Year 2, No. 2, December 2015

Auto-immunity

CLOSE UP

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A.MENARINI diagnostics

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Year 2, No. 2, December 2015
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Aims & Scope
Autoimmunity Close Up is the new A. Menarini Diagnostics publication in the field of autoimmunity. The magazine establishes an interdisciplinary forum connecting experts involved in all aspects of the complex world of autoimmunity diagnosis.
Autoimmunity Close Up provides our customers and colleagues with important product information and updates, insights into issues of general interest in autoimmunity and the latest findings in autoimmune diseases.
The magazine encompasses a wide range of topics including connective tissue diseases, rheumatoid arthritis, antiphospholipid syndrome, celiac disease, IBD, vasculitis, autoimmune thyroid and liver diseases, as well as POCT, ITC & health economics.
Each issue features reviews, editorials, and interviews with leading scientists who actively participate in building the content.
If you have any questions or require further information about *Autoimmunity Close Up*, please contact your local A. Menarini Diagnostics Affiliate or Distributor.

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In this issue:

EDITOR'S NOTE

Research, standardisation, patient care & management: the next pillars in Autoimmunity

This is the third issue of our company magazine, and this time we turn our attention to some topics that are frequently discussed during congresses, fairs and exhibitions, workshops and other relevant activities concerning Autoimmunity.

Starting from the importance of research and its impact on the progress of this specialised field, we draw attention to the many significant congresses taking place during this period. One of the most visible events is the 10th International Congress on Autoimmunity that will be held in Leipzig in April 2016. This event is usually quite rich in scientific insights often related to the world of research and translational medicine but with an eye to the parallel world of the industry.
In the same spirit, Menarini Diagnosticos SA organised an event in Madrid, Spain, on November 5th, which covered numerous topics including a highlight on standardisation processes applied to autoantibody detection and three talks on the importance of monitoring biological drugs in RA, IBD, etc., testifying to Menarini Diagnostics' belief in the value of research and innovation. Further information is included in the Company Pinboard section.

Standardisation is, however, a concept that cuts across different areas and engages many of the major players, including companies, research and academic bodies, all committed to this objective, which is still quite far from being a reality in Autoimmunity. Standardisation was dealt with in the last issue of the magazine, but the subject appears increasingly often. The next 10th international Congress on Autoimmunity will also host the Conference of the European Autoimmunity Standardisation Initiative (EASI), a network founded 6 years ago to improve diagnostics in chronic rheumatic disorders by strengthening the collaboration and exchange of information and experience of clinical and laboratory scientists responsible for autoimmune diagnostics in Europe and whose Conference is held biannually at the International Congress on Autoimmunity.
Different committees and organisations have been set up with a view to achieving a better and more standardised approach to autoantibody testing, including the previously mentioned EASI (http://www.easi-network.com), the European Consensus Finding Study Group on autoantibodies (ECFSG - EULAR) and the Working Group on Harmonization of Autoantibody Tests (WG-HAT) in the framework of the International Federation of Clinical Chemistry and Laboratory Medicine, and the International Union of Immunology Specialties (IUIS) Autoantibody Standardization Committee (http://asc.dental.ufl.edu/home.html), as evidence of the fact that standardisation and harmonisation in Autoimmunity are a challenge.

Finally, on the subject of patient care, what better example than coeliac disease.
In this issue, you will find a contribution from academia. Professor Carlo Catassi, historical pioneer in coeliac disease, explains, together with other clinicians and the support of Luigi Farina as the representative of industrial R&D, how a new analytical approach to the

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detection of tTg IgA may sustain this diagnosis that often proves challenging because of the unclear clinical presentation.

So, to summarise, we have seen that research, standardisation, and patient care represent a crucial part of the workflow and pathways associated with the diagnosis, treatment and follow-up of the autoimmune patient. These three pillars – research, standardisation, and patient care – will therefore increasingly play a pivotal role in this scenario.

Menarini Diagnostics, with its product line entirely dedicated to Autoimmunity, persistently pursues these objectives, through its initiatives, closeness to the different scientific realities, and launching of innovative products to be compliant with such vision.

Massimo Donnini
International Product Manager Autoimmunity
A. Menarini Diagnostics

TECHNICAL INSIGHTS

IgA antibodies anti-transglutaminase type 2: an essential tool in the diagnosis and follow-up of coeliac disease

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Introduction

Coeliac disease (CD), according to the recent European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) definition, is an immune-mediated systemic disorder elicited by gluten in genetically susceptible individuals that is characterised by the presence of a variable combination of gluten-dependent clinical manifestations, CD-specific antibodies, HLA-DQ2 or DQ8 haplotypes and enteropathy.

The cornerstone of laboratory-based CD diagnosis is represented by autoantibodies against the tissue transglutaminase, also known as transglutaminase type-2 (TG2). They include IgA anti-TG2 antibodies (anti-TG2), quantitatively measured by enzyme linked immunosorbent assay, and IgA anti-endomysial antibodies (EMA), measured by means of indirect immunofluorescence. The quantitative TG-2 immunoassay has been an attractive tool because it showed a good performance, equivalent to the EMA assay, but was operator-independent, easier to standardise and did not use animal or human tissue.

The diagnostic performance of anti-TG2

The first commercial kits of anti-TG2, based on guinea pig or human extractive enzyme, showed good diagnostic accuracy. Methods based on recombinant human enzyme, developed subsequently, showed a higher diagnostic accuracy and became almost a standard. In the ESPGHAN report on CD antibodies, it was not possible to obtain pooled performance estimates on sensitivity and specificity of anti-TG2 due to the heterogeneity of the studies evaluated, but for 11 of 15 study populations, sensitivity was greater than or equal to 90%, and for 13 of 15 study populations, specificity was greater than or equal to 90%. Sensitivity of EMA tests ranged lower compared with anti-TG2 (7/11 studies presented ≥90% sensitivity), but the specificity was more stably higher (all but one study reached ≥95% specificity). The main limitation of these tests is that in young children both anti-TG2 and EMA may be initially negative. For this reason, the ESPGHAN concluded that anti-TG2 assay is the test with a better diagnostic performance for identifying CD in children older than 2 years. The highly specific EMA test should be used as a confirmatory test to identify positive tests as true patients with CD. In patients suspected of CD, it is important to preliminarily exclude IgA deficiency by measuring serum total IgA levels, and IgA-deficient patients can be evaluated on the basis of IgG class tests.

The significance of anti-TG2 levels at diagnosis

The identification of villous atrophy with crypt hyperplasia at small bowel biopsy has been the confirmatory cornerstone test for CD diagnosis in the last 30 years. However, patchy villous atrophy or poor quality of the biopsy specimens can complicate or even hinder the correct diagnosis. Further, small intestinal villous atrophy is not specific for CD and can also be found in association with other diseases. Therefore, with improvement of diagnostic antibody tests, a diagnosis of CD without the intestinal biopsy has been strongly advocated in recent years. Omitting biopsies reduces the burden of endoscopy and of general anaesthesia for the affected children, saves costs, and avoids the potential adverse effects of these procedures.

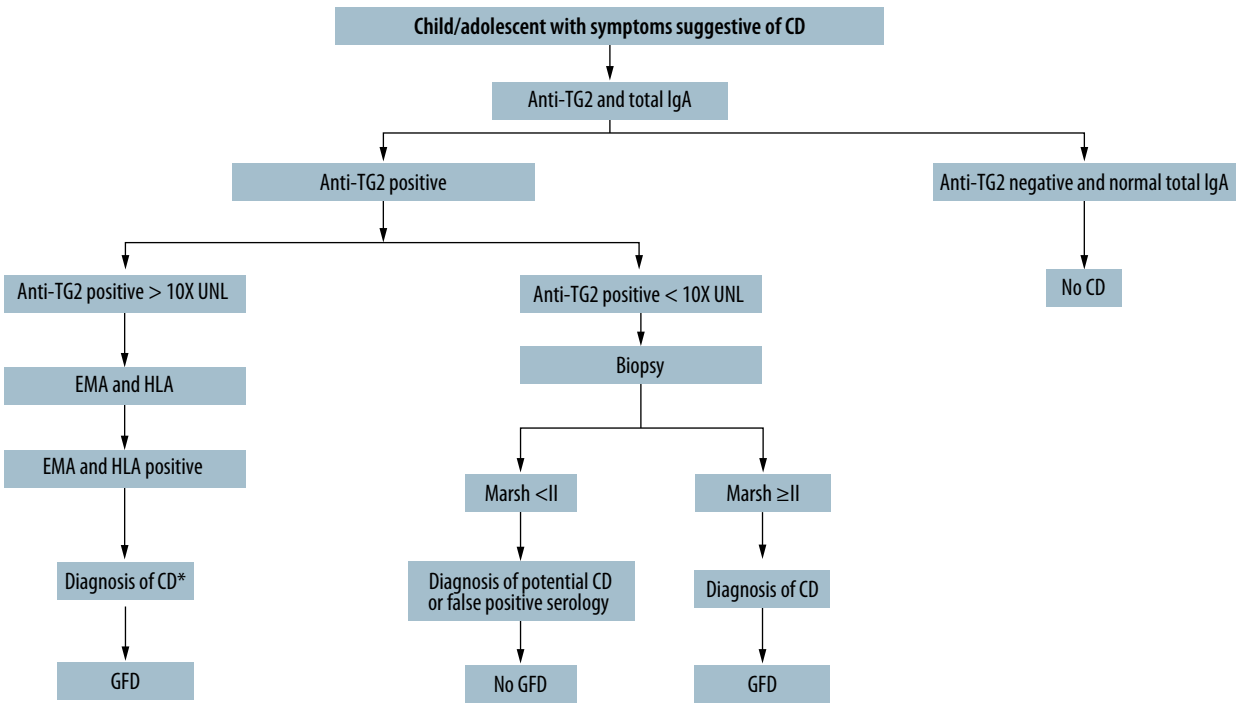
Several research studies have shown that the presence of villous atrophy can be predicted if the levels of circulating anti-TG2 are high. In such cases, intestinal biopsy would be unnecessary. Currently, anti-TG2 only can be measured in relative units, so numerical

values for such "high" values are kit-specific and show considerable variations. In addition, the calculation of results (number and value of calibrators) also differs. There are two main ways to calculate serum antibody results: the majority of currently available commercial anti-TG2 tests calculates test results by comparison with a dilution curve prepared from the serial dilutions of a positive sample that correspond to fixed concentrations (standard curve). Such values are proportional to the serum concentration of antibodies. A few tests use a more simple calculation that is dividing the specific test signal (absorbance after subtracting the background) by the signal obtained with an internal, kit-specific positive sample. These values are logarithmic; in consequence, numeric values are higher than those by standard curve calculations for samples with values below the positive control but lower for samples exceeding the positive calibrator. In other words, the dynamic range of a logarithmic test is narrower than that of a standard curve-based immunoassay.

To compare results obtained with different kits in the absence of an international standard, Hill et al. proposed the use of the ratio between the value of anti-TG2 of the patient and the decisional cut-off, instead of the absolute value. Their study showed that in 148 adults anti-TG2 values were always associated with villous atrophy when they exceeded 10 times the upper limit of normal for a test calculating antibody concentration from a standard curve. A further study by Dahlbom et al. showed that similar serum IgA-anti-TG2 levels and test result calculations as reported by Hill could predict villous atrophy in children as well. Other three studies investigated the performance of serum antibody tests at various cut-off levels: 1) 1.5 times the upper normal limit (Agardh et al.); 2) 5 times (Barker et al.); and 3) 2.5 times (Poddar et al.). However, none of the tests studied were standard curve based and may thus give different results in different runs. The validity of the ratio value of 10 times the upper normal limit, useful to identify patients with intestinal atrophy in whom it would be possible to avoid the biopsy, has been confirmed in several further adults and children studies evaluating tests based on standard curve.

On the basis of this evidence, the recent guidelines by ESPGHAN have suggested that biopsies could be avoided in patients who have very high titres of anti-TG2 (>10 X) in the presence of suggestive symptoms, a positive EMA and susceptible HLA haplotype as shown in Figure 1. Therefore, the quantitative measurement of anti-TG2 is currently considered an essential tool for the diagnosis of CD.

Figure 1: Algorithm proposed by the European Society of Paediatric Gastroenterology, Hepatology and Nutrition for the diagnosis of coeliac disease in symptomatic children and adolescent



Mod. from 2

CD: Coeliac disease; Anti-TG2: Antibodies anti-transglutaminase type 2; UNL: Upper normal limit; EMA: anti-endomysial antibodies; GFD: gluten-free diet; HLA: Human leucocyte antigen. * to be confirmed by normalisation of serology and clinical symptoms after a gluten-free diet

The significance of anti-TG2 levels in the follow-up

According to the ESPGHAN criteria, a "non biopsy" diagnosis should be confirmed by symptoms and serology normalisation after starting a gluten-free diet (GFD). For doctors who take care of patients with CD, as well as for the patients, it is important to know what time interval is expected to achieve a significant decrease and normalisation of CD antibodies after starting a GFD. It has been recently shown that within 3 months of GFD the mean concentration of anti-TG2 showed a 74% decrease, and that ~80% of patients will be seronegative for anti-TG2 after 2 years of the diet. The persistence of positive anti-TG2 therefore should induce to consider a poor compliance with the GFD, an incorrect diagnosis, or a refractory CD. However, high levels of anti-TG2 at diagnosis (>10 X) have been demonstrated to be associated with a longer time of serology normalisation, with a delayed recovery of anti-TG2 for up to three years despite strict adherence to a GFD. Therefore, the quantitative measurement of anti-TG2 may also be a useful tool in the follow-up of celiac patients, and a prolonged elevation should be taken into account when considering children with a "non-biopsy" diagnosis. Noteworthy, anti-TG2 were found to be not sensitive enough to detect slight dietary mistakes and/or moderate mucosa lesions to asses compliance with the diet.

Take home message

- IgA class anti-TG2 is the test with a better diagnostic performance for screening patients with suspected CD.
- Anti-TG2 titres correlate positively with severity of small intestinal damage, with high titres (>10 times the upper normal limit) being predictive of villous atrophy.
- According to the ESPGHAN guidelines, biopsies could be avoided in patients who have very high titres of anti-TG2 (>10 times the upper normal limit) in the presence of suggestive symptoms, a positive EMA and susceptible HLA haplotype. Diagnosis should be confirmed by symptoms and serology normalisation after starting a GFD.
- High titres of anti-TG2 at diagnosis (>10 times the upper normal limit) are associated with a long time for serology normalisation despite strict adherence to a GFD.

Suggested reading

1. *Giersiepen K, Lelgemann M, Stuhldreher N, Ronfani L, Husby S, Koletzko S, Korponay-Szabó IR; ESPGHAN Working Group on Coeliac Disease Diagnosis. Accuracy of diagnostic antibody tests for coeliac disease in children: summary of an evidence report. J Pediatr Gastroenterol Nutr. 2012;54(2):229-41.*
2. *Husby S, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Shamir R, Troncone R, Giersiepen K, Branski D, Catassi C, Lelgeman M, Mäki M, Ribes-Koninckx C, Ventura A, Zimmer KP; ESPGHAN Working Group on Coeliac Disease Diagnosis; ESPGHAN Gastroenterology Committee; European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr. 2012;54(1):136-60.*
3. *Brusca I. Overview of biomarkers for diagnosis and monitoring of celiac disease. Adv Clin Chem. 2015;68:1-55.*

AUTOIMMUNITY LAB

Solid-phase chemiluminescence immunoassay in the detection of antinuclear and cytoplasmic autoantibodies.
A comparison with the gold standard techniques

Anti-nuclear antibodies (ANA), more precisely referred to as anti-nuclear and cytoplasm antibodies, are a typical feature of systemic autoimmune diseases, where a systemic immunological response occurs against widely distributed self-antigens.

The precise characterization of ANA is crucial in diagnosis. In ANA analysis, methods such as enzyme immunoassay and Western blot or immunoblot have generally replaced older methods like indirect immunofluorescence (IIF); however, their reliability depends on the characteristics of the technique and the antigen source. Furthermore, IIF is still a valuable tool for the initial screening and intracellular mapping of target antigens.

Other methods are needed to more precisely characterize ANA autoantibody specificity. Immunoprecipitation is currently the gold standard method to characterize the specificity of ANA directed against small nuclear and small cytoplasmic ribonucleoprotein (sn/scRNP) and proteins that are not RNA-associated.

Menarini Diagnostics (Florence, Italy) recently **launched a chemiluminiscent immunoassay (CLIA) system to be used on the dedicated ZENIT RA analyzer (Zenit RA)** and based on autoantigen-coated magnetic particles as solid phase and dimethyl acridinium ester-labeled antibodies as detection markers. Specifically, the Zenit RA CLIA consists of six kits, each containing a specific solid-phase antigen.

The **Zenit RA CLIA was recently compared with immunoprecipitation in terms of sensitivity and specificity by Gelpì et al.**,[1] from the Hospital de la Santa Creu i Sant Pau in Barcelona, Spain.

The comparison was performed on 114 serum samples obtained from 98 patients with autoimmune connective tissue diseases and 16 blood donor volunteers.

The samples were tested through gold standard techniques for their ability to precipitate subsets of small RNA and proteins (U1snRNP, Sm, Ro/SS-A, La/SS-B, Jo-1, and Scl-70) from extracts of cultured cell, based on the presence of autoantibodies. In parallel, samples were tested through the Zenit RA CLIA for the presence of antibodies against the following antigens: native Sm, recombinant 70, A and C U1 RNP antigenic proteins, recombinant Ro60 and Ro52 proteins of the Ro/SS-A antigen, recombinant 48 kDa La/SSB antigenic protein, recombinant 58 kDa Jo-1 tRNA antigenic protein, and recombinant 72 kDa protein of the Scl-70 antigen.

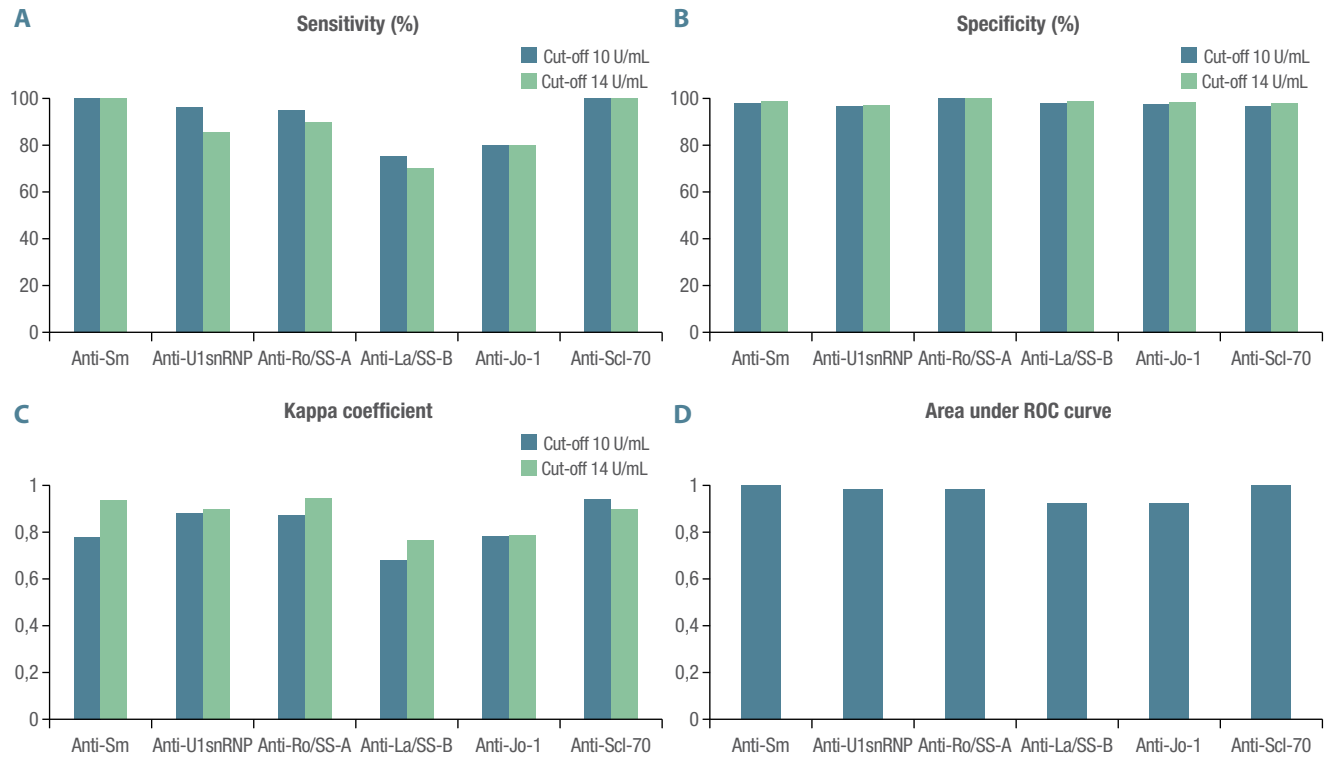
Overall, at the antibody concentration cut-off recommended by the manufacturer, i.e. 10 arbitrary units/mL (10 U/mL), the Zenit RA CLIA had a good agreement with immunoprecipitation to determine the presence of the above antibodies. The values of the Kappa coefficient, a measure of agreement, ranged from to 0.689 for anti-La/SS-B to 0.948 for anti-Scl-70 (Figure 1). Raising the cut-off to 14 U/mL led to even higher Kappa coefficients, ranging from 0.775 for anti-La/SS-B to 0.947 for anti-Ro/SS-A (Figure 1).

The area under the receiver operating characteristic (ROC) curve of each parameter indicated a discrimination capacity ranging from 0.929 for anti-La/SS-B to 1 for anti-Scl-70 (Figure 1) (discrimination is deemed acceptable when the area under the curve is > 0.7).

Intra-laboratory reproducibility and precision using the Zenit RA CLIA kits were good for all the assays. The inter-assay and intra-assay coefficients of variation were lower than 3.4% and lower than 1.2%, respectively.

The results of this study show that the **Menarini Zenit RA CLIA method for the detection of the six above autoantibodies is specific and sensitive, and provides an easy, precise and useful test for screening purposes.** However, the Authors suggest the cut-off value recommended by the manufacturer should be increased to obtain an even higher agreement with gold standard techniques. Also, they recommend using IIF as a first step algorithm that will provide a second assay to confirm diagnosis.

Figure 1: Profile of the Zenit RA CLIA: sensitivity, specificity, agreement with the gold standard assays and area under the ROC curve



Sensitivity (panel **A**) and specificity (panel **B**) of Zenit RA CLIA in detecting anti-Sm, anti-U1snRNP, anti-Ro/SS-A, anti-La/SS-B, anti-Jo-1 and anti-Scl-70 autoantibodies were determined through ROC analysis at the cut-off values of 10 U/mL (recommended by the manufacturer) and 14 U/mL. The Kappa coefficient (panel **C**) is a measure of the agreement between Zenit RA CLIA and the gold standard assays in detecting the above antibodies at the two different cut-offs. The area under the receiver operating characteristic (ROC) curve (panel **D**) is a measure of the discrimination capacity of the above antibodies.

1. *Gelpí C, Pérez E, Roldan C. Efficiency of a solid-phase chemiluminescence immunoassay for detection of antinuclear and cytoplasmic autoantibodies compared with gold standard immunoprecipitation. Auto Immun Highlights. 2014; Jul 18;5(2):47-54.*

COMPANY PINBOARD

Latest Marketing & Scientific Events

The "Reunión de Autoinmunidad" took place in Madrid, Spain, on November 5th 2015

The following is the programme of the meeting, which featured an intervention by A. Menarini Diagnostics on quality control in autoimmunity labs.

- **CE-IVD SMART ELISA kits from Sanquin Reagents for biologicals. Comparison with the golden standard and use for treatment optimization**
  H. T. Velthuis
  Sanquin, Amsterdam – Netherlands
- **Clinical implications of immunogenicity of TNF inhibitors**
  T. Van Rispens
  Sanquin, Amsterdam – Netherlands
- **Monitoring biological drugs in clinical practice**
  D. P. Salcedo
  Hospital La Paz, Madrid – Spain
- **Evaluation of new assays for the determination of antinuclear antibodies. Automatization and integration in the diagnostic process**
  M. C. Gelpì
  Hospital de Sant Pau, Barcelona – Spain
- **ISO 15189 accreditation of immunology-autoimmunity laboratories.**
  I. de la Villa
  Departamento de Sanidad ENAC
- **New QC perspectives using automated IIF analysis**
  C. Bonroy
  Department of Laboratory Medicine, Ghent University Hospital, Ghent – Belgium
- **Tools for the implementation of an internal quality control system in autoimmunity labs**
  P. Falcó
  A. Menarini Diagnostics

The 10th International Congress on Autoimmunity to be held next spring (Leipzig, Germany, April 2016)

Established by Professor Yehuda Shoenfeld, the congress brings together international leaders on immunology, rheumatology and related fields, with their diverse contributions to the "family of autoimmunity."
This congress provides the opportunity to gain multifaceted research-based and clinical insights into more than 80 autoimmune diseases.
The newest therapeutic techniques and diagnostic tools as well as the most up-to-date research on the genetic, aetiological, diagnostic and clinical aspects and the novel therapies for autoimmune diseases will be discussed.

MAI
The Mosaic of Autoimmunity Award

The 10th International Congress on Autoimmunity will also feature the **Mosaic Autoimmunity Award Ceremony**, whereby a prize established to encourage progress in autoimmunity research will be awarded to a young scientist who has enriched this field through outstanding, creative and independent studies.

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autoimmunity

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- **Full traceability** (barcode reading for all reagents and samples; continuous control of reagent levels; quality control based on Levey-Jenning & Westgard charts)

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