Year 3, No. 1, March 2016

Auto-immunity

CLOSE UP

Special issue on the occasion of the
10th International Congress on Autoimmunity
Leipzig, Germany, April 6-10, 2016

A.MENARINI diagnostics

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Year 3, No. 1, March 2016
ISBN 978 88 6756 269 5

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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.

Publisher
Springer Healthcare Italy
Via PC Decembrio, 28
20137 Milan (Italy)
www.springerhealthcare.it

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Printed in March by Lazzati Industria Grafica Srl (Casorate Sempione - Varese - Italy)

Publication not for resale. Intended for specialists only. Registered in Milan - Registration n. 313 - 1/10/2014

MENCIM1791

In this issue:

EDITOR'S NOTE

A tale about Autoimmunity

Great things are done by a series of small things brought together, said Vincent Van Gogh. This tale is about an adventure that started out in the late 90s. **A. Menarini Diagnostics** decided to take up the challenge of becoming a pivotal player on the IVD scene, trusting in the intrinsic values of the autoimmunity niche, such as its potential and the importance and prevalence of autoimmune diseases and their impact on daily life.

With hindsight, a promising niche that has led to a strong presence throughout Europe and a clear identity as "innovation promoters" according to a patient-centered viewpoint.

This year we plan to celebrate our presence in this niche with different and impactful initiatives.

To begin with, **A. Menarini Diagnostics** is attending, as a platinum sponsor, this 10th *International Congress on Autoimmunity*, for which we decided to put together this special issue of Autoimmunity Close up. Our strong presence at this very exciting event for our community will give us the opportunity to make ourselves known from both the scientific and marketing perspectives, since participants visiting our booth will learn about our innovative systems and solutions and have the chance to attend our scientific symposia featuring international key opinion leaders.

With regard to AICU, we start highlighting one of the current issues facing modern laboratory medicine, the significance of anti-DFS70 (dense fine speckled) antibodies and possible screening improvements, by presenting two contributions on the topic. The section "Autoimmunity Lab" is dedicated to **Zenit Uniqo**, the state-of-the-art platform for complete automation of immunofluorescence about to be launched by **A. Menarini Diagnostics**. The introduction of **Zenit Uniqo** represents a true challenge for us, a challenge to possibly improve an already advanced automation system and to increase the standardization of this methodology.

2016 is also the year in which **A. Menarini Diagnostics** will release **Zenit Lite**, a small, flexible, and powerful system to run ELISAs and IFAs for small or small-to-medium routines. **Zenit Lite** is the latest of a long series of analytical platforms introduced by Menarini since the early 2000s to improve standardization - and the closely related concept of "harmonization" - in the testing process. At that time, Plato Autoimmunity was probably the first liquid handling system designed to perform immunofluorescence in complete automation.

Finally, we are planning to schedule a single-sponsor scientific symposium on Autoimmunity for the end of the year. **A. Menarini Diagnostics** is accustomed to organizing this type of event and the next one will be on the 11th. The symposium will take place in Athens and is expected to attract the participation of more than 250 attendees including lab physicians, technologists, and experts from about 10 different European countries.

An exciting year ahead of us, where we dare to achieve great things through a series of small things, as the famous quote of the Dutch painter says.

Massimo Donnini
International Product Manager Autoimmunity
A. Menarini Diagnostics

TECHNICAL INSIGHTS

Optimizing the significance of anti-DFS70 antibodies in autoimmune diagnostics

The presence in human serum of autoantibodies directed against intracellular antigens, specifically antinuclear antibodies (ANA), is a hallmark of systemic autoimmune rheumatic disease (SARD) and of several other conditions.[1]

Concerning SARD, the American College of Rheumatology recommended indirect immunofluorescence (IIF) as a routine test for ANA detection.[2] A positive result on IIF ANA assay affects the triage of patients with possible SARD as well as diagnosis; for instance, a positive ANA test is one of the criteria used in the diagnosis of systemic lupus erythematosus (SLE),[1] since 98% of patients with SLE have a positive ANA test. Furthermore, the pattern of staining may be useful in making a diagnosis of a connective tissue disease.[1]

Since laboratory tests are often not based on a comprehensive clinical work-up, misinterpretation of test results may lead to inappropriate therapeutic choices, have a negative psychological impact on patients, families and physicians[2], and economically burden healthcare systems. In fact, some authors have implemented algorithms aiming to enhance the effectiveness and the efficiency of antibody testing.[3,4]

Among the abovementioned staining patterns (usually reported as nuclear, centromere, or nucleolar), the nuclear dense fine speckled (DFS) one is commonly observed in patients without an evident diagnosis of ANA-associated SARD, and who have been referred for ANA testing based on non-specific complaints and symptoms.[5]

The roles of DFS70 and anti-DFS70 antibodies

The DFS pattern has been recognized as mostly due to autoreactivity against a protein initially named DFS70 and later identified as the 75-kDalton *lens epithelium-derived growth factor* (LEDGF75) (hereafter DFS70). The anti-DFS70 IIF pattern typically consists of a dense, fine speckled fluorescence uniformly staining the interphase nucleus and chromatin in mitotic cells.[6]

The biological and clinical significance of DFS70, a protein which is ubiquitous in mammalian cells, are still actively investigated. Available data indicate that DFS70 is mainly involved in cell protection against stressful conditions. However, variants from alternative gene splicing seem to play opposite roles: while ectopic overexpression of DFS70 is associated with cell survival, that of the shorter (52 kDa) splice variant has a pro-apoptotic effect.[7]

One possible interpretation of the somewhat contrasting data is that in genetically susceptible individuals, changes in the immunogenicity of DFS70 following its overexpression in stressful conditions, or its apoptotic cleavage in severely stressful conditions, may elicit the production of anti-DFS70 autoantibodies. These could play pathogenic, protective, or sensing roles (Figure 1).[7]

Anti-DFS70 antibodies in health and disease

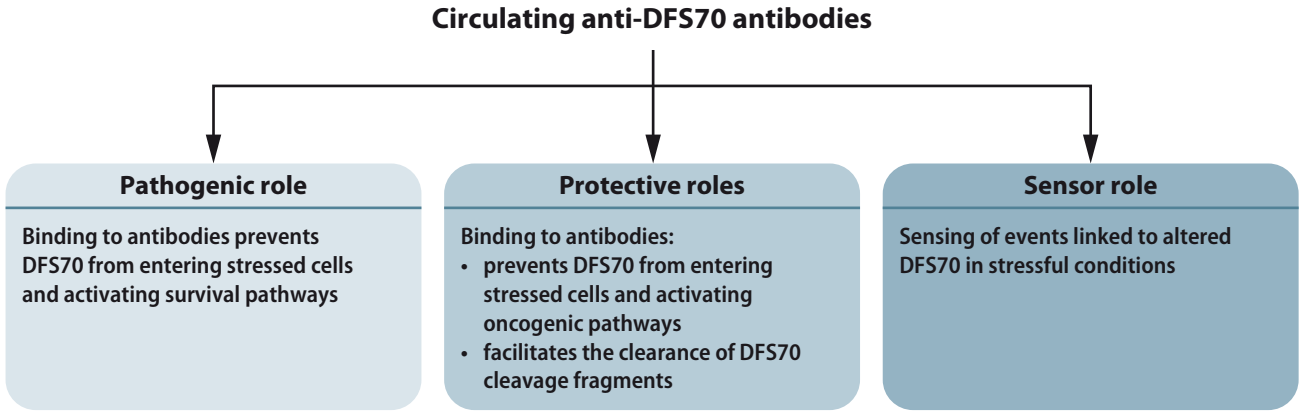
High titers of anti-DFS70 IgG have been reported in sera from apparently healthy individuals and in several different non-SARD conditions (interstitial cystitis, atopic dermatitis, alopecia areata, cataracts and other eye diseases, chronic fatigue syndrome in children, and prostate cancer). Conversely, the prevalence of anti-DFS70 antibodies is low in patients with SARD, and anti-DFS70 reactivity in SARD is usually associated with the presence of other autoantibodies identified as markers of SARD.[5] Also, anti-DFS70 positive individuals did not develop SARD during a follow-up of 4 years from the initial IIF positive test.[2]

In the study by Mahler et al., 53 of 3263 (1.62%) unselected serum samples showing a DFS staining pattern upon routine IIF ANA testing on HEp-2 (human epithelial cell 2) cell substrates were identified as anti-DFS70 positive by enzyme-linked immunosorbent assay (ELISA) and chemiluminescence assay (CIA).[2] The point prevalence of anti-DFS70 antibodies as assessed by CIA was significantly higher in healthy individuals (8.9%) than in patients with SARD, namely SLE (2.8%; $p < 0.001$) (Table 1).[2]

The presence of anti-DFS70 was not significantly associated with clinical features or other autoantibodies typical of SLE. Seven of 251 sera (2.8%) from patients with SLE were positive for anti-DFS70 antibodies on CIA, but only one had isolated anti-DFS70 reactivity. The study therefore confirmed the reported prevalence of anti-DFS70 antibodies in healthy individuals and patients with SLE.[2]

In the study by Miyara et al., 91 of 100 sera with a DFS pattern on IIF were positive for anti-DFS70 on CIA compared to 3 of 100 sera with other IIF patterns ($p < 0.0001$); titers of anti-DFS70 antibodies as assessed

Figure 1: The role played by anti-DFS70 autoantibodies (pathogenic, protective, or sensor) may depend on the condition in which they are produced (modified from ref. 7)



by CIA and IIF were highly correlated ($p < 0.0001$, rho = 0.89, 95% CI: 0.84–0.92). Based on the area under the ROC curve (receiver operating characteristics curve) (0.981; 95% CI: 0.960-1.000), anti-DFS70 antibodies allowed discrimination between samples with the DFS pattern and other IIF ANA patterns.[8]

Only 5 of 91 patients whose sera were positive for anti-DFS70 antibodies and negative for other ANA had SARD.[8] Based on ROC analyses, the presence of anti-DFS70 antibodies allowed discrimination between SARD and non-SARD patients (the latter having higher titers), with an area under the curve of 0.73 (95% CI: 0.66–0.80; $p < 0.0001$).[8]

The authors of the two above studies concluded that because compared with other ANA patterns, the DFS pattern (anti-DFS70) may represent a biomarker for differentiating SARD from non-SARD individuals,[2,8] testing for anti-DFS70 should be included in diagnostic algorithms[8] and "there is a need for a reliable assay to ensure reactivity to DFS70".[2]

The HEp-2 DFS70-KO substrate in ANA detection

The ANA HEp-2 DFS70-KO substrate is composed of approximately 90% DFS70-knock-out HEp-2 cells and 10% wild-type HEp-2 cells. The former have been genetically engineered to lack the gene (*psip1*) codifying for the LEDGF, responsible for the DFS70 pattern, whilst the latter express a high level of the DFS70 antigen.

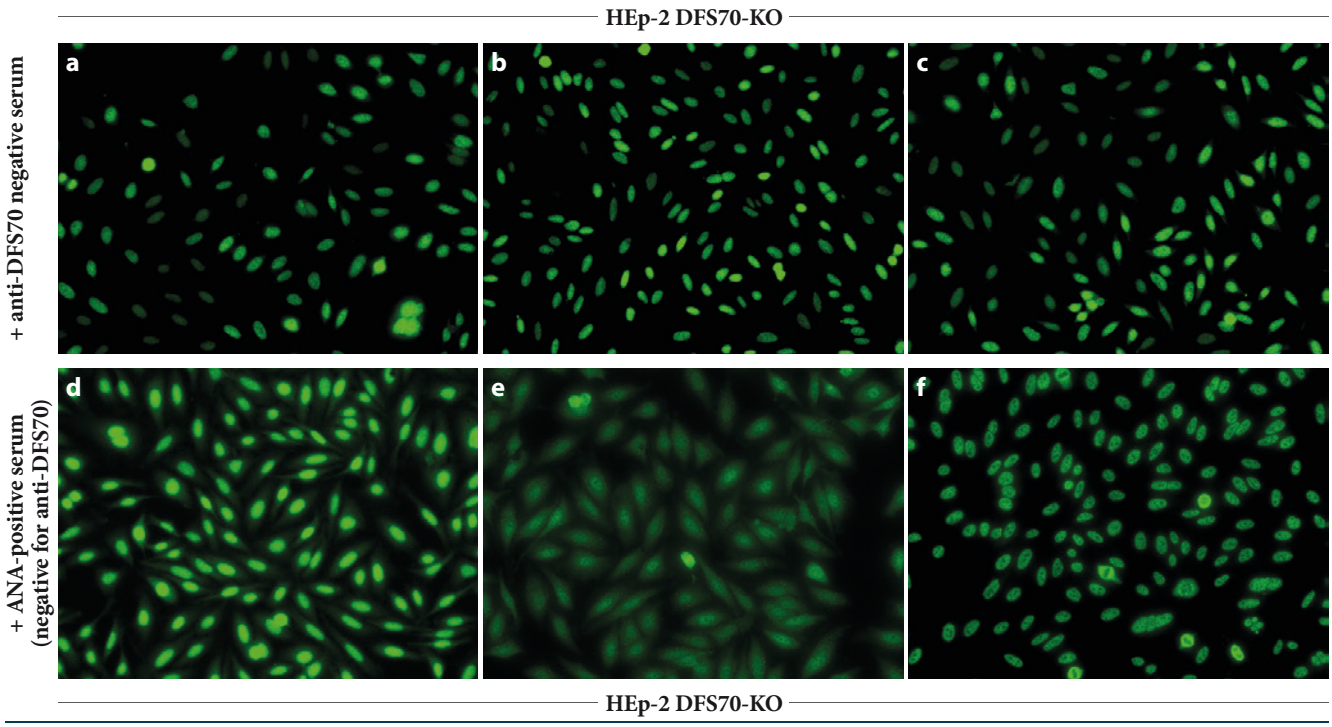
When this mixed substrate is incubated in the presence of an anti-DFS70 positive serum, the less intense reactivity of DFS70-KO cells compared to that of wild-type cells can unmask any underlying ANA patterns possibly masked by a high-titer DFS70 reaction. This substrate therefore aids in the identification of the DFS70 pattern and its distinction from other, possibly concomitant, similar patterns (e.g., fine speckled or homogeneous).

The novel substrate HEp-2 DFS70-KO, which will be launched soon by A. Menarini Diagnostics, will allow a single step identification of anti-DFS70 as well as other ANA reactivities (Figure 2), thus representing a time- and cost-saving option for autoimmune diagnostics.

Table 1: Point prevalence of anti-DFS70 antibodies in different cohorts determined by chemiluminescence immunoassay (modified from ref. 2)

Cohort	Point prevalence (%)
Healthy individuals (n = 124)	8.9
Hashimoto's thyroiditis (n = 67)	6.0
Interstitial cystitis (n = 40)	5.0
Asthma (n = 25)	4.0
Systemic lupus erythematosus (n = 251)	2.8
Rheumatoid arthritis (n = 39)	2.6
Graves' disease (n = 60)	1.7
Systemic sclerosis (n = 29)	0.0
Atopic dermatitis (n = 16)	0.0
Inflammatory bowel disease (n = 34)	0.0
Other diseases (n = 53)	0.0
Sjögren's syndrome (n = 7)	0.0
Infections (n = 20)	0.0
Cancer (n = 40)	0.0
Multiple sclerosis (n = 10)	0.0

Figure 2: IIF staining of HEp-2 KO substrate incubated in the presence of serum samples positive for anti-DFS70 (a-c) and negative for anti-DFS70 but positive for other ANAs (d-f) (Courtesy of Dr. Malyavantham)



Take home message

- High titers of autoantibodies against DFS70 have been reported in sera from apparently healthy individuals and in several different non-SARD conditions
- The prevalence of anti-DFS70 antibodies is low in patients with SARD, and anti-DFS70 reactivity in SARD is usually associated with the presence of other autoantibodies identified as markers of SARD
- Anti-DFS70 monoreactivity can be used as a negative marker for the diagnosis of SARD
- The novel HEp-2 KO substrate is a convenient option for a one-step detection of both anti-DFS70 antibodies and other antinuclear antibodies

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RESEARCH UPDATES

Improved HEp-2 substrate and impact on ANA (antinuclear antibody) screening and interpretation of anti-DFS70 (dense fine speckled) antibodies

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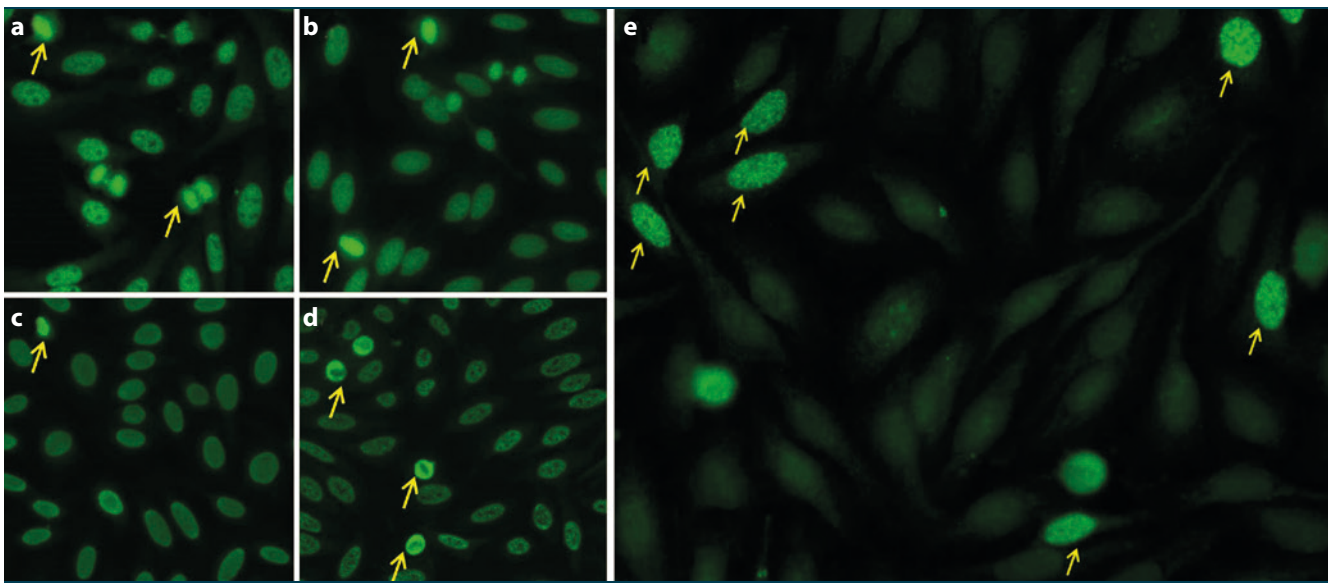
Background

The recommended screening method for detection of antinuclear antibodies (ANA) is indirect immunofluorescence (IIF) employing HEp-2 substrate.[1,2] Among the patterns seen on HEp-2 IIF, antibodies producing a dense fine speckled (DFS70) pattern (Figure 1a and 1b) have been the topic of attention during the past decade. Major reasons for this interest are: a) presence of anti-DFS70 antibodies in up to 4%[3-6] of the ANA screening population; b) unclear clinical associa-

Figure 1: (a and b) show examples of DFS70 pattern on conventional HEp-2 IIF. (c and d) depict homogeneous and speckled patterns on conventional HEp-2 IIF. (e) depicts the DFS70 pattern on novel engineered HEp-2 DFS70-KO IIF, which is a mixture of conventional and engineered cells in 1:9 ratio. Arrows indicate cells undergoing mitosis (a-d) and conventional HEp-2 cells stained positively for DFS70 (e)



tion of DFS70 antibodies; c) high prevalence of DFS70 antibodies in apparently healthy population without any systemic autoimmune diseases; d) challenges associated with discerning DFS70 pattern from classic homogenous and/or fine speckled patterns (Figure 1c and 1d); e) lack of simple and sensitive confirmatory assays; f) challenges associated with discriminating DFS70 in case of mixed patterns, and g) negative impact accompanying DFS70 false positive or false negative reporting.[7-12]

The recent recommendation from the 12th International workshop on autoantibodies and autoimmunity concluded that DFS70 pattern should be reported while screening for ANA employing HEp-2.[13] Nevertheless, DFS70 pattern could be difficult to interpret when observed on conventional HEp-2 substrates (Figure 1a-d).[9] Alternative confirmatory assays in the form of ELISA/EIA, LIA (line immunoassay), CLIA (chemiluminescence immunoassay) and immunoadsorption-IIF are available. However, recent studies report insufficient sensitivity for anti-DFS70 solid phase assays.[8] Hence there is a need for simpler and sensitive assays for detection and confirmation of DFS70 antibodies.

We developed a novel HEp-2 substrate (HEp-2 DFS70-KO) composed of a mixture of conventional HEp-2 cells and engineered HEp-2 cells for a simple and sensitive screening of DFS70 antibodies. The novel HEp-2 substrate is composed of cells disrupted for *psip1/LEDGF* gene. Approximately, 90% of the HEp-2 on each well of this type of substrate are cells that do not produce any DFS70/LEDGF antigen responsible for DFS70 pattern (Figure 1e). The other 10% of the HEp-2 cells are conventional unmodified cells which naturally express high levels of this antigen (Figure 1e).

In this study, we evaluated a novel engineered HEp-2 substrate in comparison to the conventional HEp-2 for sensitivity, specificity and ease of use for detection of ANA patterns, including DFS70 pattern. We also studied the impact on lab time, resources, and quality of results for DFS70 reporting, comparing algorithms employing conventional HEp-2 and a novel engineered HEp-2 substrates (Figure 2).

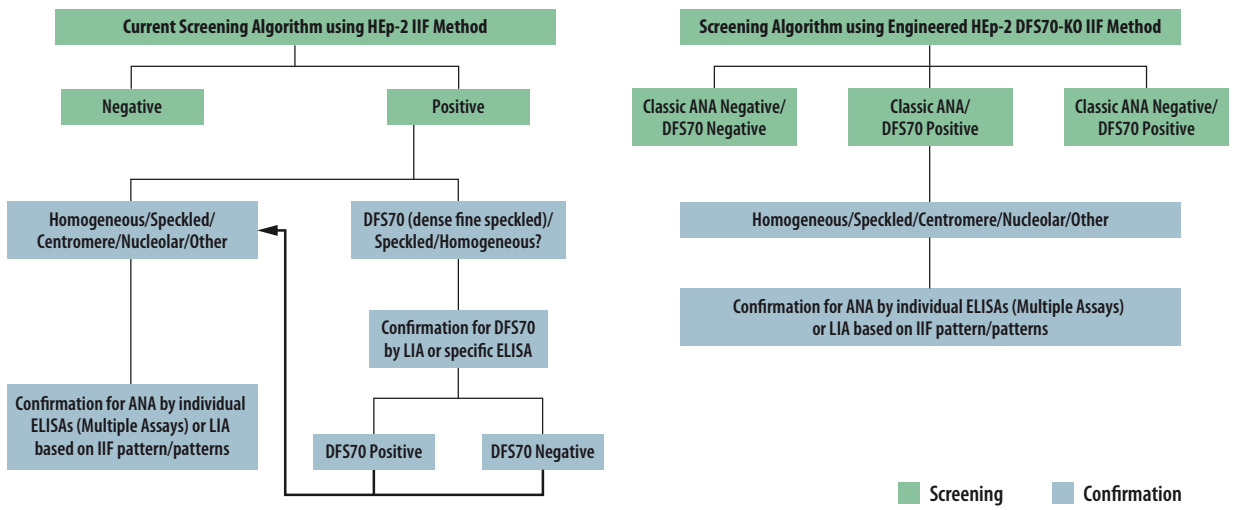
Methods

Approximately 1200 samples received for routine screening of ANAs during a period of 2 months were tested using ImmuGlo™ ANA HEp-2 Substrates (Immco Diagnostics, Buffalo, NY, USA). DFS70 suspect samples were confirmed using LIA (ImmcoStripe™ ANA) and an in-house ELISA. HEp-2 DFS70-KO IIF was employed as part of a new ANA screening algorithm.

Results

Of the 1200 samples received for ANA screening in our reference lab, 18 (1.5%) were suspected to be DFS70 positive (Table 1). Along with these 18 samples, six classic ANA controls and 2 negative sera were included in the DFS70 specific studies. Out of the 18 suspected samples, 50%[9] were identified as DFS70 with high confidence by HEp-2 IIF. Other 9 sera were suspected

Figure 2: Schematic of ANA screening and confirmation algorithm/workflow using conventional HEp-2 IIF and improved HEp-2 DFS70-KO IIF. Improved IIF substrates significantly reduce the number of confirmatory assays and enhance the overall accuracy of ANA reporting



Improved Algorithm for ANA Screening: Using Novel Engineered HEp-2 IIF Substrate

Table 1: Screening and confirmation results for DFS70 suspect samples

Sample No	Sample Type	Pattern on HEp-2 IIF	DFS70 ELISA Results	DFS70 LIA Results	HEp-2 DFS70-KO IIF ANA screening and confirmation of DFS70 pattern	Pattern on HEp-2 DFS70-KO	HEp-2 DFS70-KO Results for DFS70	HEp-2 DFS70-KO Results for classic ANA patterns
1	DFS70 Suspect	DFS/Homo?	Positive	Positive		DFS70	Positive	Negative
2	DFS70 Suspect	DFS/Homo?	Positive	Positive		DFS70	Positive	Negative
3	DFS70 Suspect	DFS70	Positive	Positive		DFS70	Positive	Negative
4	DFS70 Suspect	DFS70	Positive	Positive		DFS70	Positive	Negative
5	DFS70 Suspect	DFS70	Positive	Positive		DFS70	Positive	Negative
6	DFS70 Suspect	DFS70	Positive	Positive		DFS70	Positive	Negative
7	DFS70 Suspect	DFS70	Positive	Positive		DFS70	Positive	Negative
8	DFS70 Suspect	DFS70	Negative	Negative		DFS70	Positive	Negative
9	DFS70 Suspect	DFS70	Positive	Positive		DFS70	Positive	Negative
10	DFS70 Suspect	DFS70	Positive	Positive		DFS70	Positive	Negative
11	DFS70 Suspect	DFS70	Positive	Positive		DFS70, Nucleolar	Positive	Positive
12	DFS70 Suspect	DFS/Homo?	Negative	Negative		Homo	Negative	Positive
13	DFS70 Suspect	DFS70/Homo/Speckled?	Positive	Positive		Speckled	Negative	Positive
14	DFS70 Suspect	DFS70/Homo/Speckled?	Positive	Negative		DFS70	Positive	Negative
15	DFS70 Suspect	DFS70/Homo/Speckled?	Positive	Positive		DFS70	Positive	Negative
16	DFS70 Suspect	DFS70/Homo/Speckled?	Positive	Negative		DFS70	Positive	Negative
17	DFS70 Suspect	DFS70/Homo/Speckled?	Positive	Positive		DFS70	Positive	Negative
18	DFS70 Suspect	DFS70/Homo/Speckled?	Negative	Negative		Fine Speckled	Negative	Positive
19	Control	Fine Speckled	Negative	Negative		Fine Speckled	Negative	Positive
20	Control	Fine Speckled	Negative	Negative		Fine Speckled	Negative	Positive
21	Control	Homo	Negative	Negative		Homo	Negative	Positive
22	Control	Homo	Negative	Negative		Homo	Negative	Positive
23	Control	Negative	Negative	Negative		Negative	Negative	Negative
24	Control	Negative	Negative	Negative		Negative	Negative	Negative
25	Control	Speckled	Negative	Negative		Speckled	Negative	Positive
26	Control	Speckled	Negative	Negative		Speckled	Negative	Positive

Screening results for DFS70 suspect samples using conventional HEp-2 IIF followed by confirmation by DFS70-specific ELISA and LIA. Screening and confirmation using novel HEp-2 DFS70-KO IIF which also revealed classic ANAs in 4 samples (#11, #12, #13 and #18) with or without DFS70. Homogeneous ANA pattern is abbreviated as "Homo". Screening results with a "?" at the end indicate suspect pattern/patterns with lower level of confidence

to be positive for DFS70 or homogeneous or fine speckled patterns. ELISA and LIA assays confirmed 15 of 18 (83.3%) and 13 of 18 (72.2%) respectively to be positive for DFS70. All the controls were negative for DFS70 as expected.

HEp-2 DFS70-KO IIF (Figure 1e) can be used to screen and confirm DFS70 mono-positive cells with high confidence levels. HEp-2 DFS70-KO IIF produced a sensitivity of 83.3% for DFS70 in one step screening (ANAs) and confirmation (for DFS70) (Table 1). Serum #8 was negative on ELISA and LIA but detected as DFS70 positive on HEp-2 DFS70-KO IIF. Serum #11 suspected as DFS70 positive only by conventional HEp-2, revealed an underlying nucleolar ANA pattern using HEp-2 DFS70-KO IIF. Serum #13 was DFS70 positive on ELISA and LIA but read as speckled on HEp-2 DFS70-KO IIF indicating the co-existence classic speckled pattern along with DFS70.

Discussion

Based on the results, the screening and confirmation algorithm employing HEp-2 DFS70-KO IIF not only aids diagnostic labs in DFS70 reporting but saves considerable time and resources associated with running confirmatory assays for suspected patterns (Figure 2). Improved substrates eliminate the need to run DFS70-specific confirmatory assays. Results demonstrate the unmasking effect of HEp-2 DFS70-KO IIF in

2 out of 18 DFS70 suspect samples, where classic (speckled and nucleolar) ANA patterns previously concealed by strong DFS70 reaction were revealed.

Therefore, the new approach not only improves the sensitivity/specificity for DFS70 screening in one step, but it also improves the sensitivity for co-existing classic ANA patterns.

Additional benefits associated with this methodology include minimal end-user training due to the adaptation of standard IIF procedure, increased confidence levels in discerning the challenging DFS70 pattern from homogeneous or speckled, cost and time savings on the required confirmatory assays and improved overall accuracy and lab workflow for ANA reporting.

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AUTOIMMUNITY LAB

An all-in-one workstation for IIF automated procedure

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VISIA imaging S.r.l., San Giovanni Valdarno (AR), Italy

The indirect immunofluorescence (IIF) technique on HEp-2 cells is still the recommended method to screen for antinuclear antibodies (ANA). Indeed, well-established autoantibody testing algorithms suggest performing highly sensitive screening by immunofluorescence on HEp-2 followed by second-level antigen-specific immunoassays only on positive IIF ANA samples. Due to its **central role in the diagnostic algorithm**, a high accuracy and reproducibility of the IIF procedure are crucial. However, the IIF method presents some unfavorable features such as a low degree of standardization, the need for skilled operators and subjectivity of interpretation.

In order to improve the performance of the IIF method, technological solutions have been developed and the partial or full automation of two main steps of IIF procedure (**slide processing and slide reading**) has been implemented. Currently, there are several devices available in the market that are able to automatize the slide processing or slide reading steps separately and only one device combines both stages. Slide processors consist of the assembly of a robotic arm to a liquid handling system whereas slide readers are mainly based on the assembly of an automated microscope with a motorized slide tray, high-sensitivity CCD camera and specific software for the acquisition and analysis of digital images. Nevertheless, har-

Zenit Uniqo automated system

monization of the results obtained by the combined use of the available (semi or fully) automated systems is still not accomplished. The high variability of results observed by visual or automatic reading of similar slides is mainly due to the processing step. Several factors can determine such heterogeneous readouts: reagent preparation, washing conditions, temperature and humidity conditions during incubations and, importantly, coverslip mounting over the processed slide, where air bubbles or damage to the substrate should be avoided.

In order to improve standardization of the whole IIF procedure, a new solution has been developed. **Zenit Uniqo** is a brand-new system that provides the laboratory with an all-in-one device that completes automated IIF slide preparation with **coverslipping**, whole-well slide scanning, image analysis, image archiving and data sharing through connection to LIS or laboratory middleware. **Zenit Uniqo** is a new high-performance automated system that aims to standardize each critical step of the biological protocol, eliminating the inconsistency of human intervention and maximizing the accuracy of the overall IIF process, from accurate sample dispensation, controlled incubation conditions, efficient washing and **coverslipping**, to a fast and reliable **whole-well digitization** and image analysis. Innovative software manages and controls the entire experimental workflow for full traceability and an easy and efficient data management. Moreover, the integrated **virtual microscope** tool allows full navigation and remote viewing of the digitized slide, eliminating the problem of slide storage, signal preservation over time and uncoupling the laboratory from the validation workstations.

Full automation of the overall IIF procedure provided by an all-in-one device provides the laboratory with a cost effective, reliable, versatile, secure and streamlined solution to screen for diagnostically relevant autoantibodies, reducing intra- and inter-laboratory variability, improving standardization of the procedure and giving a valuable support to speed up the routine laboratory workflow.

COMPANY PINBOARD

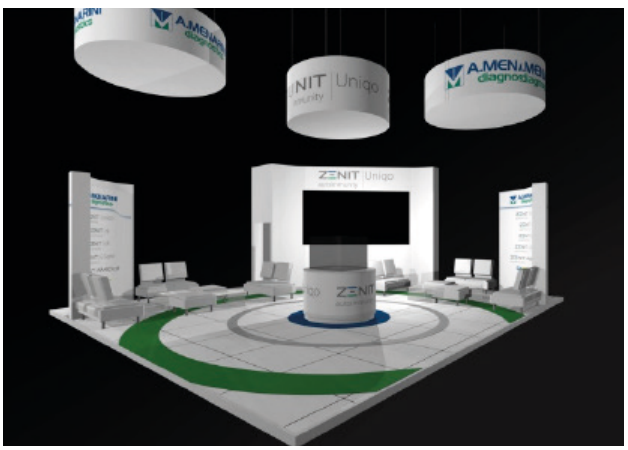
Latest Marketing & Scientific Events

The 10th International Congress on Autoimmunity, the biggest multidisciplinary meeting in the field, is ongoing (6 to 10 April 2016) in Leipzig, Germany

Attendees at the meeting are brought up to date on a large number of autoimmune diseases and gain insights from different research-based and clinical perspectives. The meeting is an opportunity to discuss the newest therapeutic techniques and diagnostic tools as well as the latest research on autoimmune diseases. Furthermore, it establishes a network of international leaders on immunology, rheumatology and related fields, each contributing their unique knowledge to the "family of autoimmunity".

Participants are welcome to take advantage of the following contributions provided by **A. Menarini Diagnostics**, a *Platinum Sponsor* of the meeting:

- A. Menarini Diagnostics' **72-m² stand**, displaying the latest technical achievements in immunodiagnostics. Visitors have the opportunity to discuss specific issues raised by their research and other activities in the field.
- An **oral presentation**, "ANA DIAGNOSTICS AND IMMUNOFLUORESCENCE", by Dr. K. Malyavantham, Director of Research & Development at IMMCO Diagnostics
- An **oral communication** "IMPROVED ANTINUCLEAR ANTIBODY (ANA) SCREENING USING A NOVEL HEp-2 SUBSTRATE", by K. Malyavantham, V. Ramsperger, E. Berleth, R. Paramanathan, M. Herold, W. Klotz, L. Suresh (USA) (abstract AUT16-0415), included in the **NOVEL AUTOANTIGENS: DFS70, 14-3-3η** session (see details below)



- A **poster,** "EVALUATION OF A NEW TEST FOR THE DETECTION OF AMA-M2 ANTIBODIES", by K. Malyavantham **(abstract** AUT16-0449**)**
- Two parallel **scientific sessions**, supported by unconditional grants:

NOVEL AUTOANTIGENS: DFS70, 14-3-3η

Chairpersons: Nicola Bizzaro (Italy), Carlos Casiano (USA), Stanley Naides (USA)

The session aims at increasing knowledge about the pathophysiological role of the DFS70/LEDGFp75 protein/antigen and related autoantibodies, and discussing the use of DFS70/LEDGFp75 immunoassays as a diagnostic tool. A new diagnostic marker for rheumatoid arthritis will also be illustrated.

The session programme is as follows:

- **The DFS70/LEDGFp75 antigen**
  Invited speaker: C. Casiano (USA)

- **Diagnostic accuracy of new immunoblot methods for the detection of anti-dense fine speckles (DFS70) antibodies**
  N. Bizzaro, E. Tonutti, F. Cucchiaro, F. Pesente, M. Fabris, M. Infantino, M. Tampoia, D. Villalta (Italy)

- **Improved antinuclear antibody (ANA) screening using a novel HEp-2 substrate**
  K. Malyavantham, V. Ramsperger, E. Berleth, R. Paramanathan, M. Herold, W. Klotz, L. Suresh (USA)

- **Comparison of fully automated IIF system and chemiluminescent immunoassay for the detection of anti-DFS70 antibodies in patients with and without systemic autoimmune rheumatic diseases**
  O. Shovman, B. Gilburd, C. Chayat, H. Amital, A. Watad, A. Guy, C. Bentow, M. Mahler, Y. Shoenfeld (Israel)

- **Clinical relevance of anti-DFS70 autoantibodies**
  K. Conrad, N. Röber, U. Höpner, M. Aringer, S. Rudolph, A. Gräßler, K. Lüthke, L. Unger, M. Mahler (Germany)

- **The use of anti-DFS70 antibodies in the study of patients with SARD suspicion is cost-effective**
  S. Gundín, J. Irure-ventura, E. Asensio, D. Ramos, M. Mahler, V. Martínez-Taboada, M. López-Hoyos (Spain)

- **Anti-DFS antibodies in a population**
  Invited speaker: M. Infantino (Italy)

- **14-3-3η, a new diagnostic marker, state-of-the-art**
  S. Naide (USA)

AUTOIMMUNE LIVER DISEASES

Chairpersons: M. Eric Gershwin (USA); Pietro Invernizzi (Italy); Luigi Muratori (Italy)

The session addresses both the pathophysiological and diagnostic aspects of autoimmune hepatopathies.

The session programme is as follows:

- **Novel treatments in primary biliary cholangitis**
  Invited speaker: P. Invernizzi (Italy)

- **Diagnostic and prognostic significance of autoantibodies in autoimmune hepatitis**
  L. Muratori (Italy)

- **Autoimmune liver diseases: an update in immunopathological aspects**
  Invited Speaker: D. Bogdanos (Greece)

- **Dysregulation of interferon interplay leads to female predominant autoimmune cholangitis: implications for the etiology of human AC**
  H. Young, J. Valencia, S. Kim, T. Back, M. Karwan, D. Feng, B. Gao, O. Park, K. Tsuneyama, P. Leung, M.E. Gershwin, H. Bae (USA)

- **Overlap syndromes and autoimmune liver disease: a five year revised experience of an autoimmunity diagnosis laboratory**
  M. Albuquerque, M.J. Sousa, R. Ribeiro, M.F. Menezes, J.G. Sousa, G. Sousa (Portugal)

- **Combined determination of autoimmune liver disease specific autoantibodies by Cytobead® assay**
  M. Sowa, J. Scholz, K. Grossmann, R. Hiemann, N. Röber, B. Glauche, K. Conrad, D. Roggenbuck (Germany)

- **Diagnostic accuracy of two tests for determination of anti-M2 in the diagnosis of primary biliary cirrhosis. Is it possible to predict the course of the disease?**
  A.M. Alfano, M. Battistini, G. Crotti, A. Ferrini, C. Mancinetti, T. Manetta, C. Marchese, P. Merlach, A. Romito, M.T. Tambuzzo (Italy)

- **Fructosylation of human serum albumin and its possible role in liver diseases**
  A. Zaman, K. Alam (India)

A two-day symposium on Autoimmunity will be held next November in Athens

The 11th International Symposium on Autoimmunity fully sponsored by **A. Menarini Diagnostics**, will be chaired by Nicola Bizzaro. The expected attendance is 250 guests from all over Europe.

Need flexibility and high specialization?

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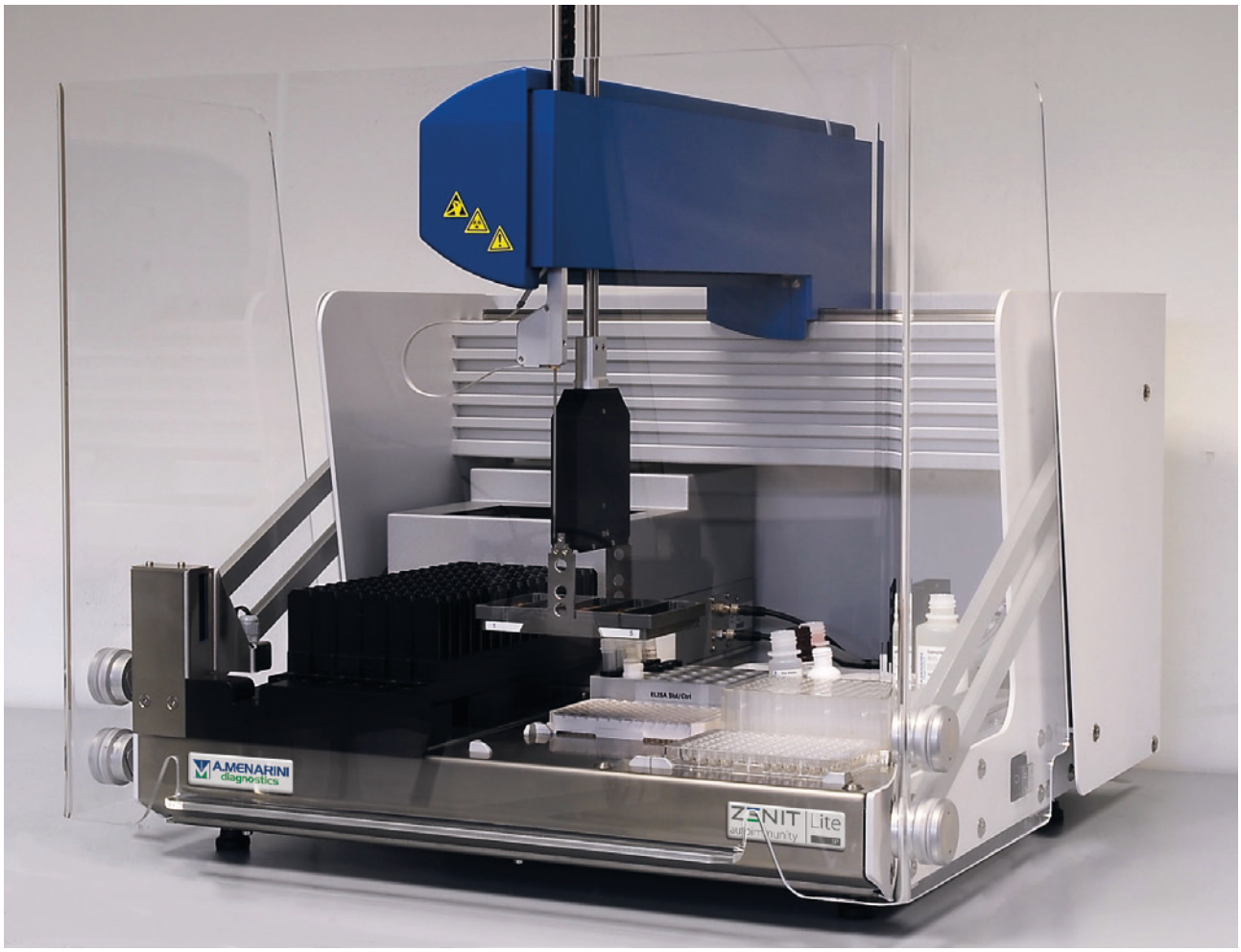

Zenit Lite is the new and last compact and flexible walk-away system for ELISA and IFA methods, an advanced state-of-the-art robotic workstation providing a complete laboratory automation solution for processing both ELISA and IFA technologies.

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