Immunol Res (2015) 61:110–116
DOI 10.1007/s12026-014-8601-5

DIAGNOSTICS AND ENVIRONMENTAL FACTORS

Clinical performance evaluation of a novel, automated chemiluminescent immunoassay, QUANTA Flash CTD Screen Plus

Chelsea Bentow · Gabriella Lakos · Rachel Rosenblum · Cassandra Bryant · Andrea Seaman · Michael Mahler

Published online: 25 November 2014

Chelsea Bentow

Abstract The QUANTA Flash® CTD Screen Plus is a chemiluminescent immunoassay (CIA) for the detection of the major antinuclear antibodies (ANA) on the BIO-FLASH® platform. NOVA View® is an automated fluorescence microscope that acquires digital images of indirect immunofluorescent assay (IFA) slides. Our goal was to evaluate the clinical performance of the two automated systems and compare their performance to that of traditional IFA. Sera from patients with systemic autoimmune rheumatic diseases (SARD, $n = 178$), along with disease and healthy controls ($n = 204$), were tested with the CTD CIA and with NOVA Lite® HEp-2 ANA, using both the manual method of reading the IFA slides and the NOVA View instrument. The CTD CIA showed 78.1 % sensitivity for SARD, coupled with 94.1 % specificity. Manual IFA and NOVA View showed somewhat higher sensitivity (81.5 and 84.8 % in SARD, respectively), but significantly lower specificity (79.4 and 64.7 %, respectively). Both automated systems displayed somewhat different performance, due to the different principals of ANA detection: IFA with NOVA View digital image interpretation had higher sensitivity, while the CTD CIA showed higher specificity. With the added benefits of full automation, the new CTD CIA is an attractive alternative to traditional ANA screening.

Keywords Antinuclear antibodies · Chemiluminescent immunoassay · Indirect immunofluorescence · Solid phase assay · Connective tissue disease · Systemic autoimmune rheumatic disease

Abbreviations

ALBIA	Addressable laser bead assays
ANA	Antinuclear antibodies
AMR	Analytical measuring range
CIA	Chemiluminescent immunoassay
CTD	Connective tissue disease
LIA	Line immunoassays
ROC	Receiver operating characteristics
SARD	Systemic autoimmune rheumatic disease
SLE	Systemic lupus erythematosus
SjS	Sjögren's syndrome
SPA	Solid phase assay
SSc	Systemic sclerosis
UCTD	Undifferentiated connective tissue disease

C. Bentow (✉) · G. Lakos · R. Rosenblum · C. Bryant · A. Seaman · M. Mahler
Inova Diagnostics, Inc., 9900 Old Grove Road, San Diego, CA 92131-1638, USA
e-mail: cbentow@inovadx.com

Introduction

Antinuclear antibodies (ANA) represent a hallmark in the diagnosis of systemic autoimmune rheumatic diseases (SARD) [1–4]. The presence of ANA is used as an aid in the diagnosis of SARD such as systemic lupus erythematosus (SLE), Sjögren's syndrome (SjS), systemic sclerosis (SSc), idiopathic inflammatory myopathy (IIM), and mixed connective tissue disease (MCTD) in conjunction with clinical finding and other laboratory tests. Typically, ANA have been detected by indirect immunofluorescence assay (IFA) using HEp-2 cells as the substrate [4]. However, performing IFA is labor intensive, subjective, and prone to reader bias [5–9]. Many other variables affect the IFA result such as the HEp-2 substrate, conjugate, microscope, type of bulb, and bulb life [7, 10–14]. In recent years, automated IFA readers have been developed to alleviate these limitations of manual IFA testing and several studies

Table 1 Qualitative agreement between methods

Methods	% PPA (95% CI)	% NPA (95% CI)	% TPA (95% CI)	κ (95 % CI)
CIA vs. manual IFA	70.1 (62.9–76.5)	89.7 (84.6–93.6)	80.1 (75.7–84.0)	0.60 (0.52–0.68)
CIA vs. NOVA view	59.6 (52.9–66.1)	88.7 (82.7–93.2)	71.7 (66.9–76.2)	0.45 (0.37–0.54)
NOVA view vs. manual IFA	96.8 (93.1–98.8)	78.5 (72.0–84.0)	87.4 (89.7–96.7)	0.75 (0.68–0.81)

PPA positive percent agreement, *NPA* negative percent agreement, *TPA* total percent agreement

have shown the benefits of the various automated systems in the industry [15–22]. NOVA View is an automated digital image analysis system, which is used for acquiring, analyzing, and interpreting ANA testing on HEp-2 cells, based on measured light intensity units (LIU) and pattern recognition. Previous studies have also evaluated the performance of ANA detection by enzyme-linked immunosorbent assay (ELISA), fluorescence immunoassay (FEIA), addressable laser bead assays (ALBIA), line immunoassays (LIA), or immunoprecipitation to identify specific autoantibodies in the sera [7, 12, 23]. Although the American College of Rheumatology (ACR) recommends the detection of ANA by IFA on HEp-2 cells, the use of solid phase assays (SPA) has recently become more popular due to various new technologies with full automation [4, 24–27]. The QUANTA Flash® CTD Screen Plus is a fully automated chemiluminescent immunoassay (CIA) for the qualitative detection of the major ANA on the BIO-FLASH® platform, a rapid-response chemiluminescent analyzer. The assay detects antibodies against dsDNA, Sm/RNP, Ro52, Ro60, SS-B, Scl-70, centromere, Mi-2, Ku, Th/To, RNA Pol III, Pm/Scl, PCNA, Jo-1, and ribosomal-P. Our goal was to evaluate the clinical performance of the two automated systems, NOVA View and QUANTA Flash CTD Screen Plus, on a clinically characterized cohort and to compare their performance to that of traditional IFA.

Materials and methods

Sera

Sera from patients with SARD ($n = 178$), including SLE ($n = 98$, Rheumatology Clinic, Neuss, Germany), SjS ($n = 30$, Bioreclamation Resources, Baltimore, MD, USA), SSc ($n = 30$, Scripps Research Institute, San Diego, CA, USA), and MCTD ($n = 20$, Scripps Research Institute, San Diego, CA, USA), along with sera from disease controls ($n = 204$), including rheumatoid arthritis (RA, $n = 30$, Rheumatology Clinic, Neuss, Germany), infectious disease ($n = 28$, ProMedDx, Norton, MA, USA), and blood donors from apparently healthy individuals ($n = 146$, ProMedDx, Norton, MA, USA), were tested with the QUANTA Flash CTD Screen Plus (CTD CIA) and with NOVA Lite® HEp-2 ANA (both from Inova Diagnostics Inc., San Diego, CA, USA), where the same technician read the IFA slides using both the manual method and interpretation of digital images captured by the NOVA View. The diagnoses were established as described before [28] or according to the standard disease criteria.

This study meets and is in compliance with all ethical standards in medicine, and informed consent was obtained from all patients according to the Declaration of Helsinki.

QUANTA Flash® CTD Screen Plus

The QUANTA Flash CTD Screen Plus assay is a novel CIA that is used on the BIO-FLASH® instrument (Biokit s.a., Barcelona, Spain), fitted with a luminometer, as well as the hardware and liquid-handling accessories necessary to fully automate the assay. The principle of the BIO-FLASH system has recently been described [29, 30]. The QUANTA Flash assay for this study was developed using recombinant Scl-70, Jo-1, Ro52, Ro60, SS-B (La), centromere, RNA Pol III, Mi-2, Ku, Th/To, PCNA, native Sm and RNP, synthetic Pm/Scl and ribosomal-P peptides, and synthetic dsDNA coupled to the surface of paramagnetic beads. Prior to use, the lyophilized beads are resuspended using the resuspension buffer. A patient serum sample is pre-diluted with the BIO-FLASH® sample buffer in a small disposable plastic cuvette. Small amounts of the diluted patient serum, the beads, and the assay buffer are combined into a second cuvette, mixed and then incubated for 9.5 min at 37 °C. The magnetized beads are sedimented using a strong magnet in the washing station and washed several times followed by addition of isoluminol-conjugated anti-human IgG and again incubated 9.5 min at 37 °C. The magnetized beads are sedimented and washed repeatedly. The isoluminol conjugate is oxidized when sodium hydroxide solution and peroxide solutions ("Triggers") are added to the cuvette, and the flash of light produced from this reaction is measured as Relative Light Units (RLUs) by the BIO-FLASH® optical system. The RLUs are proportional to the amount of isoluminol conjugate that is bound to the human IgG, which is in turn proportional to the amount of autoantibodies bound to the antigen on the beads.

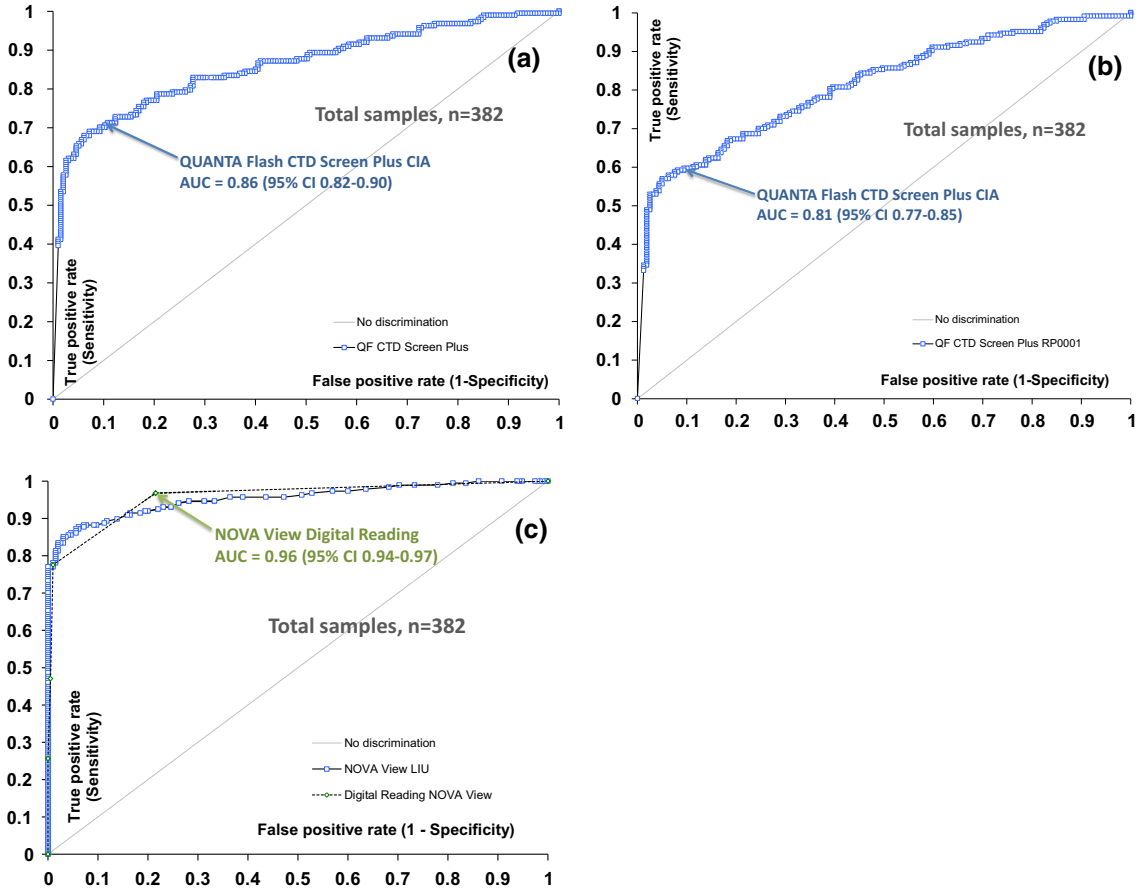

Fig. 1 ROC analysis comparing different antinuclear antibody (ANA) tests. **a** QUANTA Flash CTD Screen Plus chemiluminescent immunoassay (CIA) compared to manual indirect immunofluorescence assay (IFA)-positive ($n = 187$) and IFA-negative ($n = 195$) samples. **b** QUANTA Flash CTD Screen Plus CIA compared to NOVA View-positive ($n = 223$) and NOVA View-negative ($n = 159$) samples. **c** NOVA View compared to manual IFA-positive ($n = 187$) and IFA-negative ($n = 195$) samples. *QF* QUANTA Flash. *Arrows* indicate positive and negative agreements between methods

NOVA Lite® HEp-2 performed manually and with NOVA View®

NOVA Lite® HEp-2 is an IFA for the screening and semi-quantitative determination of ANA in human serum. The NOVA Lite HEp-2 employs human epithelial cells as a substrate. The presence of ANA can be used in conjunction with other serological tests and clinical findings to aid in the diagnosis of SARD. For this IFA, samples are incubated with antigen substrate and unreactive antibodies are washed off. The substrate is incubated with specific fluorescein-labeled conjugate, and then, unbound reagent is washed off. When viewed through a fluorescence microscope, autoantibody-positive samples will exhibit an apple green fluorescence corresponding to areas of the cell or nuclei where autoantibody has bound. Results were graded from 0 to 4 according to the intensity (see also direction insert of the kit); 4 = brilliant apple green fluorescence; 3 = bright apple green fluorescence; 2 = clearly distinguishable positive fluorescence; 1 = lowest specific fluorescence that enables the nuclear and/or cytoplasmic staining to be clearly differentiated from the background fluorescence; 0 = negative. The assay was performed at Inova Diagnostics according to the direction insert by the same technician for both the manual method and NOVA View. Therefore, reactivity grades were given for both the manual reading result and digital image analysis for NOVA View. However, the NOVA View software also outputs light intensity units (LIU), and each sample is interpreted as negative or positive based on a preset cutoff. The automated scan is followed by visual verification of the digital images, allowing for either confirmation or revision of results by the operator. The proprietary process produces three to five images per patient sample. NOVA View software recognizes five basic patterns: homogeneous, speckled, centromere, nucleolar, and nuclear dots. Pattern recognition is based on a software algorithm that analyzes the intensity and distribution of the fluorescent light over the area of the nuclei based on specific

Table 2 Performance characteristics for QUANTA Flash CTD Screen Plus and NOVA Lite HEp-2 IFA performed both manually and with the NOVA View system

	QUANTA Flash CTD Screen Plus	Manual HEp-2 IFA	NOVA View Digital reading
Sensitivity in SARD % (95 % CI)	78.1 (71.3–83.9)	81.5 (75.0–86.9)	84.8 (78.7–89.8)
Specificity % (95 % CI)	94.1 (90.0–96.9)	79.4 (73.2–84.7)	64.7 (57.7–71.3)
Area under the curve (AUC) (95 % CI)	0.92 (0.89–0.95)	0.86 (0.82–0.90)	0.85 (0.81–0.89)
LR+	13.3	3.3	2.4
LR-	0.23	0.23	0.23
Odds ratio	57.7	14.3	10.4
Sensitivity at 95.1 % specificity (95 % CI)	76.4 (69.5–82.4)	68.5 (91.2–97.6)	N/A[a]

[a] Sensitivity for digital reading results is not available at this specificity level

criteria. Mixed patterns may not be recognized by the software and may be reported as "unrecognized." In these cases, the final pattern is determined by the user during the revision and confirmation of the digital images. Although the LIU generated by the NOVA View system is not the final result, the LIU values were used in the analysis for this study as a scientific tool, since this quantitative result given by the instrument offers better discrimination than the grading scale (0–4), which uses categorical variables and therefore offers lower resolution.

Statistical analyses

The data were statistically evaluated using the Analyse-it software (Version 1.62; Analyse-it Software, Ltd., Leeds, UK). Spearman's correlation and Cohen's *kappa* agreement test were carried out to analyze the agreement between portions, and p values <0.05 were considered significant. Receiver operating characteristics (ROC) analysis was used to analyze the discriminatory ability of different immunoassays. Differences between performance characteristics of the assays were calculated using BDTcomparator as described previously for all statistical methods [31, 32].

Results

Qualitative and quantitative agreements between methods

QUANTA Flash CTD Screen Plus CIA was compared to the results obtained by manual reading of NOVA Lite HEp-2 slides and NOVA View digital image results. Additionally, qualitative agreement between manual IFA and NOVA View was also calculated. Moderate to good qualitative agreements were found between the three methods, with total percent agreements varying between 71.7 % (95 % CI 66.9–76.2 %, CIA vs. NOVA View) and 87.4 % (95 % CI 83.7–90.6 %, NOVA View vs. manual IFA) (see Table 1). The correlation according to *kappa* among methods ranged from moderate to substantial [33] and data can be found in Table 1. ROC curve analysis comparing CIA to manual IFA using manual IFA as the reference method resulted in an area under the curve (AUC) value of 0.86 (95 % CI 0.82–0.90) (see Fig. 1a). Similarly, ROC curve analysis comparing CIA to NOVA View results as reference method and NOVA View to manual IFA can be found in Fig. 1b, c, respectively.

Clinical performance of the assays

The CTD CIA showed 78.1 % (95 % CI 71.3–83.9 %) sensitivity for diagnosing SARD coupled with a specificity of 94.1 % (95 % CI 90.0–96.9 %). Manual IFA had a sensitivity of 81.5 % (95 % CI 75.0–86.9 %) and specificity of 79.4 % (73.2–84.7 %) and NOVA View digital image results had a sensitivity of 84.8 % (95 % CI 78.7–89.8 %) and specificity of 64.7 % (57.7–71.3 %) (see Table 2). When analyzing the difference in clinical performance between the CTD CIA and IFA methods for all SARD and controls, the CTD CIA showed significantly higher specificity ($p < 0.0001$) than the two IFA methods, while there was no significant difference between the sensitivities in SARD. Additionally, positive and negative likelihood ratios (LR) and odds ratio (OR) were calculated for the methods and can be found in Table 2. Using ROC curve analysis for the discrimination between SARD patients and disease controls, the AUC values were 0.92

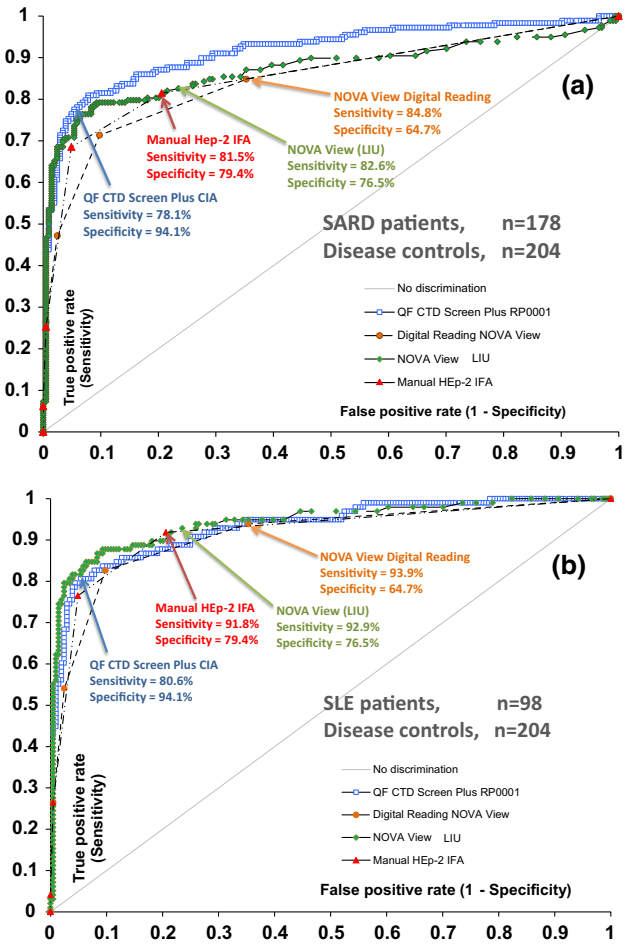

Fig. 2 Comparison of manual NOVA Lite HEp-2 IFA, QUANTA Flash CTD Screen Plus, and NOVA View using receiver operating characteristics (ROC) analysis. The ROC curves show the discrimination between **a** SARD patients ($n = 178$) and controls ($n = 204$) and between **b** SLE patients ($n = 98$) and controls ($n = 204$). *Note*: The NOVA Lite HEp-2 IFA is a semiquantitative assay using both the manual and automated NOVA View method (grades 0–4 were given by operator). However, light intensity unit (LIU) values generated by the NOVA View instrument were also plotted to show the discrimination of the instrument as a scientific tool. No ROC analysis is shown for the other SARD groups due to low sample numbers

(95 % CI 0.89–0.95) for CIA, 0.86 (95 % CI 0.82–0.90) for manual IFA, and 0.85 (95 % CI 0.81–0.89) for NOVA View (See Fig. 2a). When analyzing the difference in the CIA AUC value vs. the AUC values for the IFA methods, it was found that CTD CIA had a significantly higher AUC value than the IFA methods ($p = 0.0016$ between CIA vs. manual IFA, $p = 0.0005$ between NOVA View vs. CIA). In the individual disease groups, the CIA had higher sensitivity in SjS, SSc, and MCTD (p value not significant for these SARD groups combined), while IFA showed significantly higher sensitivity in SLE ($p = 0.0055$ between CIA vs. manual IFA, $p = 0.0036$ between CIA and NOVA View, see Table 3). To further analyze the higher sensitivity of IFA vs. CIA in SLE, ROC curve analysis was performed and showed that CIA had an AUC value of 0.93 (95 % CI 0.90–0.96), while manual IFA had an AUC value of 0.92 (95 % CI 0.88–0.95) and NOVA View had an AUC value of 0.91 (95 % CI 0.87–0.95) (see Fig. 2b).

Discussion

ANA represent a hallmark in the diagnosis of SARD [1–4]. Although the ACR recommends the detection of ANA by IFA on HEp-2 cells, the use of SPA have recently become more popular due to various new technologies with full automation [4, 24–27]. During the last decades, several novel technologies have been developed for ANA detection including the conventional ELISA, and more recently, LIA, FEIA, CIA, and ALBIA assays [26, 27]. As ELISAs are only moderately fast and labor intensive with assay times between 1.5 and 3 h, the focus has lately shifted toward a decrease in assay time and ease of use. This study is the first to evaluate the clinical performance of the new QUANTA Flash® CTD Screen Plus assay on a clinically characterized cohort and to compare its performance to that of traditional IFA and NOVA View. Like other assays on the BIO-FLASH [30, 34, 35], the CTD CIA delivers results in as little as 30 min. The use of SPAs for ANA screening is still subject for debate; some would recommend that SPAs should be used in conjunction with traditional ANA screening by IFA, while others have evaluated the option of full replacement [4, 23, 25]. When comparing the CTD CIA results to manual IFA, good qualitative agreement was found with total agreement equal to 80.1 % ($\kappa = 0.60$). This finding holds promise that the CTD CIA could be used in conjunction with or as an alternative to IFA for the detection of ANA and thus the diagnosis of SARD. The CIA and IFA had similar sensitivity among all SARD patients (78.1 vs. 81.5 %), but the CIA had a much higher specificity (94.1 vs. 79.4 %, $p < 0.0001$, see Table 2). From ROC analysis among the SARD patients and controls (Fig. 2), it can be demonstrated that the CIA had a higher AUC than IFA ($p = 0.0016$ between CIA vs. manual IFA, $p = 0.0005$ between NOVA View vs. CIA). However, it is important to note that since the IFA-grading result is 5 points (0–4), this limits the AUC obtained by ROC analysis. When analyzing positive and negative LRs and ORs, the CIA had a higher LR + and OR than the IFA (see Table 2). In the individual disease groups, the CIA had higher sensitivity in SjS, SSc, and MCTD (p value not significant for these SARD groups combined), while manual IFA showed higher sensitivity in SLE (91.8 vs. 80.6 %, $p = 0.0055$, see Table 3). However, after performing ROC curve analysis in Fig. 2b, it was found that the curves were not significantly different and therefore the higher sensitivity in SLE is based on the cutoff selection, where the CTD CIA shows significantly higher specificity ($p < 0.0001$). The

Table 3 Overview of positive rates in each disease group for QUANTA Flash CTD Screen Plus and NOVA Lite HEp-2 IFA

Disease group	$n = 382$	QUANTA Flash CTD Screen Plus No. pos (%pos)	Manual HEp-2 IFA No. pos (%pos)	NOVA View Digital reading No. pos (%pos)
Systemic lupus erythematosus	98	79 (80.6 %)	90 (91.8 %)	92 (93.9 %)
Sjögren's syndrome	30	24 (80.0 %)	23 (76.7 %)	26 (86.7 %)
Systemic sclerosis	30	21 (70.0 %)	19 (63.3 %)	18 (60.0 %)
Mixed connective tissue disease	20	15 (75.0 %)	13 (65.0 %)	13 (65.0 %)
Rheumatoid arthritis	30	4 (13.3 %)	8 (26.7 %)	15 (50.0 %)
Infectious disease	28	2 (7.1 %)	3 (10.7 %)	9 (32.1 %)
Healthy donors	146	6 (4.1 %)	31 (21.2 %)	48 (32.9 %)

higher sensitivity in SSc is of particular interest since recently developed SPA for ANA detections showed satisfactory results in SLE, MCTD, and SjS, but lacked sensitivity in SSc [3, 36, 37]. This major improvement might be attributed to the inclusion of Rpp25 (Th/To) as one of the antigens in the novel CIA. Rpp25 has recently been demonstrated to represent an important autoantibody target in SSc patients [38, 39]. Although the SSc population in this study is small and future studies are warranted for further validation, another recent study also demonstrated high sensitivity in SSc for CTD CIA, comparable in performance to IFA [40]. The most striking difference in positivity in the control groups between CIA and IFA was the healthy population (4.1 vs. 21.2 %, see Table 3). It is desirable for a screening assay to have high sensitivity, but at the same time maintain good specificity in the control population (especially healthy individuals), to avoid costly follow-up testing where it is not needed. This is of particular importance in light of the recent change in referral patterns. While in the 1960s, when the IFA test was introduced, only rheumatologists and immunologists ordered ANA tests, but there is a long and growing list of clinical disciplines ordering ANA today. This change has tremendous impact on the pretest probability and consequently the requirement for a more specific ANA testing is increasing [41]. With the development of automated digital imaging systems, such as the NOVA View, some of the limitations of IFA HEp-2 have been overcome [41, 42]. In our study, we confirmed the usefulness of NOVA View in the interpretation of ANA testing showing an 87.4 % agreement with the manual readings. In other studies, the agreement between NOVA View and the manual interpretations was even higher [43]. In our study, we did not analyze the ability of NOVA View to recognize patterns since this was not the scope of the study. Besides for diagnosing SARD, IFA HEp-2 can also guide clinicians in the diagnosis of other diseases such as autoimmune liver disease [41] or in the follow-up of juvenile idiopathic arthritis patients. The serum of patients with autoimmune hepatitis may contain anti-smooth muscle (SMA), anti-liver/kidney microsomal (LKM-1, LKM-2, LKM-3), anti-soluble liver antigen (SLA/LP), anti-mitochondrial (AMA), or anti-SP100 antibodies which generate characteristic staining patterns in IFA. When switching from ANA HEp-2 IFA to a SPA, clinicians need to be aware that such non-SARD antibodies will not be detected. A significant limitation of the present study is the lack of patients with IIM. An additional limitation is the relatively small number of patients with the individual forms of SARD. Therefore, further studies are needed to validate the new CIA in larger cohorts of all SARD subpopulations.

Conclusion

The new QUANTA Flash CTD Screen Plus CIA demonstrated similar sensitivity, but significantly higher specificity compared with HEp-2 IFA. With the added benefits of full automation, the new CIA is an attractive alternative to traditional ANA screening.

Conflict of interest C. Bentow, G. Lakos, R. Rosenblum, C. Bryant, A. Seaman, and M. Mahler are employed at Inova diagnostics selling autoantibody assays.

References

1. Mahler M, Fritzler MJ. Epitope specificity and significance in systemic autoimmune diseases. Ann NY Acad Sci. 2010;1183:267–87.
2. 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.
3. Mahler M, Dervieux T. Comments on recent advances and recommendations for the assessment of autoantibodies to cellular antigens referred as antinuclear antibodies. Ann Rheum Dis. 2014;73:e36.
4. Agmon-Levin N, Damoiseaux J, Kallenberg C, 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.
5. Copple SS, Giles SR, Jaskowski TD, Gardiner AE, Wilson AM, Hill HR. Screening for IgG antinuclear autoantibodies by HEp-2 indirect fluorescent antibody assays and the need for standardization. Am J Clin Pathol. 2012;137:825–30.

6. Van BM, Van CC, Bossuyt X, et al. Current practices in antinuclear antibody testing: results from the Belgian External Quality Assessment Scheme. Clin Chem Lab Med. 2009;47:102–8.
7. Tan EM, Smolen JS, McDougal JS, et al. A critical evaluation of enzyme immunoassays for detection of antinuclear autoantibodies of defined specificities. I. Precision, sensitivity, and specificity. Arthritis Rheum. 1999;42:455–64.
8. Peterson LK, Wells D, Shaw L, Velez MG, Harbeck R, Dragone LL. Novel method for quantitative ANA measurement using near-infrared imaging. J Immunol Methods. 2009;349:1–8.
9. Sack U, Conrad K, Csernok E, et al. Autoantibody detection using indirect immunofluorescence on HEp-2 cells. Ann NY Acad Sci. 2009;1173:166–73.
10. Burlingame RW, Peebles C. Detection of Antibodies. In: Pollard KM, editor. Autoantibodies and autoimmunity: molecular mechanisms in health and disease. Weinheim: Wiley-VCH; 2006. p. 159–88.
11. Egner W. The use of laboratory tests in the diagnosis of SLE. J Clin Pathol. 2000;53:424–32.
12. Fenger M, Wiik A, Hoier-Madsen M, et al. Detection of antinuclear antibodies by solid-phase immunoassays and immunofluorescence analysis. Clin Chem. 2004;50:2141–7.
13. Bradwell AR, Hughes RG, Karim AR. Immunofluorescent antinuclear antibody tests. In: Detrick B, Hamilton RG, Folds JD, editors. Manual of clinical laboratory immunology. 7th ed. Washington: ASM press; 2006. p. 995–1006.
14. Emlen W, O'Neill L. Clinical significance of antinuclear antibodies: comparison of detection with immunofluorescence and enzyme-linked immunosorbent assays. Arthritis Rheum. 1997;40:1612–8.
15. Egerer K, Roggenbuck D, Hiemann R, 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.
16. Hiemann R, Buttner 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.
17. Willitzki A, Hiemann R, Peters V, et al. New platform technology for comprehensive serological diagnostics of autoimmune diseases. Clin Dev Immunol. 2012;2012:284740.
18. Voigt J, Krause C, Rohwader E, et al. Automated indirect immunofluorescence evaluation of antinuclear autoantibodies on HEp-2 cells. Clin Dev Immunol. 2012;2012:651058.
19. 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:e9–11.
20. Bonroy C, Verfaillie C, Smith V, et al. Automated indirect immunofluorescence antinuclear antibody analysis is a standardized alternative for visual microscope interpretation. Clin Chem Lab Med. 2013;51:1771–9.
21. Bossuyt X, Cooreman S, De BH, et al. Detection of antinuclear antibodies by automated indirect immunofluorescence analysis. Clin Chim Acta. 2013;415:101–6.
22. Roggenbuck D, Hiemann R, Bogdanos D, Reinhold D, Conrad K. Standardization of automated interpretation of immunofluorescence tests. Clin Chim Acta. 2013;421:168–9.
23. Tonuttia E, Bassetti D, Piazza A, et al. Diagnostic accuracy of ELISA methods as an alternative screening test to indirect immunofluorescence for the detection of antinuclear antibodies. Evaluation of five commercial kits. Autoimmunity. 2004;37:171–6.
24. Meroni PL, Schur PH. ANA screening: an old test with new recommendations. Ann Rheum Dis. 2010;69:1420–2.
25. Bossuyt X, Fieuws S. Detection of antinuclear antibodies: added value of solid phase assay? Ann Rheum Dis. 2014;73:e10.
26. Shovman O, Gilburd B, Barzilai O, et al. Evaluation of the BioPlex 2200 ANA screen: analysis of 510 healthy subjects: incidence of natural/predictive autoantibodies. Ann NY Acad Sci. 2005;1050:380–8.
27. Shovman O, Gilburd B, Zandman-Goddard G, Yehiely A, Langevitz P, Shoenfeld Y. Multiplexed AtheNA multi-lyte immunoassay for ANA screening in autoimmune diseases. Autoimmunity. 2005;38:105–9.
28. Mahler M, Fritzler MJ, Bluthner M. Identification of a SmD3 epitope with a single symmetrical dimethylation of an arginine residue as a specific target of a subpopulation of anti-Sm antibodies. Arthritis Res Ther. 2005;7:R19–29.
29. Mahler M, Radice A, Yang W, et al. Development and performance evaluation of novel chemiluminescence assays for detection of anti-PR3 and anti-MPO antibodies. Clin Chim Acta. 2012;413:719–26.
30. Bentow C, Swart A, Wu J, et al. Clinical performance evaluation of a novel rapid response chemiluminescent immunoassay for the detection of autoantibodies to extractable nuclear antigens. Clin Chim Acta. 2013;424:141–7.
31. Fijorek K, Fijorek D, Wisniowska B, Polak S. BDTcomparator: a program for comparing binary classifiers. Bioinformatics. 2011;27:3439–40.
32. Nofuentes JA, Del Castillo JD. Comparison of the likelihood ratios of two binary diagnostic tests in paired designs. Stat Med. 2007;26:4179–201.
33. Viera AJ, Garrett JM. Understanding interobserver agreement: the kappa statistic. Fam Med. 2005;37:360–3.
34. Infantino M, Bentow C, Seaman A, et al. Highlights on Novel Technologies for the Detection of Antibodies to Ro60, Ro52, and SS-B. Clin Dev Immunol. 2013;2013:978202.
35. Bentow C, Seaman A, Swart A, Shikhman A, Goodman N, Mahler M. Evaluation of a novel chemiluminescent assay (QUANTA Flash® CTD Screen Plus) for the rapid detection of autoantibodies in systemic autoimmune rheumatic diseases. In: 11th symposium on autoantibodies, Dresden, Germany 2013.
36. Parker JC, Bunn CC. Sensitivity of the Phadia EliA connective tissue disease screen for less common disease-specific autoantibodies. J Clin Pathol. 2011;64:631–3.
37. De Op, Beeck K, Vermeersch P, Verschueren P, et al. Detection of antinuclear antibodies by indirect immunofluorescence and by solid phase assay. Autoimmun Rev. 2011;10:801–8.
38. Mahler M, Gascon C, Patel S, et al. Rpp25 is a major target of autoantibodies to the Th/To complex as measured by a novel chemiluminescent assay. Arthritis Res Ther. 2013;15:R50.
39. Mahler M, Satoh M, Hudson M, et al. Autoantibodies to the Rpp25 component of the Th/To complex are the most common antibodies in patients with systemic sclerosis without antibodies detectable by widely available commercial tests. J Rheumatol. 2014;41:1334–43.
40. Bossuyt X. Antinuclear antibody detection by automated indirect immunofluorescence: opportunities for value added reporting. In: 9th international congress on autoimmunity, Nice, France 2014.
41. Mahler M, Meroni PL, Bossuyt X, Fritzler MJ. Current concepts and future directions for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. J Immunol Res. 2014;2014:315179.
42. 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.
43. Copple SS, Jaskowski TD, Giles R, Hill HR. Interpretation of ANA indirect immunofluorescence test outside the darkroom using NOVA View compared to manual microscopy. J Immunol Res. 2014;2014:149316.