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Evaluation of Anti-Nuclear Antibody (ANA) Measurement Methods

Güler Buğdaycı,[1] MD, Mualla Polat,[2]* MD

Address: [1]Department of Medical Biochemistry, [2]Abant Izzet Baysal University, School of Medicine, Department of Medical Dermatology, Bolu, Turkey

E-mail: polatmualla@gmail.com

** Corresponding Author:* Dr. Mualla Polat, Abant Izzet Baysal University, School of Medicine, Department of Medical Dermatology, Bolu, Turkey

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Abstract

Background: Anti-nuclear antibody (ANA) is used as a screening test for autoimmune diseases as well as being utilized for diagnostic and classification purposes. As a gold standard for ANA, manual indirect immunofluorescence (IIF) technique is used; although it has high sensitivity, it is time-consuming and requires skilled operators. For ANA tests, clinical and research laboratories mainly use manual indirect immunofluorescence method and for antibodies enzyme linked immunosorbent assay (ELISA) is used. During recent years, in an attempt to replace the manual method, several comparative studies have been performed for using automated immunofluorescence method and fully automated multiplex immunoassay method. Automated methods have high costs, these systems also have disadvantages like giving false negative results and certain technical problems experienced at the stage of identification; however, as they provide the users with the opportunity to standardize the test results and to rapidly run and report the test results it is possible to see more widespread use of these systems in clinical laboratories thanks to the technical advancements in the field.

Introduction

The most critical function of the immune system is to discriminate self from non-self. Autoimmunity develops as a result of disregulations of the immune tolerance mechanisms [1]. Independent of the mechanism of autoimmune disease concerned, there are circulating autoantibodies in the blood stream in these disorders. Although the autoantibodies are important serological features of autoimmune diseases, their presence is not exclusive for these conditions. The indications for use are to establish a diagnosis of autoimmune disease in patients having suggestive clinical symptoms, to exclude a diagnosis of autoimmune disease in patients with few or uncertain clinical signs, to subclassify patients with a known diagnosis, and to monitor disease activity [2]. Detection of serum autoantibodies in clinical practice has

Table 1. Anti-Nuclear (ANA) Antibody Assay

	Sensitivity (%)	Specifity (%)
Systemic Lupus Erythematosus	>95	60
Romatoid Artrit	45	60
Scleroderma	60	50
Polymyositis Dermatomyositis	60	60
Sjögren's Sydrome	50	50

Table 2. Some Technical Specifications of Automated Indirect Immunofluorescence System

System	Throughput (Sample/Hour)	Automated pattern recognization type	Microscope Type
Aklides	48-60	Homogeneous, speckled, nucleolar, centromeric, nuclear dots, mitotic, cytoplasmic	Olympus IX81, Microscope
EUROPattern	90	Homogeneous, speckled, nucleolar, centromeric, nuclear dots, mitotic, nuclear membrane, cytoplasmic	EUROPattern Microscope
Helious	150	None	Helmed IFA Processor, Nikon Microscope
Imagine Navigator	90	None	Imagine Microscope
Nova View	48-60	Homogeneous, speckled, nucleolar, centromeric, nuclear dots	Olympus IX81
Zenit G-Sight	14-48	Homogeneous, speckled, nucleolar, centromeric, cytoplasmic/mitochondrial	Zenit G-Sight Microscope

become more available to clinicians worldwide while providing them with a powerful diagnostic tool [**2**].

Autoantibody testing has some limitations during its use [**3**]. Autoantibodies should only be considered as markers of disease. They are also found in normal individuals in the absence of any definable disease and with increasing prevalence in the aging population. This lack of specificity makes autoantibody testing only a part of a diagnostic panel at best. A multitude of kits is now available for the detection and quantification of autoantibodies. Unfortunately, there are few reliable national or international standards, and there is a huge variation between reagent producers in the preparation and source of antigens and the methods. Results are often reported in arbitrary units, and every method will have different cut-off values, reference ranges and measuring ranges. Overall, this makes comparing methods, interpreting published data and carrying out multicenter studies difficult [**3**].

As a historical note; the nucleus was the first intracellular structure identified by *Franz Bauer* in 1802 and in 1943, nearly a century later, serum reactivity against nuclear structures, i.e. antinuclear antibodies (ANA), was observed in a positive LE cell test [**4**]. But it was not until 1964 that the reality of autoimmunity as an important cause of human disease received public acknowledgment and consensus during an International Conference on Autoimmunity, assembled by the New York Academy of Sciences [**5**].

ANA is the brand name of the antibodies towards nuclear and cytoplasmic structures of the cell. ANA is a screening test used for rheumatologic and nonrheumatologic autoimmune diseases [**6**]. When compared with other antibodies ANA assay has the highest sensitivity in the diagnosis of autoimmune diseases (**Table 1**) [**7**]. Both microscopy and immunoassay methods have significant importance in laboratory medicine. The methods for detecting ANA are manual and automated indirect immunofluorescence (IIF) methods as well as enzyme linked immunoassay (ELISA) [**8**]. During recent years, the use of fully automated BioPlex 2200 (Bio-Rad) which is a Multiplex immunoassay increased significantly and it is regarded as the most current technique to detect ANA and antibodies [**9**].

Conventional methods of testing ANA

In a patient with a suspicion of autoimmune disease ANA positivity/negativity is first assessed by indirect immunofluorescence technique. A positive IIF result leads to further investigations using ELISA to detect specific markers such as anti-dsDNA antibodies and/or anti-extractable nuclear antigens (anti-ENA) antibodies. ELISA uses specific nuclear antigens (SS-A, SS-B, Sm, Sm/RNP, Jo-1, and Scl-70) coated on a multi-well plate to detect antibodies in a patient's serum [**10**].

Table 3. Autoimmune Disease Associated with Antibodies That can be Detected by BioPlex 2200

Disease	Antibodies
Systemic Lupus Erythematosus	dsDNA, SSA-60 kD, SSA-52kD, SSB, SM, Am/RNP, RNP-A, RNP-60kD, Chromatin, Centromere B, Ribosomal P
Scleroderma	dsDNA, SSA-60 kD, SSA-52kD, SSB, Chromatin, Scl-70, Centromere B, Ribosomal P
Sjögren's Syndrome	SSA-60 kD, SSA-52kD, SSB, Chromatin
Polymoyositis/Dermatomyositis	SSA-60 kD, SSA-52kD, Jo-1
Mixed Connective Tissue Disease	dsDNA, , SSA-60 kD, SSA-52kD, Sm, Chromatin, Scl-70

Serum samples are then added to the wells and ANA, if present, binds to the wells. Following washing, labeled ligand is added to the wells to detect bound antibody. The concentration of antibody is determined by comparison to a standard curve generated by known concentrations of ANA [**11**].

Indirect Immunofluorescence Assay

The gold standard for ANA detection is still IIF on human epithelial (HEp-2) cells. This is a manual technique which is time consuming; it also requires advanced training and creates standardization and harmonization problems that is why automated indirect immunofluorescence methods started to be used during recent years [**12**].

Automated reading of ANA IIF

Currently, at least six commercial systems for the automated reading of ANA IIF are available: Aklides (Medipan, Dahlewitz, Germany), EUROPattern (Euroimmun AG, Luebeck, Germany), Helios (Aesku Diagnostics, Wendelsheim, Germany), Image Navigator (ImmunoConcepts, Sacramento, CA), NOVA View (Inova Diagnostics, San Diego, CA), and Zenit G-Sight (A. Menarini Diagnostics, Florence, Italy). These systems are based on a composition of different hardware modules combined with mathematical pattern-recognition software algorithms, enabling fully automated image acquisition, analysis, and evaluation of IIF ANA tests. Certain technical features of these systems and the microscope types they are equipped with are summarized on **Table 2** [**13, 14**].

For automation in indirect immunofluorescence systems, positive/negative agreement percentages and/or percentage intervals that have been published so far are 90-99% for Aklides, 93-100% for Nova View, 94% for Europattern, 96% for Zenit G _Sight, 98% for Helious and 97% for Imagine Navigator [**15, 16, 17, 18, 19, 20**].

BioPlex 2200 (Bio-Rad); a Multiplex Platform

BioPlex 2200 is a system whose use has increased significantly during recent years; it is fully automated and is developed as a luminex-based system, it can detect 13 different autoantibodies and has a capacity to read 96-well plates in 35 minutes. The flow fluorometry includes two different lasers, the first laser is for identification and the second is for quantification. With BioPlex 2200, qualitative ANA screening and quantitative detection of 13 antibodies is possible. The antibodies that can be detected with this technique are dsDNA, chromatin, ribosomal protein, SSA-52, SSA-60, SSB, Sm, SmRNP, RNP-A, RNP-68, Scl-70, Jo-1, and centromere B, the list of diseases in which these antibodies have been detected has been given on **Table 3** [**21**]. In a laboratory that receives 70-80 samples a day, the whole procedure can be completed within 90 minutes, in other systems manual procedures require at least 7-8 hours for the reporting of the results. ANA testing by multiplexing has good concordance with the comparative methods [**22**].

In conclusion manual IIF that is used as a gold standard has cost related disadvantages as well as requiring time consuming procedures and necessitating reporting by

two well trained staff members. There are limitations in front of using IIF in automation, these are: having high costs at the moment and the need for further technical development at the stage of cellular identification and differentiation. The advantages that can be anticipated from this system are having fast output in the laboratory workflow with these tests, decreased frequency of false positive and negative results, the reduction of intra- and inter-laboratory variability and the ability to achieve harmonization. As a fully automated system using multiplex technology BioOne 2200 can be utilized by laboratories having high number of samples.

References

1. Jiang S, Lechler RI. Regulatory T cells in the control of transplantation tolerance and autoimmunity. Am J Transplant. 2003; 3: 516-524. PMID: 12752307
2. Jacobe HT, Sontheimer RD. Autoantibodies Encountered in Patients with Autoimmune Connective Tissue Diseases. In: Bolognia JL, Jorizzo J, Rapini RP, Callen JP, Horn TD, Mancini AJ et al. eds. Dermatology 2 nd ed. Spain: Mosby Elsevier; 2008: 549-560.
3. Sheldon J. Laboratory testing in autoimmune rheumatic diseases. Best Pract Res Clin Rheumatol 2004; 18: 249-269. PMID: 15158740
4. Miescher P, Fauconnet M. Absorption of L. E. factor by isolated cell nuclei. Experientia 1954; 10: 252–253. PMID: 13183062
5. Lleo A, Invernizzi P, Gao B, Podda M, Gershwin ME. Definition of human autoimmunity--autoantibodies versus autoimmune disease. Autoimmun Rev 2010; 9: A259-266. PMID: 19963079
6. Fritzler MJ, Wiik A, Fritzler ML, Barr SG. The use and abuse of commercial kits used to detect autoantibodies. Arthritis Research and Therapy. 2003; 5: 192–201. PMID: 12823850
7. Castro C, Gourley M. Diagnostic testing and interpretation of tests for autoimmunity. J Allergy Clin Immunol; 2010; 2: S238-247. PMID: 20061009
8. Ulvestad E, Kanestrom A, Madland TM, Thomassen E, Haga HJ, Vollset SE. Evaluation of diagnostic tests for antinuclear antibodies in rheumatological practice. Scand J Immunol 2000; 52: 309-315. PMID: 10972909
9. Tozzoli R, Bonaguri C, Melegari A, Antico A, Bassetti D. Current state of diagnostic techologies in the autoimmunology laboratory. Clin Chem Lab Med 2013; 51: 129-138. PMID: 23092800
10. https:// www.aacc.org/ publications/ cln/ articles/2014/june/ana-testing
11. Kivity S, Gilburd B, Agmon-Levin N, Carrasco MG, Tzafrir Y, Sofer Y, et al. A novel automated indirect immunofluorescence autoantibody evaluation. Clin Rheumatol 2012; 31: 503-509. PMID: 22057233
12. Bonroy C, Verfaillie C, Smith V, Persijn L, De Witte E, De Keyser F, et al. Automated indirect immunofluorescence antinuclear antibody analysis is a standardized alternative for visual microscope interpretation. Clin Chem Lab Med 2013; 51: 1771-1779. PMID: 23612554
13. Meroni PL, Schur PH. ANA screening: an old test with new recommendations. Ann Rheum Dis 2010; 69: 1420-1422. PMID: 20511607
14. Vazzana I, Borghi MO, Tincani A. Autamated tests of ANA immunfluorescence as throughput autoantibody detection technology: strengths and limitations. BMC Medicine 2014; 12: 38. PMID: 24589329
15. Tozzoli R, Antico A, Porcelli B, Bassetti D. Automation in indirect immunofluorescence testing: a new step in the evalution of the outoimmonology laboratory. Auto Immun Highlights 2012; 3: 59-65. PMID: 26000128
16. 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. PMID: 20214808
17. Melegari A, Bonaguri C, Russo A, Luisita B, Trenti T, Lippi G. A comparative study on the reliability of an automated system for the evaluation of cell-based indirect immunofluorescence. Autoimmun Rev 2012; 11: 713-716. PMID: 22269861
18. Voigt J, Krause C, Rohwäder E, Saschenbrecker S, Hahn M, Danckwardt M, et al. Automated indirect immunofluorescence evaluation of antinuclear autoantibodies on HEp-2 cells. Clin Dev Immunol 2012; 2012: 651058. PMID: 23251220
19. Bossuyt X, Cooreman S, De Baere H, Verschueren P, Westhovens R, Blockmans D, et al. Detection of antinuclear antibodies by automated indirect immunofluorescence analysis. Clin Chim Acta 2013; 415: 101-106. PMID: 23026234
20. 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: 292-298. PMID: 24220268
21. Shovman O, Gilburd B, Barzilai O, Shinar E, Larida B, Zandman-Goddard G,et al. Evaluation of the BioPlex 2200 ANA screen: analaysis of 510 healthy subjects: incidence of natural/predictive autoantibodies. Ann NY Acad Sci 2005; 1050: 380-388. PMID: 16014555
22. Desplat-Jego S, Bardin N, Larida B, Sanmarco M. Evaluation of the BioPlex 2200 ANA screen fort he detection of antinuclear antibodies and comparison with conventional methods. Ann NY Acad Sci 2007; 1109: 245-255. PMID: 17785313