

Evaluation of the BioPlex 2200 ANA Screen for the Detection of Antinuclear Antibodies and Comparison with Conventional Methods

SOPHIE DESPLAT-JEGO,^a NATHALIE BARDIN,^a BRUNO LARIDA,^b
AND MARIELLE SANMARCO^a

^a*Laboratoire d'Immunologie, Hôpital de la Conception, Marseille, France*

^b*Bio-Rad Laboratories, Hercules, California, USA*

ABSTRACT: BioPlex 2200 multiplexed assays system is an automatic method allowing detection of antinuclear antibodies (ANA). The aim of our study was to evaluate the determination of 13 autoantibodies against chromatinic and nonchromatinic nuclear antigens by the BioPlex 2200 system and to compare the results achieved by this method to those obtained with our routinely used immunoassays. One thousand and four serum samples consecutively sent for ANA detection were routinely tested by indirect immunofluorescence (IIF) on HEp2 cells. Among them, 321 were also analyzed by dsDNA enzyme immunoassay (EliA) test and 657 by double immunodiffusion (DID) for extractable nuclear antigen (ENA) antibodies. All the sera were evaluated by the BioPlex 2200 ANA screen kit allowing simultaneous detection of antibodies against the following antigens: dsDNA, chromatin, SSA-52 kDa, SSA-60 kDa, SSB, Sm, Sm/RNP, RNP-A, RNP-68 kDa, Scl70, centromere B, Jo-1, and P ribosomal proteins. The kappa coefficient between BioPlex 2200 and routine tests for detection of ANA on HEp2 cells, anti-dsDNA, and anti-ENA antibodies was, respectively, 0.31, 0.66, and 0.61. The comparison with our routine tests showed numerous discrepancies between IIF ANA screening and BioPlex but a good concordance for detection of anti-dsDNA and anti-ENA specificities. BioPlex 2200 system is a rapid and sensitive method for simultaneous quantitative detection of several autoantibodies. It is perfectly well adapted to determine ANA antigenic specificities of samples found positive using initial IIF screening. The capability of this multiplexed technology to analyze simultaneously 13 ANA autoantibodies leads to the rapid availability of an "autoimmune connective tissue disease serologic profile."

Address for correspondence: Sophie Desplat-Jego, Laboratoire d'Immunologie, Hôpital de la Conception, 147, Bd. Baille, Marseille, France. Voice: 33-4-91-38-39-07; fax: 33-4-91-38-36-33.
sophie.jego-desplat@ap-hm.fr

Ann. N.Y. Acad. Sci. 1109: 245–255 (2007). © 2007 New York Academy of Sciences.
doi: 10.1196/annals.1398.030

KEYWORDS: antinuclear antibodies; Luminex-based assay; anti-dsDNA antibodies; antiextractable nuclear antigen antibodies

INTRODUCTION

Antinuclear antibodies (ANA) are a common feature of autoimmune connective tissue diseases (ACTD), such as systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), Sjögren's syndrome (SS), scleroderma (Scl), dermatomyositis (DM), and polymyositis (PM).¹⁻³ The measurement of ANA is used for screening, diagnosis, and monitoring of these diseases. The term ANA refers to a large family of antibodies that react with nuclear constituents of cells, such as the nuclear membrane, nucleoplasm, nucleoli, and nuclear organelles. All these antibodies do not have the same clinical significance: some are associated with both pathological and non-pathological conditions, while others are closely associated with ACTD.³⁻⁵ Currently, the ANA screening test is performed using an indirect immunofluorescence (IIF) assay on HEp2 cells, which allows the detection of a large number of autoantibodies that bind to a variety of nuclear antigens from all the cellular constituents listed above.⁶ A positive ANA screening leads to further laboratory evaluations in order to detect the presence of markers useful for the diagnosis of ACTD, such as anti-dsDNA antibodies or antiextractable nuclear antigens (anti-ENA) antibodies. These are mainly detected either by radioimmunoassay, by enzyme-linked immunosorbent assay (ELISA) using purified native or recombinant antigens or by immunodiffusion techniques using thymic and/or spleen extracts. These techniques used in autoantibodies identification are time-consuming and characterized by frequent discrepancies in the results between laboratories.⁶⁻⁹ Recently, new automated technologies have been introduced to screen for multiple antibodies. Recent reports suggest that automated Luminex-based systems can rapidly determine a profile of multiple antibodies having a clinical significance.¹⁰⁻¹⁹ These systems use a new approach for the measurement of autoantibodies, based on multiple dyed beads, coated with specific antigen targets, and on flow cytometry detection.

The BioPlex 2200 ANA screen is a fully automated Luminex-based assay using magnetic beads to facilitate washing before and after conjugate addition. This system allows the simultaneous detection in one sample of 13 autoantibodies (reacting with SSA (52 and 60 kDa), SSB, Sm, Sm/RNP, RNP-A, RNP-68 kDa, Scl70, centromere B, dsDNA, chromatin, Jo1, ribosomal P proteins). Recently, it has been successfully evaluated on healthy subjects.²⁰ In this study, we evaluate for the first time, the performance of the BioPlex 2200 ANA screen on a retrospective hospital cohort of 1,104 patient samples. We compare the results obtained with data from our in-house testing scheme, which includes IIF on HEp2 cells, DID and an anti-dsDNA enzyme immunoassay test.

PATIENTS

Patient Samples

Our study included all the venous blood samples consecutively sent to our hospital immunology laboratory over a 3-week period for detection of ANA. After serum separation, samples were stored at -80°C until further analysis on BioPlex 2200. Patients were submitted by the internal medicine, rheumatology, dermatology, and obstetrics units of the university teaching hospital in Marseilles. Our study was not a clinical study and clinical charts were reviewed only for a few patients in case of discrepancies in the results between the multiplex method and routine tests.

METHODS

BioPlex 2200 Automated System

The BioPlex 2200 system employs multiplexed technology to perform measurements of 13 autoimmune antibodies simultaneously in one tube. For convenience in presenting results from the BioPlex 2200 system, these antibodies have been divided into three groups: (a) antibodies against chromatinic nuclear antigens (CNA); (b) antibodies against nonchromatinic nuclear antigens (NCNA); and (c) antibodies against cytoplasmic antigens (CyA). The autoantibodies detected with BioPlex 2200TM system and ANA screen kit (Bio-Rad, Hercules, CA) and the source of antigens precoated on dyed beads are the following: dsDNA (produced by PCR from plasmid), chromatin (purified from calf thymus), centromere B, Scl70, RNP-68 kDa, RNP-A, SSA-52 kDa, and Jo1 (recombinant), SSA60 kDa, SSB Sm, Sm/RNP, and P ribosomal proteins (affinity-purified).

The mixture of beads, separately coated with the listed antigens, was combined with patient sample and diluent and then allowed to incubate for 20 min at 37°C . After a wash cycle, anti-human IgG antibody conjugated to phycoerythrin (PE) was added to the dyed beads and allowed to incubate for 10 min at 37°C . Following removal of excess conjugate, the mixture passed through the detector, which identifies the bead type based on the fluorescence of the dyed bead. The amount of antibody bound to the bead was determined using a second laser based upon the fluorescence of PE. For this method, we reported sera that were positive for one or more of the 13 antibodies as "BioPlex +."

IIF on HEp2 Cells

All serum samples were diluted 1:100 in phosphate-buffered saline and tested on a single lot of commercially prepared HEp-2 cells (Bio-Rad, Marnes-la-Coquette, France) according to the manufacturer's instructions and using

the PhD automated pipetter (Bio-Rad, Hercules, CA). Antigen–antibody complexes were detected using a fluorochrome-labeled anti-human IgG, IgA, IgM antiserum (Bio-Rad, Marnes-la-Coquette, France). The same microscope and settings were used for all samples, and the slides were read by two independent readers.

dsDNA EliA Test

Anti-dsDNA antibodies were investigated in response to requests from clinicians and in the follow-up of positive samples for ANA screening test. They were measured according to the manufacturer's instructions for the dsDNA EliA™ test (Pharmacia BV Diagnostics GmbH and Co, Freiburg, Germany) using single polystyrene wells coated with double-stranded cyclic plasmid DNA of *Escherichia coli*. This test was performed for 321 sera out of 1104. Samples were automatically dispensed and processed in the Unicap® 100 instrument at 37°C (Pharmacia and Upjohn Diagnostics GmbH and Co, KG, Freiburg, Germany).

Ouchterlony Double Immunodiffusion (DID)

Anti-ENA antibodies (including in this work anti-NCNA antibodies but also anti-Scl70 and anti-Jo1 antibodies, as already described in the literature) were tested according to the clinician requests even though the ANA screening test was negative. The assay was performed as previously described,²¹ using home-made agarose gels and a lyophilized mammalian extracted nuclear antigen (Auto I.D, Immunoconcepts, Sacramento, CA). This test was performed for 657 sera out of 1104.

Statistical Analysis

The kappa agreement coefficients were calculated to estimate agreement between the various methods evaluated.

RESULTS

Prevalence and Antigenic Specificity of Autoantibodies Detected

A total of 1104 consecutive serum samples were evaluated for the presence of various ANA by the BioPlex 2200 ANA screen. However, by routine tests except IIF, not all sera were tested. Of the 1104 sera tested, our routine tests identified 411 sera positive for IIF on HEp2 cells, a majority ($n = 311$) having

TABLE 1. Prevalence and antigenic specificities of autoantibodies ($n = 221$) detected in 1104 sera with BioPlex 2200 system.

Antigenic specificity	Number of positive
Nonchromatinic nuclear	
RNP 68	10 (4.5%)
RNP A	49 (22.2%)
Sm	22 (9.9%)
Sm/RNP	41 (18.5%)
SSA52 kD	38 (17.2%)
SSA60 kD	62 (28%)
SSB	39 (17.6%)
Chromatinic nuclear	
dsDNA	39 (17.6%)
Chromatin	82 (37.1%)
Centomere B	19 (8.6%)
Scl-70	15 (6.8%)
Cytoplasmic	
Jol	4 (1.8%)
P ribosomal	11 (5%)

speckled patterns. Fifty four percent of them ($n = 222$) were associated with a titer of 200 or less. Six hundred fifty-seven sera out of 1104 were tested by DID and a positive reactivity was found in 70 cases. Moreover, EliA test for anti-dsDNA autoantibodies detection identified 45 positive sera out of the 321 analyzed.

Using the BioPlex 2200 ANA screen, 221 out of the 1104 sera tested reacted with at least one autoantigen from the available antigen panel (TABLE 1). The most frequently detected autoantibody was antichromatin (37.1%), which is not specifically identified on HEp2 cells or with other routine tests. Antichromatin antibodies were found alone in 35 out of 82 positive sera. In the remaining samples, they were mainly associated with anti-dsDNA antibodies ($n = 26$, not shown). Among NCNA antibodies detected, anti-SSA 60 kDa antibodies were the most frequent ($n = 62$, 28%), followed by anti-RNP-A antibodies ($n = 49$, 22.2%). Among the 22 anti-Sm positive sera, 20 were associated with anti-RNP antibodies. We found that 83 out of 221 positive sera showed reactivity with more than one autoantibody. Even though 52% of anti-SSA 60 kDa were detected in the absence of anti-SSA 52 kDa, the combination of these two antibodies was the most frequent.

Comparison between BioPlex2200 and Routine Tests

BioPlex results were then compared with data obtained from our routine tests (FIG. 1). The kappa agreement indices were, respectively, 0.31, 0.61, and 0.66 between BioPlex 2200 and IIF on HEp2 cells (FIG. 1A), EliA dsDNA

(FIG. 1B), and DID for ENA detection (FIG. 1C), respectively. Two hundred fifty five out of the 1104 sera reacted on HEp2 cells but were negative with the BioPlex 2200 system (FIG. 1A). The staining pattern was mainly speckled ($n = 206/255$) with a titer of 100 ($n = 83/206$). Among these 255 samples, 247 were negative for both DID and EliA tests. HEp2 cells IIF was negative for 26.8% (22/82) of sera displaying antichromatin antibodies with the BioPlex 2200 system. It was worth noting that none of the sera antichromatin positive with IIF (60/82) displayed an IIF pattern suggesting the presence of an antichromatin antibody. Concerning anticentromere B antibodies, BioPlex 2200 system detected 19 positive sera. These findings were confirmed by IIF in 17 out of the 19 cases. Conversely, two sera displayed a staining pattern of centromeres by IIF and were found negative with the BioPlex2200 system.

Analysis of Discrepant Results between BioPlex 2200 and Anti-dsDNA and Anti-ENA Routine Tests

Five sera were positive for anti-dsDNA antibodies by the use of BioPlex 2200 system and negative with EliA method (FIG. 1B). All reacted on HEp 2 cells but mainly with a speckled staining pattern. Eighteen sera were negative for anti-dsDNA antibodies by BioPlex 2200 system but positive with the EliA test (FIG. 1B). All were positive for the ANA screening test by IIF.

Regarding the detection of anti-ENA antibodies, the reactivity found with the BioPlex 2200 system was not confirmed by DID for 63 sera (FIG. 1C). Among these 63 BioPlex+/DID- sera, 24 had a negative ANA test on HEp2 cells. They were found positive with the BioPlex 2200 for the following specificity: SSA 52 kDa ($n = 3$), SSA 60 kDa ($n = 2$), SSB ($n = 6$), Sm/RNP ($n = 3$), RNP-A ($n = 5$), Sm ($n = 1$), Scl70 ($n = 3$), and Jo1 ($n = 1$). On the other hand, 39 were positive on Hep 2 cells and among them, 33 displayed a speckled staining pattern. Reactivity linked to these 33 sera was: SSA 52 kDa ($n = 1$), SSA 60 kDa ($n = 8$), Sm/RNP ($n = 11$), RNP-A ($n = 13$), Sm ($n = 3$), and Scl70 ($n = 3$). Auto-antibodies were detected by DID only for three patients (BioPlex -/DID+). All reacted against PM/Scl antigen and they were collected from three patients presenting a PM/Scl overlapping syndrome. The reactivity toward P ribosomal proteins found with BioPlex 2200 ($n = 11$) was not compared to another test; nevertheless, none of the 11 sera had an IIF pattern on HEp2 cells evoking such a specificity.

DISCUSSION

In this study, the BioPlex 2200 ANA screen, a fully automated Luminex-based assay, was evaluated for the detection of ACTD-associated antibodies. The purpose of this study was to describe the performances of BioPlex 2200 by comparison with our routine tests but not to determine the level of clinical

(A) ANA (n=1104)		BioPlex 2200™	
IIF		Positive	Negative
	Positive	156	255
	Negative	65	628
Kappa coefficient: 0.31			

(B) Anti dsDNA Ab (n=321)		BioPlex 2200™	
dsDNA ELIA™		Positive	Negative
	Positive	27	18
	Negative	5	271
Kappa coefficient: 0.66			

(C) Anti-ENA Ab* (n=657)		BioPlex 2200™	
DID		Positive	Negative
	Positive	67	3
	Negative	63	484
Kappa coefficient: 0.61			

FIGURE 1. Correlation between routine tests and BioPlex 2200 for detection of anti-nuclear antibodies (ANA) (A), antibodies against dsDNA (B), and antibodies against extractable nuclear antigens (ENA) (C). Ab = antibodies; IIF = indirect immunofluorescence; DID = double immunodiffusion. *Including anti-Scl 70 and anti-Jo1 antibodies.

concordance of autoantibody detection using various methods. The capability of this multiplexed technology to analyze simultaneously 13 ANA autoantibodies leads to the rapid determination of an “ACTD serologic profile.” Using this new system, we observed a reactivity against at least 1 of the 13 simultaneously tested autoantigens for 221 out of the 1104 consecutive sera tested (20.13%). The most frequently detected autoantibody was directed against chromatin (37.1%). The association of two or more autoantibodies, especially anti-SSA 52 kDa with SSA 60 kDa, was frequently found (83 sera out of 221). The kappa coefficient between BioPlex 2200 and routine tests for detection of anti-dsDNA and anti-ENA antibodies was good. Indeed, the kappa value for IIF was considered unacceptably low, but not surprising. The screening test on HEp2 cells allows to evaluate a larger panel of antigenic specificities than BioPlex 2200 system. Moreover, with IIF, antigens display native epitopes in their original conformation. Such epitopes are lost during purified or recombinant antigen preparation. These parameters enhance the sensitivity of IIF screening test. Moreover, the use of a polyspecific antiserum can also reveal natural autoantibodies of IgM isotype, occurring in healthy people, but in such cases titers obtained are usually low.²⁰ Finally, it is not surprising that discrepant sera IIF+/BioPlex– was mostly found with a titer of 100 corresponding to the positivity threshold of our method. In cases of discrepancies between two methods, for example, BioPlex 2200 and DID, only clinical data could allow us to draw a conclusion about the pertinence of detected results. Moreover, a significant number of patients lacked an established diagnosis at

the sampling time, therefore we could only hypothesize: (a) false positives are obtained with one of the two compared methods, and/or (b) antigens used and especially presented epitopes are not exactly the same in the two methods and so the reactivity tested is different, and/or (c) as previously described, the presence of serum antibodies precedes the clinical onset of the disease and so the follow-up of the patients should be informative.²² The BioPlex 2200 ANA screen profile includes autoantibodies against CNA, NCNA, and CyA. It can be noted that this large panel may be useful in establishing an ACTD diagnosis but may be inappropriate for following up patients displaying only one or two antibodies of well-known specificities.

In the group of CNA, chromatin was the most frequently found specificity in the tested samples. Mainly found alone, this reactivity was not correlated with a fluorescent staining pattern on HEp2 cells, which questions the significance of these antibodies and above all their clinical relevance. To our knowledge, there is not yet a large-scale prospective published study analyzing the diagnostic interest of antichromatin antibodies. Antibodies to dsDNA are highly specific for SLE but the level of clinical concordance depends on the assay used and the cut-off established. The BioPlex system and EliA had the same source of antigen but other technical points, such as the nature of the coating support, the time of coating or the molecular weight of DNA could explain discrepant results between the two methods.²³ Among the 18 sera BioPlex –/dsDNA EliA+, 9 sera were found positive just at the cutoff level in EliA test and five out of the nine patients did not suffer from an SLE (data not shown). But because of the small number of patients, further testing is required. Another study from our team enrolling a large cohort of patients suffering from SLE, and clinically well documented, is in progress. As regards the antibodies associated with Scl, antibodies against centromere B and Scl70, the BioPlex 2200 and IIF seem to be equivalent. In only one case, a diagnosis of CREST syndrome could be assigned for a patient. This patient was repeatably negative with HEp2 test and positive for the automated multiplex system.

Reactivity against NCNA could also be tested with BioPlex 2200 ANA screen. This method allows separate testing for antibodies against recombinant (52 kDa) and purified (60 kDa) forms of SSA antigen. The SSA 52 kDa mainly co-occurs with SSA 60 kDa in the tested samples, but in six cases, we found a reactivity against SSA 52 kDa only. Some authors have proposed that SSA 52 kDa represents an antigenic target strongly associated with the autoimmune response in mothers whose children have neonatal lupus and congenital heart block, but studies on this topic are discrepant especially due to differences in SSA 52 kDa antibodies detection methods, the nature of the recombinant protein used and in the selection of patients.^{24–26} Regarding anti-SSB antibodies, the BioPlex 2200 system detected six positive sera not confirmed by DID and negative for HEp2 test. Among them, three were detected alone and not associated with an autoimmune disease while the three others were associated with other antibodies, especially anti-SSA in patients suffering from

autoimmune diseases. These data suggest a higher sensitivity of BioPlex 2200 than routine tests for anti-SSB detection but also underline the necessity for the biologist to attempt as far as possible taking account of clinical context. Until recently, the detection of RNP antibodies was limited by the inability to isolate RNP antigens from the complex Sm/RNP. Using the recombinant RNP proteins used in BioPlex 2200 ANA screen, direct and reliable identification of a specific reactivity against two of the different proteins belonging to the RNP antigen (RNP-68 kDa and/or RNP-A) has become possible. Moreover, to determine the frequency of antibodies to RNP -68 kDa on the one hand and to RNP-A on the other may provide additional information for better classifying a patient between MCTD and SLE. The interest of an additional testing of reactivity against RNP-C remains to be determined.

In conclusion, we can postulate that BioPlex 2200 system is suitable as a sensitive screening test to confirm or to exclude the presence of a large number of autoantibodies simultaneously and rapidly. We propose to continue performing initial screening of ACTD suspect patients with IIF on HEp2 cells especially as various specificities are not tested by the BioPlex 2200 system, such as PM/Scl, PCNA, actin, mitochondria, and so on. Further, a positive IIF ANA result at screening should be a prerequisite for ordering further specific ANA testing, especially with the BioPlex system. BioPlex 2200 is a very useful, well-adapted and effective tool to precisely, easily, and rapidly characterize the antigenic reactivity of the sera found positive on an IIF screening test.

REFERENCES

1. CATOGGIO, L.J., R.M. BERNSTEIN, C.M. BLACK, *et al.* 1983. Serological markers in progressive systemic sclerosis: clinical correlations. *Ann. Rheum. Dis.* **42**: 23–27.
2. FERNANDEZ-MADRID, F. & M. MATTIOLI. 1976. Antinuclear antibodies (ANA): immunologic and clinical significance. *Semin. Arthritis Rheum.* **6**: 83–124.
3. TAN, E.M. 1989. Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. *Adv. Immunol.* **44**: 93–151.
4. FERNANDEZ, S.A., A.Z. LOBO, Z.N. OLIVEIRA, *et al.* 2003. Prevalence of antinuclear autoantibodies in the serum of normal blood donors. *Rev. Hosp. Clin. Fac. Med. Sao Paulo* **58**: 315–319.
5. WIIK, A.S. 2005. Anti-nuclear autoantibodies: clinical utility for diagnosis, prognosis, monitoring, and planning of treatment strategy in systemic immunoinflammatory diseases. *Scand. J. Rheumatol.* **34**: 260–268.
6. TAN, E.M. 1982. Autoantibodies to nuclear antigens (ANA): their immunobiology and medicine. *Adv. Immunol.* **33**: 167–240.
7. CHRETIEN, P., M. DAUVIN, P. HELIN, *et al.* 1994. Comparison of indirect immunofluorescence on *Crithidia luciliae* of Farr test, and immunoenzymatic methods for the screening of anti-native DNA autoantibodies. *Ann. Biol. Clin. (Paris)* **52**: 645–650.
8. DERKSEN, R.H., E.J. BAST, T. STROOISMA, *et al.* 2002. A comparison between the Farr radioimmunoassay and a new automated fluorescence immunoassay for the

- detection of antibodies against double stranded DNA in serum. *Ann. Rheum. Dis.* **61**: 1099–1102.
9. HAUGBRO, K., J.C. NOSSENT, T. WINKLER, *et al.* 2004. Anti-dsDNA antibodies and disease classification in antinuclear antibody positive patients: the role of analytical diversity. *Ann. Rheum. Dis.* **63**: 386–394.
 10. BULIARD, A., F. FORTENFANT, P. GHILLANI-DALBIN, *et al.* 2005. Analysis of nine autoantibodies associated with systemic autoimmune diseases using the Luminex technology. Results of a multicenter study. *Ann. Biol. Clin. (Paris)* **63**: 51–58.
 11. FULTON, R.J., R.L. MCDADE, P.L. SMITH, *et al.* 1997. Advanced multiplexed analysis with the FlowMetrix system. *Clin. Chem.* **43**: 1749–1756.
 12. GILBURD, B., M. ABU-SHAKRA, Y. SHOENFELD, *et al.* 2004. Autoantibodies profile in the sera of patients with Sjogren's syndrome: the ANA evaluation—a homogeneous, multiplexed system. *Clin. Dev. Immunol.* **11**: 53–56.
 13. GONZALEZ, C., B. GARCIA-BERROCAL, T. TALAVAN, *et al.* 2005. Clinical evaluation of a microsphere bead-based flow cytometry assay for the simultaneous determination of anti-thyroid peroxidase and anti-thyroglobulin antibodies. *Clin. Biochem.* **38**: 966–972.
 14. MARTINS, T.B., R. BURLINGAME, C.A. VON MUHLEN, *et al.* 2004. Evaluation of multiplexed fluorescent microsphere immunoassay for detection of autoantibodies to nuclear antigens. *Clin. Diagn. Lab. Immunol.* **11**: 1054–1059.
 15. NIFLI, A.P., G. NOTAS, M. MAMOULAKI, *et al.* 2006. Comparison of a multiplex, bead-based fluorescent assay and immunofluorescence methods for the detection of ANA and ANCA autoantibodies in human serum. *J. Immunol. Methods* **3**: 3.
 16. ROUQUETTE, A.M., C. DESGRUELLES & P. LAROCHE. 2003. Evaluation of the new multiplexed immunoassay, FIDIS, for simultaneous quantitative determination of antinuclear antibodies and comparison with conventional methods. *Am. J. Clin. Pathol.* **120**: 676–681.
 17. SHOVMAN, O., B. GILBURD, G. ZANDMAN-GODDARD, *et al.* 2005. Multiplexed AtheNA multilyte immunoassay for ANA screening in autoimmune diseases. *Autoimmunity* **38**: 105–109.
 18. YIANNAKI, E.E., E. ZINTZARAS, A. ANALATOS, *et al.* 2004. Evaluation of a microsphere-based flow cytometric assay for diagnosis of celiac disease. *J. Immunoassay Immunochem.* **25**: 345–357.
 19. ZANDMAN-GODDARD, G., B. GILBURD, O. SHOVMAN, *et al.* 2005. The homogeneous multiplexed system—a new method for autoantibody profile in systemic lupus erythematosus. *Clin. Dev. Immunol.* **12**: 107–111.
 20. SHOVMAN, O., B. GILBURD, O. BARZILAI, *et al.* 2005. Evaluation of the BioPlex 2200 ANA screen: analysis of 510 healthy subjects: incidence of natural/predictive autoantibodies. *Ann. N. Y. Acad. Sci.* **1050**: 380–388.
 21. ISENBERG, D.A., C. DUDENEY, W. WILLIAMS, *et al.* 1987. Measurement of anti-DNA antibodies: a reappraisal using five different methods. *Ann. Rheum. Dis.* **46**: 448–456.
 22. SCOFIELD, R.H. 2004. Autoantibodies as predictors of disease. *Lancet* **363**: 1544–1546.
 23. GEISERT, M., B. HEICKE, E. METZMANN, *et al.* 1975. Influence of molecular weight of DNA on the determination of anti-DNA antibodies in systemic lupus erythematosus (SLE) sera by radioimmunoassay. *Nucleic Acids Res.* **2**: 521–535.
 24. BEN-CHETRIT, E., R.I. FOX & E.M. TAN. 1990. Dissociation of immune responses to the SS-A (Ro) 52-kd and 60-kd polypeptides in systemic lupus erythematosus and Sjogren's syndrome. *Arthritis Rheum.* **33**: 349–355.

25. CHAN, E.K., J.C. HAMEL, J.P. BUYON, *et al.* 1991. Molecular definition and sequence motifs of the 52-kD component of human SS-A/Ro autoantigen. *J. Clin. Invest.* **87**: 68–76.
26. FRITSCH, C., J. HOEBEKE, H. DALI, *et al.* 2005. 52-kDa Ro/SSA epitopes preferentially recognized by antibodies from mothers of children with neonatal lupus and congenital heart block. *Arthritis Res. Ther.* **8**: R4.