Allergy and Autoimmunity **Edited by: U. Sack/K. Conrad**

Mandy Sowa, Kai Großmann, Juliane Scholz, Nadja Röber, Stefan Rödiger, Peter Schierack, Karsten Conrad, Dirk Roggenbuck and Rico Hiemann*

The CytoBead assay – a novel approach of multiparametric autoantibody analysis in the diagnostics of systemic autoimmune diseases

DOI 10.1515/labmed-2015-0036
Received November 1, 2014; accepted November 12, 2014

Abstract: If there is a suspicion of a systemic autoimmune disease, a two-step assessment of autoantibodies (AAb) is recommended for the serological diagnosis thereof. First, AAb will be determined using sensitive, cell-based indirect immunofluorescence. Then, a positive result must be confirmed with a more specific test due to the possibility of false-positive results. This gradual approach is necessary because there is currently no assay technique that fulfills the requirements for a one-stage procedure for sensitivity and specificity. For effective AAb analysis, simultaneous determination of several AAb with multiparametric confirmatory assays significantly shortens serological diagnosis, compared with conventional monoparametric testing. Yet, currently available multiparametric AAb detection techniques do not offer the combination of screening and confirmatory testing. Thus, a new approach based on digital fluorescence was developed by applying a novel CytoBead technology that is presented here. The aim was to combine the recommended stepwise approach consisting of sensitive screening and confirmation of specific diagnosis in a reaction environment and thereafter the possibility of adaptation to the serological diagnosis of several autoimmune diseases. Using standard microscopic glass slides and the combination of native cellular or tissue substrates with autoantigen-loaded fluorescent microparticles (beads) in a reaction environment, along with the possibility of manual and automatic evaluation by IIF and the quantitative measurement of fluorescent signals, the disadvantages of currently existing test systems could be overcome. This novel concept is applicable for the determination of various multiparametric AAb, e.g., the determination of antinuclear antibodies and the corresponding AAb in molecular cytoplasmic and nuclear autoantigenic structures. Further, this becomes the basis for the simultaneous multiparametric AAb determination for the serology of celiac disease or ANCA-associated vasculitides.

Keywords: autoantibody; confirmatory testing; indirect immunofluorescence; microparticle; multiparametric diagnostics; screening.

***Correspondence: Dr. Rico Hiemann,** Faculty of Natural Sciences, Brandenburg University of Technology Cottbus-Senftenberg, Großenhainer Str. 57, 01968 Senftenberg, Germany, Phone: +49-3573 85823, Fax: +49-3573 85809, E-Mail: Rico.Hiemann@b-tu.de
Mandy Sowa, Juliane Scholz and Dirk Roggenbuck: Faculty of Natural Sciences, Brandenburg University of Technology Cottbus-Senftenberg, Senftenberg, Germany; and GA Generic Assays GmbH, Dahlewitz/Berlin, Germany
Kai Großmann: GA Generic Assays GmbH, Dahlewitz/Berlin, Germany
Nadja Röber and Karsten Conrad: Institute of Immunology, Medical Faculty of the Technical University of Dresden, Dresden, Germany
Stefan Rödiger and Peter Schierack: Faculty of Natural Sciences, Brandenburg University of Technology Cottbus-Senftenberg, Senftenberg, Germany

Introduction

The serological diagnostics of systemic autoimmune diseases (SAD) includes the determination of inflammatory parameters and disease-specific autoantibodies (AAb) [1–5]. While the former parameters point to inflammatory processes irrespective of their causes, disease-specific AAb can be seen as an important sign of the autoimmune pathogenesis. AAb can, therefore, serve as a benchmark for the diagnosis and therapy of SAD [3, 4, 6]. In the majority of known SAD, more than a few AAb with diagnostic and/or prognostic relevance can be detected, and their determination has been included in classification criteria

of corresponding SAD [7–9]. For reasons of cost and time savings, there is an ongoing discussion about determining all disease relevant AAb by using multiparametric test approaches [10–14].

The significance of multiparametric biomarker analysis in autoimmune diseases

Regarding their large variability of clinical manifestations and the mostly long pre-clinical stage of SAD the analysis of biomarkers is of special importance. The clinical diagnosis of SAD is often difficult due to the non-specific and variable onset of the disease. An early identification of disease-specific AAb (e.g., anti-CCP antibodies when rheumatoid arthritis is suspected) may point towards further diagnostics and therapy strategies (Figure 1) [4, 15, 16].

A large number of diagnostic relevant AAb can be detected pre-clinically [15–18]. In contrast to the conventional stepwise diagnostics, multiparametric assays for the simultaneous determination of several AAb in one approach can reduce the time needed to get a medical diagnosis [19, 20]. The higher number of relevant parameters in one assay will correlate with the higher probability of confirmation or exclusion of a specific SAD [1, 21].

Apart from time savings, the automation and (relative to the technological solution) significant reduction of costs are key arguments in favor to use multiparametric assays. In addition, there are numerous advantages from a clinical-diagnostic point of view. The increasing number of parameters which can be detected with one assay also increases the certainty of reaching a decision in the case of suspected SAD. The antigenic diversity of a multiparametric assay can be adapted to all diagnostic investigations, which allows an extensive way a more reliable identification of certain overlapping syndromes.

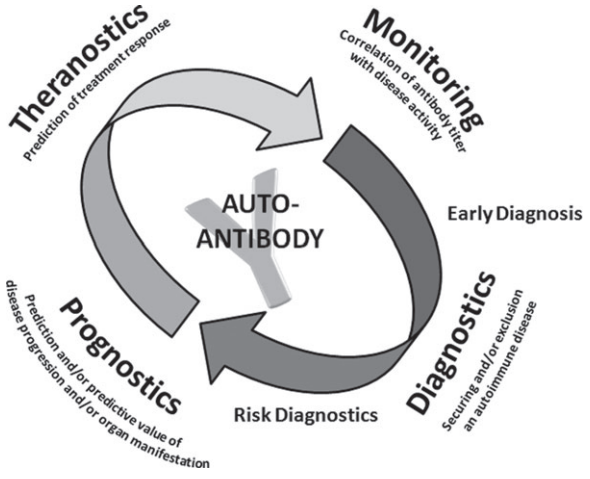

Figure 1: Schematic representation of the guideline for the diagnostics and theranostics of patient's autoantibodies.
The early detection (early diagnosis) of antibodies sets the course for further theranostics – treatment, diagnostics and monitoring of the patient.

The principle: screening and confirmation

The current standard in routine diagnostics where there is a suspicion of SAD (particularly an ANA-associated rheumatic disease, AARD) is the combination of a highly sensitive screening assays followed by specific determination of marker antibodies [22]. AARD, also known as connective tissue diseases (CTD), comprise systemic lupus erythematosus (SLE), systemic sclerosis (SSc), the Sjögren's syndrome (SjS), autoimmune myositis (AIM) and various mixed CTDs (e.g., Sharp syndrome). These systemic diseases are characterized by the production of numerous non-organ-specific, predominantly antinuclear antibodies (ANA) as well as anticytoplasmic antibodies, which, except for a few myositis-specific AAb, can be detected via immunofluorescence screening on HEp-2 cells [23–27]. Depending on the clinical suspicion, a positive fluorescence patterns on HEp-2 cells should be confirmed with antigen-specific immunoassays. This gradual approach has several advantages in contrast to the solitary testing of disease-associated AAb specificities [1, 28]: (a) Negative results of the screening assay can be used to exclude a number of AARD (especially SLE and Sharp syndrome) with high confidence. (b) The HEp-2 cell assay allows highly sensitive multiparametric screening for more than 30 clinically relevant AAb specificities, and, therefore, increases the sensitivity for the diagnosis of AARD with independently expressed marker antibodies in the cell (e.g., SSc). (c) The pattern differentiation, aside from the specific AAb diagnostics (e.g., anti-centromere antibodies), provides diverse indications about underlying clinically relevant AAb specificities, such as dsDNA and DFS70 antibodies [29]. (d) It is possible to obtain clinically relevant or even incidental findings (e.g., anti-mitochondrial antibodies in connection with primary biliary cirrhosis with initial rheumatic symptoms). (e) The screening results distinguish possible false-positive findings in the specific immunoassays (e.g., positive dsDNA antibodies with

negative ANA) and thus increase diagnostic confidence. Thus, the quality of medical diagnostics is improved by the combination of highly sensitive screening and highly specific confirmatory testing [30, 31]. Essentially, a negative result obtained with a highly sensitive screening assay potentially exclude patients under suspicion of AARD due to its high negative predictive value [32, 33]. However, a positive result provides an important indication, but does not prove the presence or allow a reliable diagnosis of an autoimmune disease. Screening is a test method to detect all AAb, where a certain proportion of false-positive findings is accepted [34]. Therefore, a positive test result of a screening assay-has to be confirmed with a specific immunoassay. The confirmation assay has a significantly higher diagnostic specificity and a higher positive predictive value than the screening assay, but does not rule out false-positive findings completely [35, 36]. In the case of emergency situations like ANCA-associated vasculitis involving the kidneys, a maximum diagnostic confidence is needed which requires screening for C/P-ANCA via IIF on neutrophil granulocytes along with the specific determination of myeloperoxidase (MPO)- and proteinase 3 (PR3)-ANCA [1, 4, 33].

Multiparametric methods for detecting autoantibodies

Today, there are many multiparametric methods for detecting autoantibodies, which differ mainly in the test matrix and the measurement method (Table 1). The basic principle is based on immobilized biomolecules which are detected employing different methods.

Test matrix

A test matrix is the substrate and format on which proteins or peptides are immobilized. The most common are applications of proteins in the form of spots or lines on membranes – so-called line dot assays. Furthermore, it is also possible to immobilize proteins to multiwell plates or glass slides. The advantages of these protein-coated matrices are derived from the easy handling and very efficient manual or automated analysis reader systems with evaluation software. However, measurement accuracy due to the lack of calibrators (semi-quantitative) and low sensitivity due to the densitometric determination of a color change reaction are disadvantageous. Various manufacturers managed to improve the latter through the fluorescence labeling of detector molecules and the associated light quantum detection.

Alternatively, microparticles (beads), consisting of polystyrene (PS) or polymethyl methacrylate (PMMA), with a diameter of up to 20 µm are used as solid phase for the development of the reaction environment [13, 14, 19]. Polymerized fluorescence dyes and various sizes of beads allow the differentiation of individual populations. On the surface of the beads, there are immobilized highly purified autoantigens in native or recombinant form. They allow the specific detection of AAb. Furthermore, the possible individual surface modification of each bead population, generates the optimal immobilization strategy for each autoantigen. This creates opportunities for adapting the reaction environment with respect to protein folding, as well as for the targeted modulation of other performance parameters. Given their small sizes, many beads can be combined into individual measuring points and guarantee solid statistical distributions in calculating the measured value. The measurement of fluorescence, or also chemiluminescence, is very sensitive. Additionally, attached calibrators allow real quantitative measurements via lot-specific calibration curves.

A disadvantage in this context are manufacturer-specific proprietary measurement systems. Until now, manual analyses of bead-based assays were not possible.

Measuring systems

Measuring systems for antibody detection include scanners for color change reactions on line dot assays, flow cytometers for beads, as well as fluorescence microscopes for protein spots and beads.

Scanner systems are easy to use and cost efficient but the documentation for line dot assays causes a problem. Semi-quantitative measurements and analyses are possible, but these systems cannot achieve the high precision of fluorescence or chemiluminescence-based systems due to their densitometric evaluation.

Cytometers detect and measure beads in terms of size and fluorescence intensity in the flow and allow accurate quantitative measurements. Measurements are taken in standard vials or special manufacturer-designed cartridges, sequentially as single beads or by parallel multiplex measurements of a bead mixture. The fluorescence on the bead surface is analyzed whereby the fluorescence intensity correlates with the concentration of bound AAb. However, the high acquisition costs for the measurement system and the one-time bead measurement which

Table 1: Overview of multiparametric methods for detecting autoantibodies.

Method	Chemiluminescence	CytoBead	Individual ELISA	Mosaic biochip	Screen ELISA	SeraSpot	Strip test	Cellular assays
Company	Inova (Bioflash) Menarini (Zeuss)	Medipan (Aklides)	Various	Euroimmun	Various	Seramun	Various	Various
Principle	Protein-packed beads	Monolayer of cells/ tissue, combined with protein-loaded beads	Individual proteins combined in multiwell plates	Monolayer of cells/ tissue, combined with protein spots	Protein mix in multiwell plate	Protein spots in multiwell plate	Protein spots on membrane	Monolayer of cells or tissue
Detection	Confirmation	Screening (cells/ tissue), confirmation (beads)	Confirmation	Screening (cells), confirmation (protein spots)	Screening	Confirmation	Confirmation	Screening
Method	Sequential	Parallel	Sequential	Parallel	Parallel	Parallel	Parallel	Parallel
Measuring system	Manufacturer-specific	Manual microscope, Aklides	ELISA reader	Manual microscope	ELISA reader	Manufacturer-specific	Scanner	Manual microscope
Evaluation	Automated	Manual, automated	Automated	Manual	Automated	Automated	Manual, automated	Manual, automated
Result	Quantitative	Semi-quantitative (cells/tissue), quantitative (beads)	Quantitative	Semi-quantitative	Semi-quantitative	Semi-quantitative	Semi-quantitative	Semi-quantitative
Degree of multiplexing	1	Variable	1	Variable, usually <12	High	Up to 24	Variable, up to 24	Very high (e.g., HEp-2 cell: >3000)
Time required	~30 min	~1.5 h	Variable, ~2 h	Variable, ~1.5 h	Variable, usually 1–2 h	~2 h	Variable, usually 1–2 h	Variable, ~1.5 h
Literature	Pelkum et al. 2014 [31]	Sowa et al. 2014 [11]		Sayegh et al. 2014 [37]				Hiemann et al. 2009 [28]

Real quantitative measurement results are possible only when calibrators for each parameter are used.

cannot be repeated are shortcomings. The possibility of using more complex autoantigenic substrates e.g., tissue sections, for the AAb determination has so far not been realized by using flow cytometry.

Microscopes that are either controlled manually or automatically via appropriate software are used for the analysis of fluorescence-based assays. Beads and protein spots are analyzed on planar surfaces in standard formats, such as multiwell plates and glass slides. Apart from artificial substrates, such as antigen-coated beads, native substrates like cells and tissues, which are used in autoantibody screening, can be detected and measured [28, 38]. The wide availability of fluorescence microscopes and their manufacturer independent and flexible uses are the main benefits. Quantitative measurements as well as sample control by repeated measurements are possible.

However, all currently available multiparametric methods for detecting autoantibodies, given their limitation to a single detection system, do not allow the combined analysis of screening and confirmation assays.

The CytoBead principle

Based on experience with existing methods with respect to the advantages and disadvantages mentioned, the CytoBead principle was developed to be a more advanced diagnostic tool. The aim was to generate a simple detection system which combines the stepwise diagnostics of AAb with sensitive screening and specific confirmatory testing that furthermore is adaptable for various autoantibody determinations. By combining innovative and new approaches as described below, it is now possible to overcome the disadvantages of existing test systems.

Multiple parted wells

Stepwise diagnostics requires the detection of AAb on different autoantigenic substrates. To allow these test systems to be combined, wells on conventional glass slides were divided into compartments by Teflon barriers (Figure 2A). This creates test environments for disease-specific combinations allowing specific detection systems for profile diagnostics. Regarding the test performance, there are no differences compared to conventional IIF, as used for ANA and ANCA diagnostics [11]. The fluidics of the serum and conjugate drop is equivalent to the standard glass slide with traditional wells. The regular distance in accordance with the 96-cavity grid enables manual and automated test processing.

Cells, tissues + beads

The creation of multiple compartments makes it possible to combine different methods. Regarding the detection of ANA, HEp-2 cells are used as a sensitive screening system with a repertoire of more than 30 clinically relevant autoantigens. In addition, granulocytes can be used as a substrate for ANCA detection, and *Crithidia luciliae* as a specific target for anti-dsDNA-AAb [11, 20, 39, 40]. For the

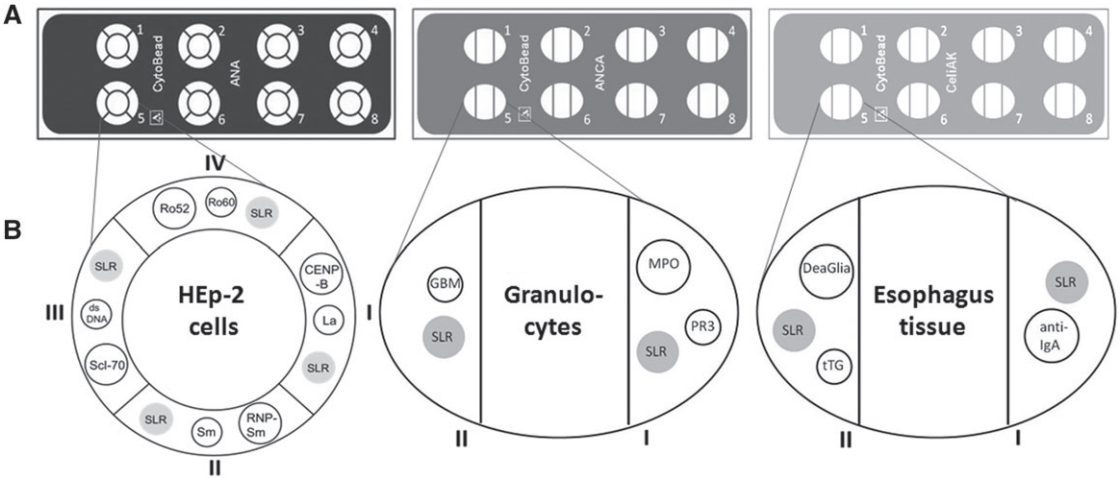

Figure 2: CytoBead slides with eight application points for different test profiles.
Combination of screening with native substrate of cells or tissue (center compartment) and artificial substrate through antigen-loaded fluorescent micro-particles (peripheral compartments). CytoBead ANA (left), ANCA (center) and celiac disease (right). SLR is the designation of reference beads for the manual bead classification.

detection of organ-specific AAb, primate or rat tissue sections from the esophagus, liver, stomach or kidney as well as recombinant autoantigens expressed in specific cell lines are used [41, 42].

Through adapted surface modifications, cells or tissue sections can be immobilized on to the center compartment, which, together with the beads at the outer compartments, create a test profile. As a result, the test system allows sensitive screening on native structures and specific confirmation by solid-phase assay, represented as protein-loaded beads (Figure 2B).

Manual and automated evaluation

New measurement methods are often based on new evaluation systems, which enable the readout of measured data. High investment costs and a lack of laboratory space usually prevent the introduction of new methods. However, almost every diagnostic routine autoimmune laboratory has a fluorescence microscope with a green filter for fluorescein isothiocyanate (FITC), which is used for the manual, traditional analysis of the IIF tests developed in the 1970s.

For the first time, the newly established and unique CytoBead slide format allows the evaluation of bead reactions with conventional manual routine microscopes due to its combination with green fluorescence (FITC wavelength range) for signal detection. This qualitative to semi-quantitative evaluation by eye does not require a special measurement system. The size differentiation of the red fluorescent, antigen-coated beads into different populations for manual evaluation is supported by reference beads. These beads of homogeneous green fluorescence serve as a size scale and enable reliable manual identification and classification.

The interpretation systems developed in recent years for the automated analysis of IIF tests can also be used equally for the analysis of the CytoBead assays [28, 43–46].

Quantification

International comparability of test results requires calibrated systems which yield semi-quantitative or quantitative results [47, 48]. To compensate for any batch-specific and device-dependent fluctuations, it is necessary to use calibrators for quantitative analyses. It follows that manually evaluated tests and tests without calibrators can produce, at best, semi-quantitative results. When evaluating CytoBead tests manually, semi-quantitative statements are comparable to statements obtained from line dot assays.

With measurements involving automated systems like Aklides (Medipan, Dahlewitz) and calibrators, lot-specific master curves can be used to produce values in international units (IU/ml), comparable with conventional ELISA [11].

In summary, the CytoBead principle combines the conventional stepwise diagnostic of different test systems in one single approach. Evaluation can be done manually by using conventional fluorescence microscopes, as well as automatically by modern microscopes. Through the inclusion of calibrators, automated evaluation allows the output of results in international units. The principle can be applied to a variety of autoantibody tests for the serological diagnosis of collagenoses (ANA screening plus determination of collagenose-associated ANA specificities), ANCA-associated vasculitis (ANCA screening plus determination of ANCA specificities), as well as organ-specific autoimmune diseases (Figure 2).

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: GA Generic Assays GmbH develops and sells diagnostic products, such as test systems to detect autoantibodies. Current research projects of R. Hiemann, S. Rödiger and P. Schierack are being supported by GA Generic Assays. However, this did not affect the planning and writing of this paper.
Employment or leadership: M. Sowa, K. Grossmann, J. Scholz and D. Roggenbuck work at GA Generic Assays GmbH.
Honorarium: None declared.
Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

References

1. Conrad K, Roggenbuck D, Reinhold D, Sack U. Autoantibody diagnostics in clinical practice. Autoimmun Rev 2012;11:207–11.
2. Conrad K, Sack U. Multiparameteranalytik in Diagnostik und Monitoring von Autoimmunerkrankungen: Stand und Perspektiven. J Lab Med 2011;35:375–82.
3. Chan EKL, Fritzler MJ, Wiik A, Andrade LE, Reeves WH, Tincani A, et al. AutoAbSC.Org -- Autoantibody Standardization Committee in 2006. Autoimmun Rev 2007;6:577–80.
4. Wiik A, Cervera R, Haass M, Kallenberg C, Khamashta M, Meroni PL et al. European attempts to set guidelines for improving diagnostics of autoimmune rheumatic disorders. Lupus 2006;15:391–6.

5. Bizzaro N. Autoantibodies as predictors of disease: the clinical and experimental evidence. Autoimmun Rev 2007;6:325–33.
6. Wiik AS. Diagnostische Strategien bei rheumatischen Autoimmunerkrankungen. Z Rheumatol 2007;66:219–4.
7. Savige J, Dimech W, Fritzler M, Goeken J, Hagen EC, Jennette JC, et al. Addendum to the International Consensus Statement on testing and reporting of antineutrophil cytoplasmic antibodies. Quality control guidelines, comments, and recommendations for testing in other autoimmune diseases. Am J Clin Pathol 2003;120:312–8.
8. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271–7.
9. Manns MP, Czaja AJ, Gorham JD, Krawitt EL, Mieli-Vergani G, Vergani D, et al. Diagnosis and management of autoimmune hepatitis. Hepatology 2010;51:2193–213.
10. Conrad K, Chan EK, Fritzler MJ, Humbel RL, Meroni PL, Shoenfeld Y. From prediction to prevention of autoimmune disease. 2011;7.
11. Sowa M, Grossmann K, Knütter I, Hiemann R, Röber N, Anderer U, et al. Simultaneous Automated Screening and Confirmatory Testing for Vasculitis-Specific ANCA. PLoS One 2014;9:e107743.
12. Roggenbuck D, Schierack P, Sack U, Lapin SV, Mazing AV, Totolian AA. Novel methods for autoantibody detection in laboratory diagnostics of autoimmune rheumatic diseases. Medical Immunology 2014;16:221–6.
13. Rödiger S, Schierack P, Böhm A, Nitschke J, Berger I, Frommel U, et al. A Highly Versatile Microscope Imaging Technology Platform for the Multiplex Real-Time Detection of Biomolecules and Autoimmune Antibodies. Adv Biochem Eng Biotechnol 2013;133:35–74.
14. Willitzki A, Hiemann R, Peters V, Sack U, Schierack P, Rödiger S, et al. New platform technology for comprehensive serological diagnostics of autoimmune diseases. Clin Dev Immunol 2012;2012:284740.
15. Rantapaa-Dahlqvist S. What happens before the onset of rheumatoid arthritis? Curr Opin Rheumatol 2009;21:272–8.
16. Eriksson C, Kokkonen H, Johansson M, Hallmans G, Wadell G, Rantapaa-Dahlqvist S. Autoantibodies predate the onset of systemic lupus erythematosus in northern Sweden. Arthritis Res Ther 2011;13:R30.
17. Conrad K, Roggenbuck D, Reinhold D, Dörner T. Profiling of rheumatoid arthritis associated autoantibodies. Autoimmun Rev 2009;9:431–5.
18. Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. N Engl J Med 2003;349:1526–33.
19. Grossmann K, Roggenbuck D, Schröder C, Conrad K, Schierack P, Sack U. Multiplex assessment of non-organ-specific autoantibodies with a novel microbead-based immunoassay. Cytometry A 2011;79:118–25.
20. Knütter I, Hiemann R, Brumma T, Büttner T, Grossmann K, Cusini M, et al. Automated interpretation of ANCA patterns – a new approach in the serology of ANCA-associated vasculitis. Arthritis Res Ther 2012;14:R271.
21. Mahler M, Fritzler MJ. The clinical significance of the dense fine speckled immunofluorescence pattern on HEp-2 cells for the diagnosis of systemic autoimmune diseases. Clin Dev Immunol 2012;2012:494356.
22. Fenger M, Wiik A, Hoier-Madsen M, Lykkegaard JJ, Rozenfeld T, Hansen MS, et al. Detection of antinuclear antibodies by solid-phase immunoassays and immunofluorescence analysis. Clin Chem 2004;50:2141–7.
23. Agmon-Levin N, Damoiseaux J, Kallenberg C, Sack U, Witte T, Herold M, et al. International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. Ann Rheum Dis 2014;73:17–23.
24. Sack U, Conrad K, Csernok E, Frank I, Hiepe F, Krieger T, et al. Autoantibody Detection Using Indirect Immunofluorescence on HEp-2 Cells. Ann N Y Acad Sci 2009;1173:166–73.
25. Sack U, Conrad K, Csernok E, Frank I, Hiepe F, Krieger T, et al. Autoantikörpernachweis mittels indirekter Immunfluoreszenz an Hep-2-Zellen. J Lab Med 2012;36:135–41.
26. Van Blerk M, Van Campenhout C, Bossuyt X, Duchateau J, Humbel R, Servais G, et al. Current practices in antinuclear antibody testing: results from the Belgian External Quality Assessment Scheme. Clin Chem Lab Med 2009;47:102–8.
27. Op de Beeck K, Vermeersch P, Verschueren P, Westhovens R, Marien G, Blockmans D, et al. Detection of antinuclear antibodies by indirect immunofluorescence and by solid phase assay. Autoimmun Rev 2011;10:801–8.
28. Hiemann R, Büttner T, Krieger T, Roggenbuck D, Sack U, Conrad K. Challenges of automated screening and differentiation of non-organ specific autoantibodies on HEp-2 cells. Autoimmun Rev 2009;9:17–22.
29. Conrad K, Röber N, Rudorff S, Mahler M. DFS70-Autoantikörper – Biomarker zum Ausschluss ANA-assoziierter autoimmuner rheumatischer Erkrankungen. J Lab Med 2014;38:299–307.
30. Radice A, Bianchi L, Sinico RA. Anti-neutrophil cytoplasmic autoantibodies: methodological aspects and clinical significance in systemic vasculitis. Autoimmun Rev 2013;12:487–95.
31. Schulte-Pelkum J, Radice A, Norman GL, Hoyos ML, Lakos G, Buchner C, et al. Novel Clinical and Diagnostic Aspects of Antineutrophil Cytoplasmic Antibodies. J Immunol Res 2014;2014:1–13.
32. Meroni PL, Bizzaro N, Cavazzana I, Borghi MO, Tincani A. Automated tests of ANA immunofluorescence as throughput autoantibody detection technology: strengths and limitations. BMC Med 2014;12:38.
33. Meroni PL, Schur PH. ANA screening: an old test with new recommendations. Ann Rheum Dis 2010;69:1420–2.
34. Mierau R, Csernok E. Labordiagnostik bei Kollangenosen und Vaskulitiden. Akt Rheumatol 2014 2014;39:49–57.
35. Savige J, Gillis D, Benson E, Davies D, Esnault V, Falk RJ, et al. International Consensus Statement on Testing and Reporting of Antineutrophil Cytoplasmic Antibodies (ANCA). Am J Clin Pathol 1999;111:507–13.
36. Holle JU, Csernok E, Fredenhagen G, Backes M, Bremer JP, Gross WL. Clinical evaluation of hsPR3-ANCA ELISA for detection of antineutrophil cytoplasmatic antibodies directed against proteinase 3. Ann Rheum Dis 2010;69:468–9.
37. Sayegh J, Poli C, Chevailler A, Subra JF, Beloncle F, Deguigne PA, et al. Emergency testing for antineutrophil cytoplasmic antibodies combined with a dialog-based policy between clinician and biologist: effectiveness for the diagnosis of ANCA-associated vasculitis. Intern Emerg Med 2015;10:315–9.
38. Hiemann R, Hilger N, Michel J, Nitschke J, Böhm A, Anderer U, et al. Automatic analysis of immunofluorescence patterns of HEp-2 cells. Ann N Y Acad Sci 2007;1109:358–71.

39. Roggenbuck D, Conrad K, Reinhold D. High sensitive detection of double-stranded DNA antibodies by a modified Crithidia luciliae immunofluorescence test may improve diagnosis of systemic lupus erythematosus. Clin Chim Acta 2010;411:1837–8.
40. Conrad K, Ittenson A, Reinhold D, Fischer R, Roggenbuck D, Büttner T, et al. High sensitive detection of double-stranded DNA autoantibodies by a modified Crithidia luciliae immunofluorescence test. Ann N Y Acad Sci 2009;1173:180–5.
41. George S, Paulick S, Knütter I, Röber N, Hiemann R, Roggenbuck D, et al. Stable Expression of Human Muscle-Specific Kinase in HEp-2 M4 Cells for Automatic Immunofluorescence Diagnostics of Myasthenia Gravis. PLoS One 2014;9:e83924.
42. George S, Georgi M, Roggenbuck D, Conrad K, Küpper JH. A strategy for cell-based multiplex diagnostics of Myasthenia gravis and autoimmune encephalitis by modifying the subcellular localization of cell membrane autoantigens. Clin Hemorheol Microcirc 2014;58:211–28.
43. Bizzaro N, Antico A, Platzgummer S, Tonutti E, Bassetti D, Pesente F, et al. Automated antinuclear immunofluorescence antibody screening: A comparative study of six computer-aided diagnostic systems. Autoimmun Rev 2014;13:292–8.
44. Egerer K, Roggenbuck D, Hiemann R, Weyer MG, Büttner T, Radau B, et al. Automated evaluation of autoantibodies on human epithelial-2 cells as an approach to standardize cell-based immunofluorescence tests. Arthritis Res Ther 2010;12:R40.
45. Hiemann R, Roggenbuck D, Sack U, Anderer U, Conrad K. Die Hep-2-Zelle als Target für multiparametrische Autoantikörperanalytik – Automatisierung und Standardisierung. J Lab Med 2011;35:351–61.
46. Bonroy C, Verfaillie C, Smith V, Persijn L, De WE, De KF, et al. Automated indirect immunofluorescence antinuclear antibody analysis is a standardized alternative for visual microscope interpretation. Clin Chem Lab Med 2013;1–9.
47. Roggenbuck D, Hiemann R, Schierack P, Reinhold D, Conrad K. Digital immunofluorescence enables automated detection of antinuclear antibody endpoint titers avoiding serial dilution. Clin Chem Lab Med 2014;52:e9–e11.
48. Roggenbuck D, Hiemann R, Bogdanos D, Reinhold D, Conrad K. Standardization of automated interpretation of immunofluorescence tests. Clin Chim Acta 2013;421C:168–9.

Article note: Original German online version at: http://www.degruyter.com/view/j/labm.2014.38.issue-6/issue-files/labm.2014.38.issue-6.xml. The German article was translated by Compuscript Ltd. and authorized by the authors.