 (95% confidence interval: from 0.61 to 0.844). The strength of agreement is considered to be good.

		Phadia 2500		
		+	-	Total
Alegria	+	25	2	27
	-	22	84	106
	Total	47	86	133

Table 2: ASCA IgA. Agreements observed on 82% of the observations. Kappa=0.563 (95% confidence interval: from 0.417 to 0.709). The strength of agreement is considered to be moderate.

Prevalence of ASCA IgG and/or IgA in IBD and non IBD populations.

A positive EliA ASCA IgA + IgG result ensures better sensitivity and specificity for CD especially for the differential diagnosis with UC. However we observed that almost 50% of the celiac population had ASCA IgG+ IgA or only IgG; almost all patients who were ASCA positive had also a high anti-transglutaminase IgA titer.

We observed 22 discrepancies "Phadia positive and Alegria negative": 6/22 patients were suspected of CD, 2/22 had celiac disease. Unfortunately, we could not obtain clinical information on the other patients but we could also notice that ASCA IgA values were low (< 30 U/mL) and prescriptions were for IBD diagnosis (ASCA + ANCA).

Conclusions: The new EliA ASCA assay represents a convenient and sensitive test for ASCA determination with good analytical performances and appears to be a helpful tool for IBD diagnosis. The specificity of the test is improved when IgG + IgA are positive, especially in the differential diagnosis with UC.

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- The Phadia 5000 answers to all the quality and automation requirements of the laboratory.
- RF IgA is frequent in rheumatoid arthritis and seems to represent a subset of disease. In Sjögren's Syndrome the presence of RF IgA might give an additional hint for the development of rheumatoid arthritis.
- IIF on HEp-2 and EliA CTD Screen complement each other, and if either is used alone, negative samples should be analysed by the other method if clinically indicated.
- EliA dsDNA titers correlate with SLE disease activity.
- A „Low RU“ signal in EliA Celikey always correlates with IgA deficiency.
- The new EliA ASCA assay represents a convenient and sensitive test for ASCA determination.

thermoscientific.com/phadia

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