Year 4, No. 1, May 2017

Auto-immunity

CLOSE UP

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A.MENARINI diagnostics

Auto-immunity CLOSE UP

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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 inter-disciplinary 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

Innovation as Group Identity

This year's first issue of Autoimmunity Close Up, the sixth in sequence since its creation, is entirely devoted to a special event represented by the launch on the market of our total automation system for indirect immunofluorescence (IIF), the **Zenit PRO**.

This instrumental platform is a state-of-the-art system resulting from the spirit that animated all of us over this last period, a spirit that leads to innovation. Innovation, as we see it, stems from our group identity, a lesson that pervades our daily work.

Today, **A. Menarini Diagnostics** strives to carry that lesson forward. If I were to spell out our current rules and practices for inspiring innovation, I would say that we try to consider:

1. creating a climate where people can do their best work;
2. embracing the best ideas regardless of where they come from;
3. embarking on missions that matter with a vision that inspires;
4. exemplifying strong values in all that we do.

These values have inspired all our work leading to the launch of the **Zenit PRO**. These principles have indeed inspired the innovation that led to the development of some of the most iconic laboratory equipment in the history of laboratory medicine, such as the **Zenit PRO**. More importantly, though, I would like to remark on how these principles have been driving the development of next-generation technology.

First, to inspire innovation we must create a climate where people can do their best work. **Zenit PRO** was born with the idea of standardizing the analytical variability that preceded the automatic intepretation of the images from a IIF slide. **Zenit PRO** is new, unique, and different. Its difference lies in the application of the most advanced technologies to the field of image analysis.

So what lesson can be learned about driving this kind of innovation? At **A. Menarini Diagnostics** we have created an environment where everyone is empowered to bring ideas forward no matter how unconventional. Irrespective of the engineer's background, experience, or job title, everyone's voice is heard. Every idea is evaluated based on its merits. In short, we have created a climate where people can do their best work. And in the case of the **Zenit PRO**, that climate inspired one of the greatest innovations of in-vitro diagnostics engineering.

The second principle to inspire innovation is that we must embrace the best ideas regardless of where they come from. The **Zenit PRO** began as an effort to create an international ultimate generation IIF analyzer with a wide capability to process slides. A historical partner, Visia Imaging, has agreed to develop the **Zenit PRO**, and together we have contributed to developing the operational requirements and designing and testing programs and incorporated the expertise of a global network of scientific consultants.

The global need for standardization in autoimmunity, more specifically in the classification of the HEp2 patterns for the detection of antinuclear antibodies (ANA), spans across several continents and leads to significant initiatives such as the ICAP,

International Consensus on ANA patterns (see www.anapatterns.org). **Zenit PRO** was born with this goal, which leads me to the third principle to inspire innovation: embark on missions that matter with a vision that inspires.

The fourth principle to inspire innovation is to exemplify strong values that resonate with employees, partners, and customers alike. This principle is part and parcel of the other three. It's fundamental. It's written in our DNA. At **A. Menarini Diagnostics** our values are very simple and very clear:

- Do what's right;
- Respect others; and …
- Perform with excellence.

A company's values are what set it apart, enable it to weather the challenges that test every company, and inspire it to achieve ambitious goals.

Massimo Donnini
International Product Manager Autoimmunity
A. Menarini Diagnostics

TECHNICAL INSIGHTS

Automated Systems for Indirect Immunofluorescence

By the Editorial Team

Indirect immunofluorescence (IIF) is a key diagnostic tool for the detection of a broad spectrum of autoantibodies, and therefore for diagnosing, monitoring and establishing the prognosis of autoimmune diseases.[1]

However, IIF is burdened by intra- and inter-assay/laboratory variability and by subjectivity in result interpretation, although manual microscopic reading is considered the reference method for IIF assays.[2] Finally, the whole IIF procedure, from sample processing to result interpretation, is very labor intensive.

Standardization of IIF testing, which is critical for addressing the issue of variability, can be improved by the introduction of automation in processing of samples and/or evaluation of results. Furthermore, automated systems can contribute to reduce errors and the operational burden of IIF assays, provided they meet the basic requirement of correctly and reproducibly discriminating between positive and negative samples.

Currently, several automated systems are available on the market that can manage slide processing through proper liquid handling as well as slide reading by means of an automated microscope that also generates and analyzes digital images.

The performance of several automated IIF systems has been evaluated by a number of studies. The results of three such studies – involving the HELIOS® and the NOVA View systems – are summarized here.

The **HELIOS® system** (AESKU-SYSTEMS) is a fully automated platform featuring two barcode readers for sample and slide traceability –which ensure compliance with laboratory accreditation – and an autofocus epifluorescence microscope unit generating 1–10 pictures per analyzed well. The system yields quantitative results on a continuous scale. HELIOS® uses AESKUSLIDES® according to an immunofluorescence assay (IFA) protocol and can automatically prepare slides for analysis. It is operated by the HELMED® IFA v3.0 Software and can combine the results on all available dilutions of tested samples into one final result for each sample.[1]

Giuliani et al.[3] evaluated the reliability of the HELIOS® system for the detection – i.e. discrimination of positive and negative samples – of antinuclear antibodies (ANA), anti-double stranded DNA antibodies (anti-dsDNA) on *Crithidia luciliae*, anti-endomysium (anti-EMA), anti-mitochondrial, anti-smooth muscle, anti-parietal cell antibodies (LKS) and anti-neutrophil cytoplasmic antibodies (ANCA). Specifically, the diagnostic performance of the automated reading was compared with that of traditional visual interpretation by a laboratory expert on a total of 210 samples, of which 86 with known autoantibody titers.

In 5 of the 86 latter samples discrepant results between the automated and the visual reading were observed (Table 1, shaded areas) concerning the classification as *negative* versus *low-titer* samples, possibly due to the influence of assay variables (substrate, conjugate antibody) and cut-off setting.[3]

According to a validation on over 1000 samples, results from the HELIOS® system showed a 98.4% correlation with those from the manual IIF procedure and visual interpretation.[3]

In a similar study, Shovman et al.[1] assessed the performance of the HELIOS® system, as compared with visual interpretation, in discriminating between ANA and ANCA positivity and negativity. The study was conducted on 425 sera samples for ANA detection (218 samples that were to undergo routine testing at a reference laboratory, 137 samples from healthy subjects, and 70 ANA/ENA-positive samples) and on 170 sera samples for ANCA detection (40 samples that were to undergo routine testing, 90 samples from healthy subjects and 40 anti-PR3/anti-MPO positive samples).[1]

The visual and automated ANA IIF approaches demonstrated a *good* agreement in discriminating ANA-positive and negative samples (kappa coefficient: 0.633 for positive samples and 0.657 for negative

Table 1: Comparison between interpretations of IIF results on 210 samples by HELIOS® and a laboratory expert (modified from Giuliani et al.[3])

	HELIOS®			Expert			
	Positive	Negative	Low titer	Positive	Negative	Low titer	n
ANA	40	55	13	40	51	17	108
anti-dsDNA	8	14	2	8	15	1	24
LKS	8	10	1	8	9	2	19
anti-EMA	9	23	1	9	23	1	33
ANCA		26			26		26
Total	65	128	17	65	124	21	210

Table 2: Comparison between the interpretations of IIF results by the HELIOS® automated system and experienced examiners (ANA testing on 425 samples and ANCA testing on 170 samples) (modified from Shovman et al.[1])

	Kappa coefficient	Agreement consideration*
ANA findings		
Positive	0.633	Good
Negative	0.657	Good
ANCA findings		
Positive§	Not reported	Very good
Negative#	1.000	Very good

* Interpretation of kappa values: ≤0.20 *poor*, 0.21–0.40 *fair*, 0.41–0.60 *moderate*, 0.61–0.80 *good* and 0.81–1.00 *very good*.[4]

§ Sera positive for anti-PR3/anti-MPO antibodies.

Sera from healthy subjects and routine samples.

ones) (Table 2).[1] Furthermore, the two approaches showed a *very good* agreement in discriminating ANCA-positive and negative samples (Table 2). Specifically, agreement was 100% (that is, kappa coefficient:1.00) on sera from healthy subjects and on routine sera, and 95% on anti-PR3/anti-MPO positive sera.[1]

As reported by Shovman and coauthors, a broad spectrum of staining patterns are correctly identified by the HELIOS® system, thus providing the basis for follow-up decisions.[1] In fact, all automated systems include a short final step of approving positive results and visual pattern assignment on the basis of a pattern library.[1]

NOVA View is a computer-controlled fluorescence microscope that automatically acquires digital images of stained IIF slides, presents them for operator review, and provides a median value of fluorescent light intensity based on a statistically relevant number of measurements.[2]

The study by Lakos et al.[2] aimed to evaluate the performance of the NOVA View system in detecting anti-dsDNA antibodies with the *Crithidia luciliae* indirect immunofluorescence test (CLIFT). Due to its high clinical specificity, the CLIFT is the reference method for the detection of these antibodies and, therefore, for the diagnosis of systemic lupus erythematosus (SLE). This study was the first complete evaluation of CLIFT testing with an automated system.[2]

Stained slides were read with the NOVA View system and also interpreted by traditional fluorescence microscope (Olympus BX41) with a 40× objective (referred to as "manual reading"). Digital images were also interpreted by a technologist.[2]

Based on the light intensity cut-off value established by the manufacturer, NOVA View classifies CLIFT results as positive (≥120 LIU), indeterminate (60–119 LIU), or negative (<60 LIU).[2]

Since manual microscopic reading is considered the reference method for IIF assays, the results generated by NOVA View or by reading of digital images were compared to those generated by manual reading. Agreement was 96.0% (95% CI: 92.8–98.1%) in both comparisons (Table 3).[2] Digital image reading and interpretation by NOVA View matched in 98.4% of the cases (95% CI: 96.0–99.6%), with almost perfect correlation (kappa coefficient: 0.94).[2]

The study by Lakos et al. demonstrated the accuracy and consistency of the NOVA View CLIFT for the detection of dsDNA antibodies, with repeatability, reproducibility, concordance with other instruments and a high level of agreement with manual in-

Table 3: Agreement between manual reading, digital image interpretation, and NOVA View software interpretation of 250 clinical samples (modified from Lakos et al.[2])

Interpretation	PPA (95% CI)	NPA (95% CI)	TPA (95% CI)	kappa (95% CI)
Manual *vs* NOVA View interpretation	88.4 (74.9–96.1)	97.6 (94.5–99.2)	96.0 (92.8–98.1)	0.86 (0.77–0.94)
Manual *vs* Digital image interpretation	90.7 (77.9–97.4)	97.1 (93.8–98.9)	96.0 (92.8–98.1)	0.86 (0.78–0.95)
Digital image *vs* NOVA View Interpretation	93.3 (81.7–98.6)	99.5 (97.3–100.0)	98.4 (96.0–99.6)	0.94 (0.89–1.00)

PPA, positive percent agreement; NPA, negative percent agreement; TPA, total percent agreement.

terpretation. The high level of consistency demonstrated by NOVA View can be viewed as an important step toward harmonization of autoantibody testing and improving inter-laboratory portability of CLIFT results.[2]

Along with the above described automated systems, a brand-new, high-performance, fully automated system – **Zenit PRO** – has been developed by **A. Menarini Diagnostics**. This device aims to improve standardization of the whole IIF procedure and avoid human-borne errors in order to increase accuracy. Of note, the device performs coverslip mounting, which is generally a critical step in slide preparation because of the risk of air trapping and sample damaging.

Zenit PRO also performs whole-well slide scanning and digitization, image analysis, image archiving and data sharing by connecting to a laboratory information system/middleware. Operation of Zenit PRO is entirely software-managed and fully traceable. Moreover, a virtual microscope allows remote viewing of the digitized slides, eliminating the burden of slide storage and the problems of signal preservation in the long term.

As with other available systems, full automation of the IIF procedure through Zenit PRO all-in-one device is a reliable, secure, streamlined, cost-effective solution to screen for diagnostically relevant autoantibodies. It reduces intra- and inter-laboratory variability and considerably shortens laboratory workflow.

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RESEARCH UPDATES

Zenit PRO: Towards Total Automation and IIF Standardization

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System overview

Analysis of autoantibodies by indirect immunofluorescence (IIF) remains the hallmark of autoimmune disease diagnosis. This technology was the first "multiplex" method used to detect key autoantibodies: in the case of ANA, in fact, IIF on HEp-2 cells allows the identification of at least 28 different cellular patterns correlated to more than 60 autoantibodies[1].

Automation solutions of the IIF method have been developed to improve laboratory workflows and ensure cost-effective and more accurate screening for diagnostically relevant autoantibodies by reducing errors caused by several manual operations and subjective image evaluation.

The newly developed Zenit PRO system is a fully automated solution for autoimmune laboratories performing IIF assays, which streamlines the complete IIF protocol from slide processing to reading and interpretation of results. The project stems from the need to improve IIF standardization protocols, to reduce costs and increase the efficiency, productivity and quality of laboratory operations, with a remarkable impact on overall laboratory management.

Zenit PRO integrates an automated slide-processing module with a reading unit. The system automatically processes and aids in the interpretation of IIF tests and advanced dedicated software orchestrates multiple processes into a seamless system (Figure 1).

The technology used to process and read the slides is tailored to the requirements of the IIF technique. The system

Figure 1: Zenit PRO – the system integrates an automated slide-processing module with a reading unit. An advanced software orchestrates multiple processes into a seamless system

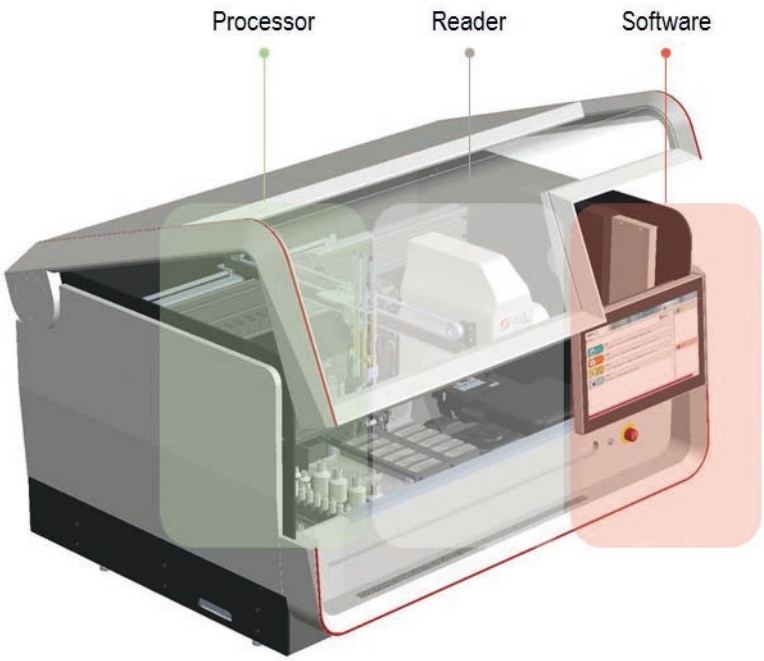
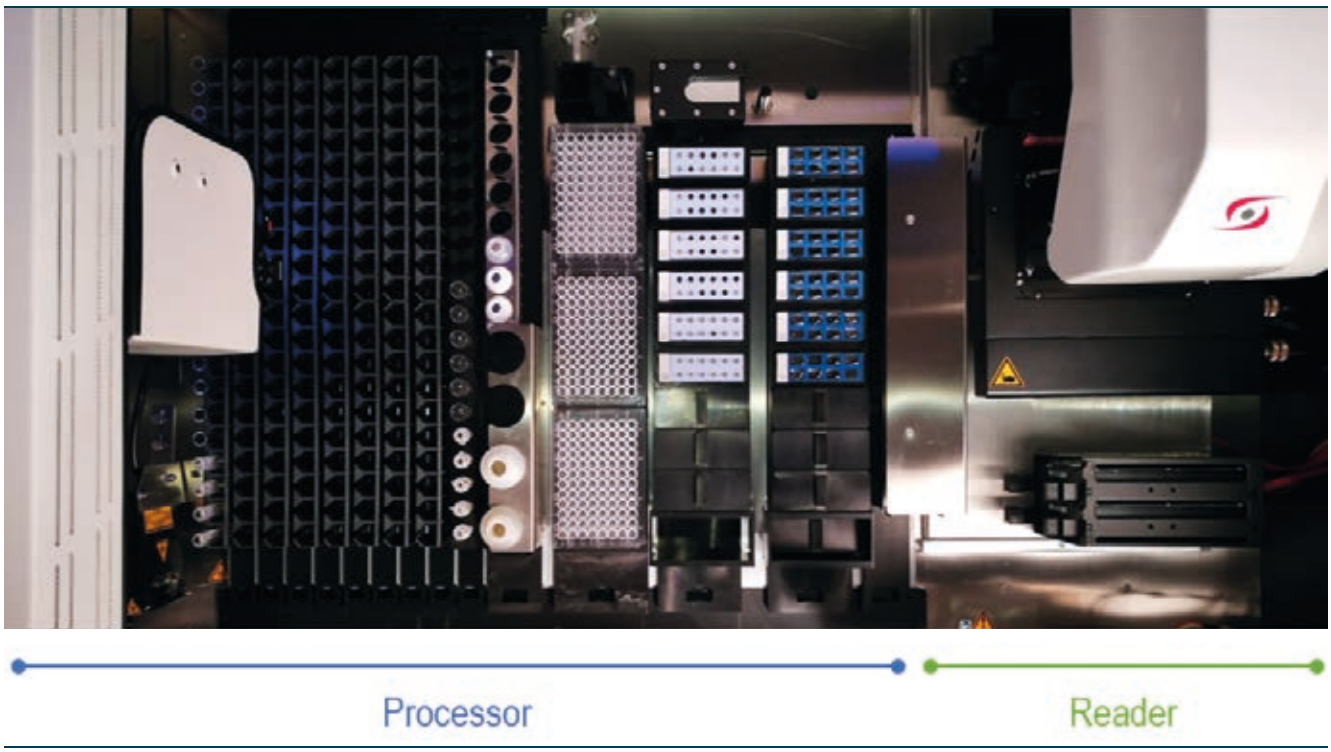


Figure 2: Zenit PRO Workplan – the system is able to prepare, scan and read 18 different slides in a single run



comprises a liquid-handling robotic unit designed for slide processing and a motorized microscope unit for image acquisition and whole well scanning. The liquid handling system aspirates and dispenses samples, reagents, controls, washing solutions and the mounting medium. The system processes 18 slides in a batch and opens up the possibility of "continuous access" to add further tests while the system is operating. The software automatically schedules and organizes the steps to ensure uniform incubation times across each slide. After the incubation and washing procedures, the system automatically mounts each processed slide: the mounting medium is carefully dispensed over the slide and a dedicated tool joined into the sampling arm places the coverslip to cover and seal the slide avoiding bubble formation. After the mounting procedure, each slide is delivered onto the microscope precision stage for automatic scanning of each well of the slide. The motorized microscope drives to the substrate positions, autofocuses and scans a square area inside the rim of each well. The digitized image is then displayed and can be navigated with the virtual microscope tool that allows the user to have a broad view of the substrate at multiple magnifications. After termination of the reading procedure, a precision clamp delivers the slide into a slide parking rack for slide disposal (Figure 2).

The system can process and scan various cellular substrates, including HEp-2, neutrophils and *Crithidia luciliae* as well as a variety of tissues such as liver, kidney, stomach and monkey esophagus. Results are interpreted on screen by the user, who can classify and report each test result from a powerful yet intuitive software interface.

The system includes an automatic classification of positive/negative results for ANA tests and identification of a number of cellular patterns (nuclear and cytoplasmic patterns including mitotic figures) even in mixed cases. The software measures the intensity of fluorescence for each positive test and provides a titer suggestion based on a wide database of reference images used to train a state-of-the-art classifier. The software for automated determination of positive/negative results is designed to efficiently classify ANCA/c-ANCA, nDNA and EMA tests as well.

The system orchestrates multiple processes in order to simplify the workflow, increase the safety of IIF testing by full traceability and to minimize technologist interaction all along a high-volume testing procedure.

Major benefits of a fully automated IIF system:

- Streamlined process for simple operation
- End-to-end management of overall IFA protocol
- Standardization of IFA protocol
- Harmonization of results

Table 1: Automatic interpretation performed by different systems on the market

Test	Interpretation	NovaView	Europattern	Image navigation	Aklides	Helios	G-Sight
ANA	Pos/Neg	Yes	Yes	Yes	Yes	Yes	Yes
	Pattern recognition	Yes	Yes	-	Yes	-	Yes
	Titer prediction	Yes	Yes	-	Yes	-	-
ANCA	Pos/Neg	-	-	-	Yes	-	Yes
	Pattern recognition	-	-	-	Yes	-	Yes
DNA	Pos/Neg	-	-	-	Yes	-	-

- Reduction of intra- and inter-laboratory variability
- Reduction of operating costs

State of the art

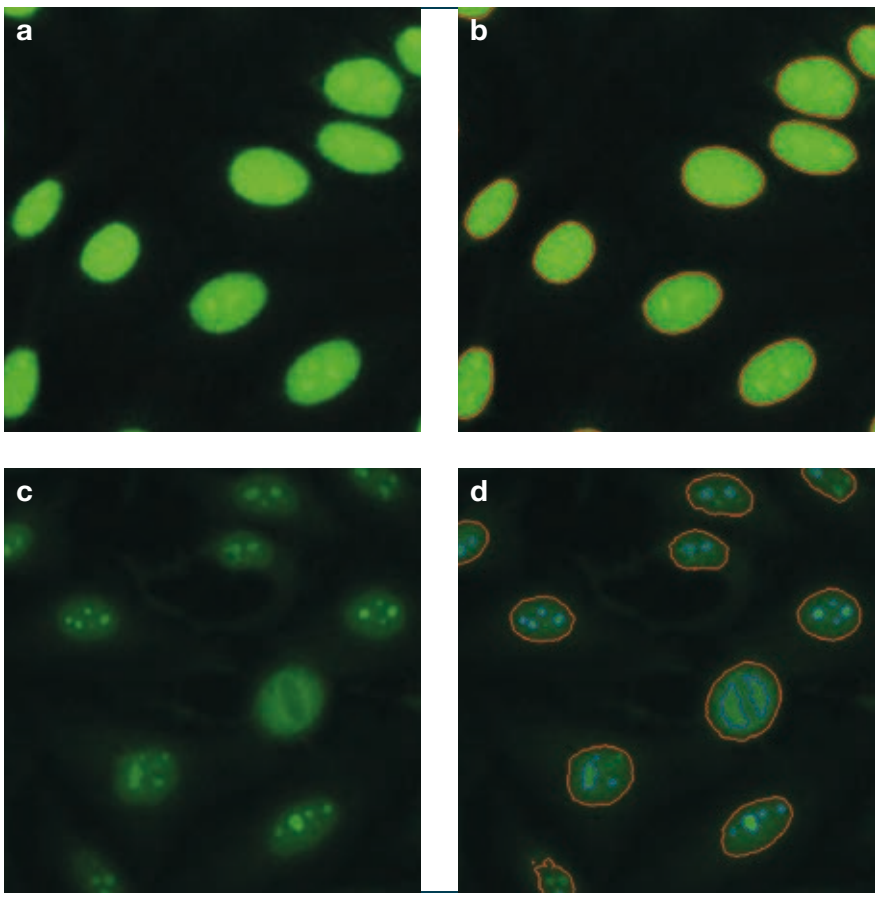
A few automated slide processors are currently available on the market, IF Sprinter by Euroimmun, Quanta-Lyser by Inova Diagnostics and HelMed by Aesku being just some examples. These systems prepare slides for manual mounting, and after that critical step the slides can be read and possibly interpreted with a manual or semi-automated microscope.

In recent years, automated slide reading and interpretation systems have been also developed and introduced. Both NOVA View of Inova Diagnostics, Europattern of Euroimmune and Image Navigator of Immunoconcepts follow the same steps: they acquire a certain number of images per well in specific areas and provide an interpretation of results[2-6].

Table 1 summarizes the list of tests automatically interpreted by each of these systems. Regarding ANA HEp-2, all devices are able to discriminate between positive and negative samples, some of them can detect one or more HEp-2 patterns, and some are able to interpret positive/negative results of ANCA and nDNA tests. Europattern of Euroimmune also performs a titer prediction that requires at least two dilutions in the screening step, making the procedure not particularly cost saving. Helios, manufactured by Aesku, is the only system integrating automated slide preparation with slide reading thanks to the presence of an integrated microscope and camera that captures a limited number of images.

Nevertheless, whenever the user is not confident with the automated results (because the number of acquired images is not enough to provide the whole picture), evaluation of the slide under a manual mi-

Figure 3: (a) Homogeneous detail image; (b) Fluorescence target detection on cells displaying a homogeneous pattern; (c) Nucleolar detail image; (d) Fluorescence target detection of nucleolar stained cells (blue profile)



croscope still remains a necessary step to complete the test.

In 2010 Visia Imaging presented a new concept to perform automated reading and interpretation of IFA tests and introduced the Zenit G-Sight system in the market. This system performs the complete digitization of the well generated by a mosaic of single images that can be navigated through a dedicated software tool called Virtual Microscope at different magnifications. Zenit G-Sight can discriminate between positive and negative samples and can give a pattern suggestion for both ANA HEp-2 and ANCA tests[7-8] (Table 1).

ZENIT PRO test interpretation

The Zenit G-Sight algorithms implemented for automated interpretation and classification of IIF test results on HEp-2 and neutrophils have been widely improved in the Zenit PRO system. Moreover, novel algorithms for positive/negative classification of nDNA and EMA tests have been implemented.

Below are listed the main features of the new software for the automated interpretation of IIF results:

1. **ANA Positive/Negative classification**
   An evaluation of fluorescence intensity is performed on every single identified cell. An average level of fluorescence is calculated according to the fluorescent structures detected inside each analyzed cell. As shown in Figure 3a and 3b, cells displaying a homogeneous pattern have a homogeneous level of fluorescence, whereas for cells displaying nucleolar staining (Figure 3c and 3d) the analysis of fluorescence is limited to the nucleolar structures. This response – according to the titration and the level of fluorescence obtained – is also calculated for other types of patterns, and two thresholds are used to classify the results into three different classes: negative, borderline and positive. Zenit PRO software displays an index value representing the level of fluorescence that gives an indication on the positivity of the sample analyzed.
2. **Recognition of 9 HEp-2 patterns** (homogeneous, fine speckled, coarse speckled, nucleolar, centromere, mitochondrial, ribosomal, few nuclear dots, multiple nuclear dots).
   The first step in the image processing algorithm consists in the use of morphological operators and threshold techniques to separate background from foreground. The segmentation of foreground is performed in order to evaluate each single cell. In a second step, a collection of texture features are analyzed to evaluate the intensity surface of the cells. Finally, a supervised learning classifier is used to classify patterns using the descriptors. Mitotic figures of recognized patterns are properly detected and shown in a dedicated gallery of the software interface and each figure can be easily identified in the digital well, automatically relocated at the center of the screen.
   In Figure 4a and 4c, two images of a speckled and a nuclear dots pattern are shown. In Figure 4b and 4d the results of cell segmentation and classification of each analyzed cell are displayed. For both patterns a pseudo-color is used to classify the cell as speckled (purple) or nuclear dots (cyan).
3. **Recognition of 3 ANCA patterns** (p-ANCA, c-ANCA, *other*-ANCA and Negative).

Figure 4: (a) Image of speckled pattern; (b) Classification of speckled image; (c) Image of multiple nuclear dots; (d) Classification of multiple nuclear dots image

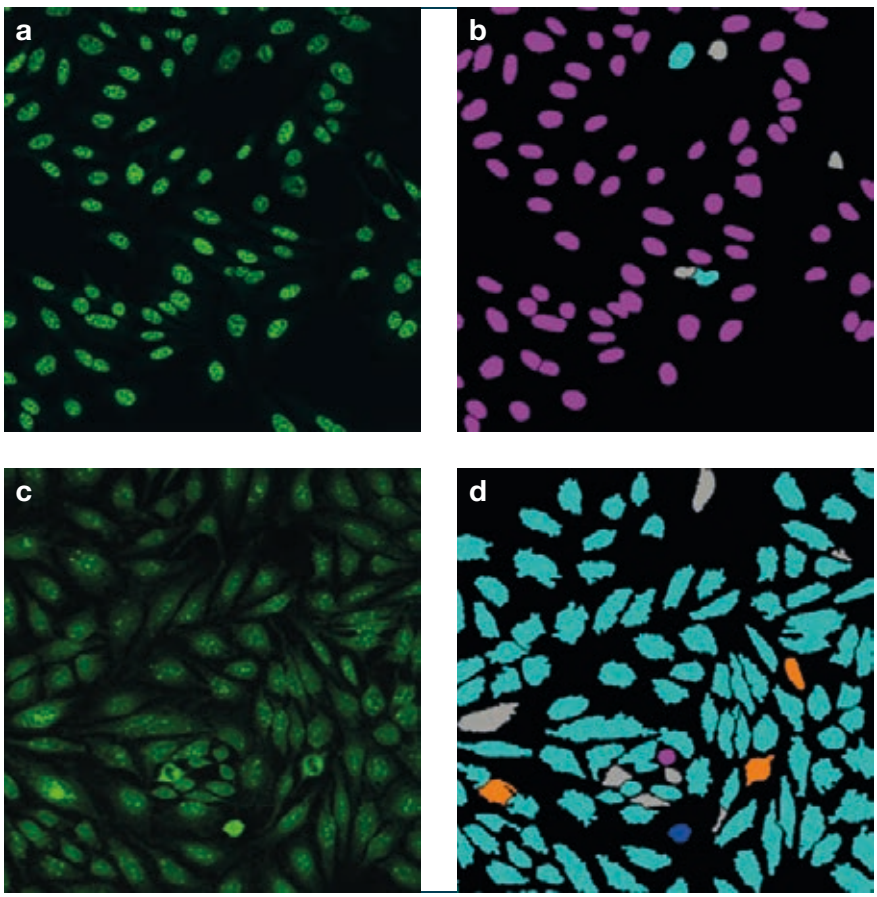
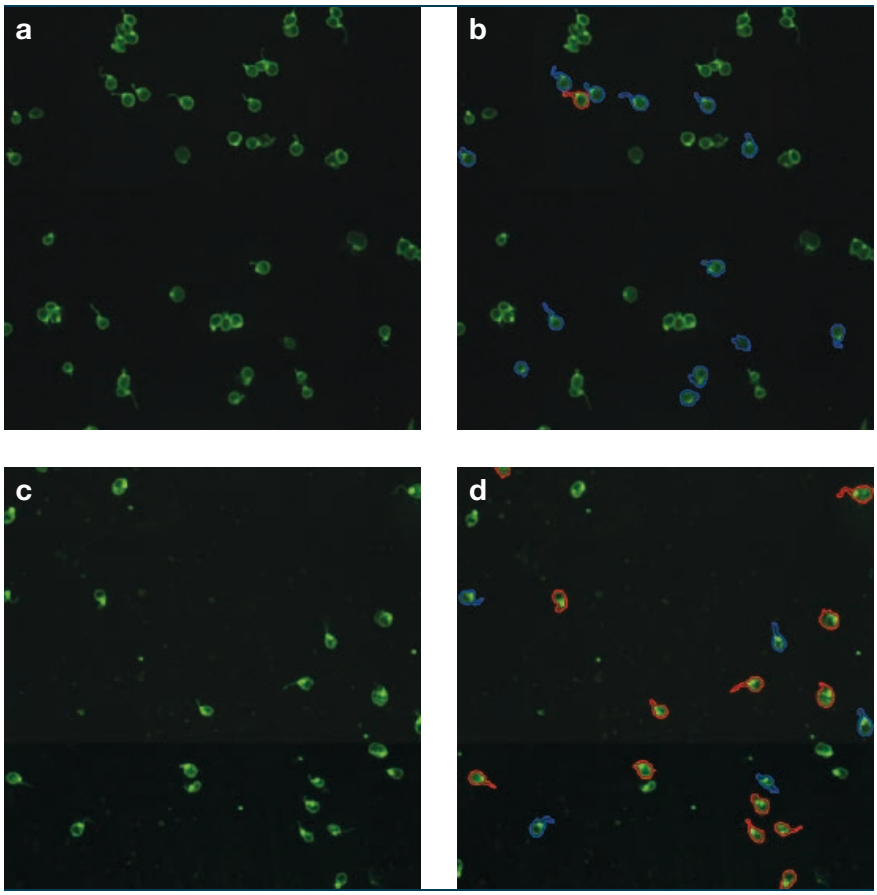


Figure 5: (a) Negative DNA image; (b) Detection of negative particles (blue outline); (c) Positive nDNA image; (d) Detection of positive particles (red outline)



At the basis of the analysis is the identification of the cells through separation of the background from the foreground and cell segmentation whenever clusters are present. For each cell, a collection of features is calculated and used by the classifier to provide a pattern suggestion. Four are the classes of interest: p-ANCA, c-ANCA, Negative and Other. A single training phase is requested for ANCA Ethanol (p-ANCA, c-ANCA, Negative and Other) and ANCA Formalin (c-Anca, Negative and Other) to optimize the classification capability.

4. **Positive/negative classification of nDNA tests on *Crithidia luciliae*.**

The discrimination of positivity is based on the detection of the hemoflagellate organisms as the first step. Then, fluorescent particles related to the basal body, the kinetoplast and the nucleus are analyzed. The presence of basal body is detected taking the flagellum as a reference point, whereas the kinetoplast and the nucleus are analyzed – if present – in terms of average fluorescence intensity. The system displays an indication of positivity (Figure 5) and a measurement of the mean intensity of the kinetoplast.

5. **Positive/negative classification of EMA test.**

For EMA testing on monkey esophagus, the morphology and the intensity of a consistent and specific part of the tissue has to be analyzed. The fixed tissue section on Zenit EMA slides has a typical arrangement which is shown in Figure 6a. In Figure 6a, a positive sample and a control case are shown. The tissue is arranged as a tubular cross-section where the inner part presents an empty area (excluding the membrane). Tissue detection begins with the identification and the removal of the central "empty" (non-informative) zone, highlighted in red in Figure 6b and 6d, and then through iterative evaluations of the variations in average fluorescence intensity. Whenever there are positive peaks or negative peaks, the inner part of the tissue is detected and a thin profile of the area of interest is extracted (blue outline in Figure 6b and 6d). Finally, the average intensity is evaluated at high magnification and target tissue recognition is performed to evaluate if the sample is positive or negative.

For each of the above tests the system performs the analysis on the whole well and not just on a limited number of pre-defined images only partially covering the area of interest.

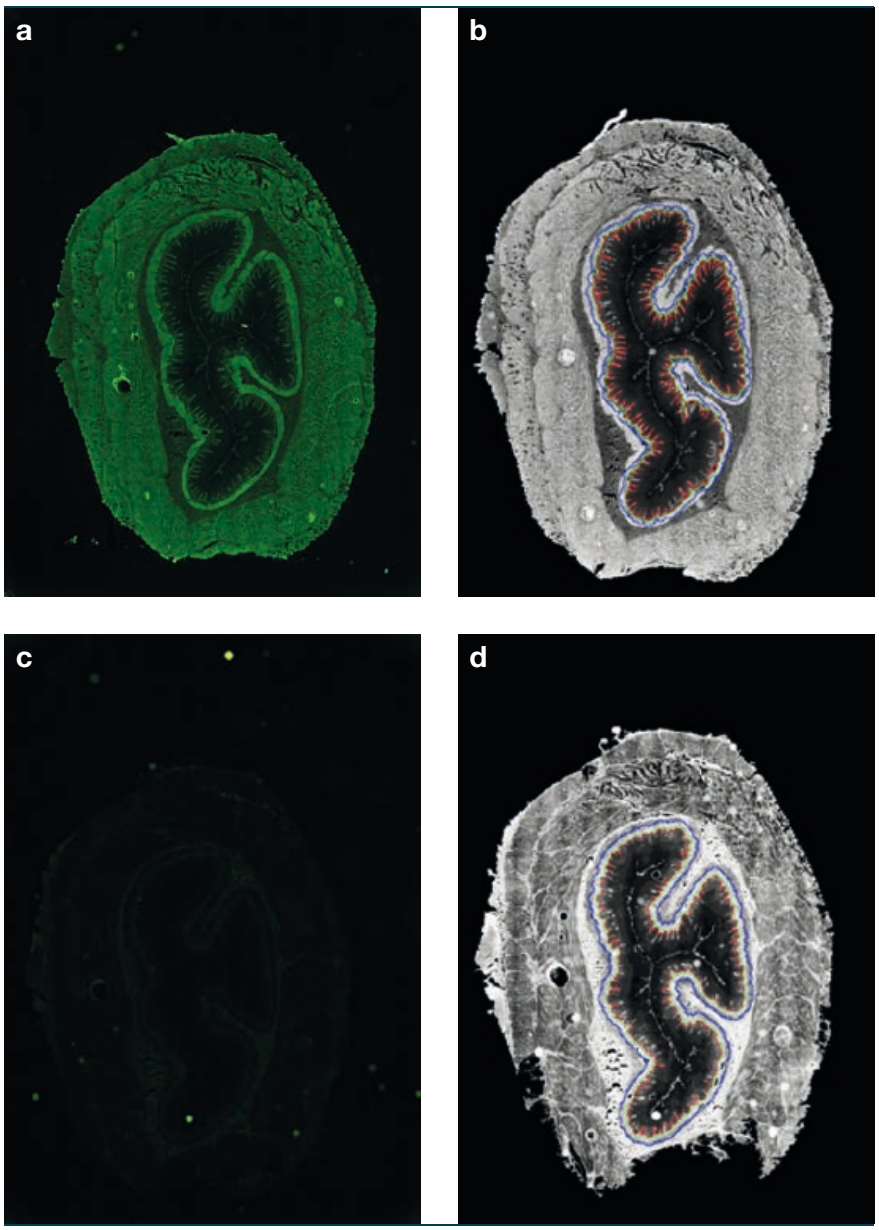
This feature offers several advantages:

- As the number of counted and analyzed cells is fairly large (greater than 3000 for each well), the results are more reliable and consistent;
- Results are robust even in the presence of microbubbles or damaged areas in the well;
- High sensitivity due to the possibility to adjust the cell count and cell identification to detect rare patterns or to detect a pattern displayed by a few cells only.

Conclusion

A. Menarini Diagnostics' partnership with Visia Imaging dates back to 2010, when Zenit G-Sight reader was first introduced in the market.

Figure 6: (a) Low magnification view of EMA-positive control; (b) Enhanced image view and detection of target tissue to be evaluated (blue outline); (c) Low magnification view of EMA-negative control; (d) Enhanced image view and detection of target tissue (blue outline)



The continuous efforts to strive for some form of standardization in a very subjective field such as Autoimmunity led the two Companies to join forces in developing, producing and distributing an automated reader in IIF. At that time, the Zenit G-Sight represented a novelty in the Autoimmunity field because manual microscopy was still considered the golden standard in IIF reading and interpretation.

After some years – during which a number of automated systems have been introduced in the market – things have changed and nowadays automated readers are quite well accepted for reading and interpreting IIF slides even by those who were initially skeptical. Many steps forward have been made over the years to make these systems increasingly reliable, reach sensitivity and specificity values in line with recommendations, include pattern suggestions among the different capabilities, obtain reproducible results, and guarantee analytic precision.

Thanks to the success obtained over the years with the Zenit G-Sight, the A. Menarini Diagnostics and Visia Imaging partnership has become even stronger in a continuous research for new technologies to be used for cost-effective screening of relevant autoantibodies, reducing intra- and inter-laboratory variability and eliminating errors caused by subjective manual preparation and evaluation of IIF slides.

Zenit PRO is the result of such a search, representing a third-generation system where not only reading and interpretation are performed automatically but where also slide preparation – including the mounting phase – is totally automatized thereby lessening the effects of inadequately expert personnel or possible differences among laboratories across Europe.

Autoimmunity has in fact always been burdened by some unfavorable aspects, mostly related to the need to have expert operators and the critical consequences of subjective interpretation but most of all to the lack of any real standardization.

A first step was made many years ago by automatizing the preparation phase, a second one has only recently been made by rendering the reading phase automatic.

It's now time for a real change to complete this standardization process and Zenit PRO is the solution.

A new way to harmonize results, guarantee full traceability and make life in the laboratories easier.

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AUTOIMMUNITY LAB

Moving Towards a Bright Future!

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Introduction

The important role of the IIF in immunological and immunometric assays has already been debated in many publications owing to its labor intensiveness, high inter-reader variability and risk of error due to manual handling/reporting.

In addition, considerable expertise remains mandatory. However, despite attempts to use new methods for the determination of certain antibodies, IIF is still the best method[1].

As a result, extensive efforts to develop technological solutions for IIF automation have been undertaken.

Semi-automation

Two decades ago the biomedical industry proposed new devices*[1] for substrate (slides) preparation to perform the "dirty work".

Semi-automation of this method has indeed given the chance to reduce the variability of results between laboratories, to increase the accuracy of results and to improve the correlation of staining patterns with corresponding autoantibody reactivities[1].

However, the main disadvantages of manual microscopy such as subjectivity and low reproducibility remained unchanged.

From manual microscope to automated scanner

To date, automated approaches for IIF reading and interpretation have become available**. These systems are based on the use of automated microscopes, robotized slide trays, high-sensitivity video cameras and software dedicated to acquisition and analysis of digital images.

The introduction of automated microscopes enables objective internal quality control procedures and should be considered an important step forward in IIF harmonization[2].

However, a study by Stefanie Van den Bremt et al.[3] revealed a large inter- and intra-run variability of results between laboratories due to preanalytical and analytical problems.

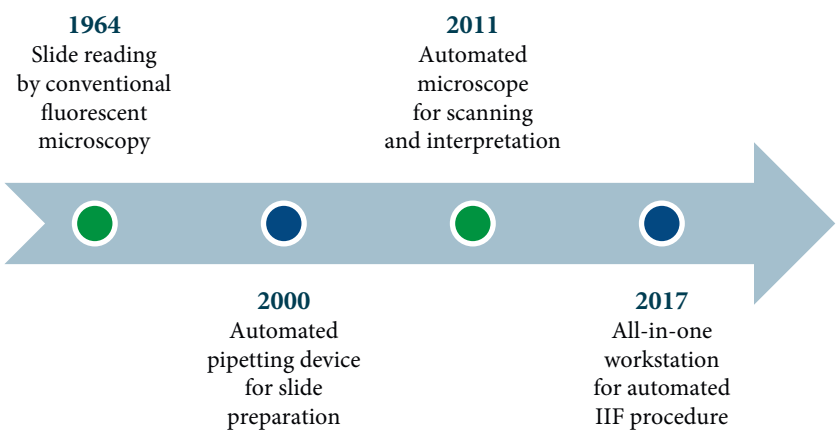
Third-generation

Today, consolidation of the existing slide processor with an automated microscope in one single instrument*** is available, eliminating the need for manual intervention for adding mounting medium and cover slip, transfer of slides from the slide processor to the microscope.

A complete paperless process and automated data transmission is no longer science fiction.

Moreover, its introduction in clinical practice should reduce inter-laboratory variability and time required to perform this test especially in medium- and high-throughput laboratories.

Figure 1: IIF Automation History Timeline



To conclude

The routine clinical laboratory has evolved to an efficient and highly automated environment. A stand-alone solution to automate IIF testing from A to Z is what we still needed in lab routine.

Now we will reach the highest standards of quality, accuracy, economy and feasibility and IIF will gain popularity once more.

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* These include Zenit UP, an accurate and reliable microplate and slide processor by A. Menarini Diagnostics, which makes the daily workflow of IIF tests efficient & flexible.

** Zenit G-Sight by A. Menarini Diagnostics is able to measure fluorescence intensity and interpret and classify the fluorescence pattern.

*** The brand-new Zenit PRO by A. Menarini Diagnostics offers a complete walk-away solution for IIF testing in one stand alone instrument for medium and high throughput laboratories.

COMPANY PINBOARD

Latest Marketing & Scientific Events

EuroMedLab 2017 at Megaron in June

The 22nd IFCC-EFLM European Congress of Clinical Chemistry and Laboratory Medicine "EuroMedLab Athens 2017" will take place on June 11-15, at Megaron, the Athens International Conference Center.
EuroMedLab Athens 2017 will cover scientific and technological aspects of Laboratory Medicine, depicting the state of the art and innovations in Laboratory Medicine through presentations, posters, symposia, open sessions and workshops, thus providing the opportunity for a fertile exchange of opinions among experts in the field.

The congress will be co-organized along with the 15th National Congress of the Greek Society of Clinical Chemistry - Clinical Biochemistry (GSCC-CB) and the 25th Balkan Clinical Laboratory Federation (BCLF) meeting, which will also encourage enriching and fruitful connections.

Among the latter, the educational workshop **Total automation of indirect immunofluorescence testing (IFA) in autoimmune diseases** (EduW #37), supported and organized by **A. Menarini Diagnostics**.

EUROMEDLAB
ATHENS 2017
11-15 JUNE

A.MENARINI diagnostics

The workshop programme is as follows:

Brief introduction by the Scientific Coordinator
M. Berth (Belgium)

An all-in-one workstation for IIF automated procedure
D. Picchioni (Italy)

A new fully automated analyser for the determination of antinuclear antibodies on HEp-2 cells
M. Berth (Belgium)

Discussion

The industrial exhibition accompanying EuroMedLab Athens 2017 will display the most recent equipment and provide information and advice on diagnostics, informatics and professional practice.

ZENIT autoimmunity | PRO

The evolution of indirect immunofluorescence

All-in-one automation, from slide processing
to reading and interpretation of results

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