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Clinical performance evaluation of a novel rapid response chemiluminescent immunoassay for the detection of autoantibodies to extractable nuclear antigens ☆

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ABSTRACT

Background: We analyzed the performance of a novel ENA screening chemiluminescent immunoassay (CIA) and the confirmation QUANTA Flash tests.
Methods: Sera (n = 1079) from patients referred to a rheumatology clinic were screened by QUANTA Flash ENA7 (INOVA Diagnostics). All positive (n = 89) and a matched control group (n = 90) were reflexed for autoantibodies to the individual antigens. Moreover, sera from patients with systemic lupus erythematosus (SLE, n = 252), systemic sclerosis (SSc, n = 64), polymyositis/dermatomyositis (PM/DM, n = 72), Sjögren's syndrome (SjS, n = 39) as well as disease controls (n = 605) were tested by ENA7 CIA and by Quanta Lite ENA6 ELISA (INOVA).
Results: 89/1079 (8.3%) samples were ENA7 CIA positive with the following reactivity profile: RNP (36.0%), Sm (13.5%), Scl-70 (9.0%), Jo-1 (0.0%), Ro60 (44.9%), Ro52 (39.3%) and SS-B (24.7%). In the negative group, the reactivity profile was: RNP (1.1%), Sm (1.1%), Scl-70 (2.2%) and 0.0% for Jo-1, Ro60, Ro52 and SS-B. The positive/negative/total agreements (ENA7 CIA vs. confirmation assays) were 95.3%/91.5%/93.3%. The sensitivity of the ENA7 CIA was 62.3% in SLE, 54.7% in SSc, 92.3% in SjS, 50.0% in PM/DM, and 61.8% in the total systemic autoimmune rheumatic disease (SARD) population (specificity 95.0%).
Conclusion: The QUANTA Flash ENA7 CIA is a reliable screening test.
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1. Introduction

Autoantibodies targeting extractable nuclear antigens (ENA) are hallmarks in the diagnosis of systemic autoimmune rheumatic diseases (SARD) such as systemic lupus erythematosus (SLE) or systemic sclerosis (SSc) [1]. The primary antigenic targets of anti-ENA antibodies are U1-ribonucleoproteins (RNP), Sm (Smith antigen), Scl-70 (topoisomerase I), Jo-1, Ro60 (SS-A), Ro52 (TRIM21) and SS-B (La). Not all of those antibodies are specific for a particular disease, but are useful to help ruling in or out SARD [1]. Among the disease specific antibodies, anti-Scl-70 are specific for SSc [2], anti-Jo-1 for polymyositis (PM) [1] and anti-Sm antibodies for SLE [1,3]. Among the most common antibodies are antibodies to Ro52, Ro60 and SS-B which therefore deserve special attention and careful evaluation. Historically, anti-Ro52 and anti-Ro60 antibodies have been detected and reported simultaneously. However, recent data suggested that both the cellular function and the clinical association of anti-Ro52 and anti-Ro60 antibodies are significantly different [4]. Anti-Ro60 antibodies are associated with SLE and SjS whereas anti-Ro52 antibodies can be found in various disease conditions including PM and SSc [5] but also in several forms of lung disease associated with autoimmunity [6]. Of high importance, about 20% of anti-Ro52/anti-Ro60 antibodies can be missed when tested using a blend of the two antigens [4]. Besides the diagnostic value of antibodies to Ro52, Ro60 and SS-B, it has been shown that those antibody specificities can precede the clinical onset of SLE for many years [7]. In addition to SARD, previous studies have reported autoantibodies to ENA in PBC [1,8]. Several methods have been developed and used for the detection of anti-ENA antibodies

Abbreviations: ALBIA, addressable laser bead assays; AMR, analytical measuring range; CIA, chemiluminescent immunoassay; CLSI, Clinical and Laboratory Standards Institute; CTD, connective tissue disease; LIA, line immunoassays; PBC, primary biliary cirrhosis; PM/DM, polymyositis/dermatomyositis; SARD, systemic autoimmune rheumatic disease; SLE, systemic lupus erythematosus; SjS, Sjögren's syndrome; SSc, systemic sclerosis.

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including ELISA, line immunoassays (LIA) [9], addressable laser bead assays (ALBIA) [10–13] and protein arrays [14]. During the last years, the chemiluminescence technology, which has been used for clinical chemistry for a long time, has been applied for autoantibody testing [15].

2. Materials and methods

2.1. Sera

Sera (n = 1079) from patients referred to a rheumatology clinic were screened for anti-ENA7 antibodies by QUANTA Flash ENA7 (INOVA Diagnostics, Inc., San Diego, CA). All positive samples (n = 89) and approximately the same number of randomly selected negative samples (n = 90) were reflexed for autoantibodies to the individual antigens (RNP, Sm, Scl-70, Jo-1, Ro60, Ro52 and SS-B) using the QUANTA Flash assays. To compare the clinical performance of the QUANTA Flash ENA7 CIA to a predicate device, sera from patients with SLE (n = 252), SSc (n = 64), PM/DM (n = 72), and SjS (n = 39), as well as various disease controls (n = 605, for details see the Results section) were tested by QUANTA Flash ENA7 CIA and by Quanta Lite ENA6 ELISA (INOVA; RNP, Sm, Scl-70, Jo-1, Ro60, Ro52 and SS-B). Additionally, sera from patients with PBC (n = 52) were screened by both methods, but were not included as part of the disease controls for analysis since PBC patients are known to have ENAs. A list of the disease cohorts and controls can be found in the Results section. To verify the specificity of the Scl-70 assay, various disease controls were tested (n = 628). The diagnoses were established as described before [3] or according to the standard disease criteria.

This study meets and is in compliance with all ethical standards in medicine. Patient identity was not disclosed and the data was anonymously used in accordance with the latest version of the Helsinki Declaration of human research ethics.

2.2. QUANTA Flash(R) assays

The QUANTA Flash assays (INOVA Diagnostics Inc., San Diego, CA) are novel CIA that are currently used on the Bio-Flash® instrument (Biokit s.a., Barcelona, Spain), a fully automated chemiluminescent immuno-analyzer. The principle of the Bio-Flash system has recently been described [15]. The QUANTA Flash assays used in this study were developed using native or recombinant antigens (INOVA Diagnostics, see Table 1) coated onto 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 all 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. The Bio-Flash system also allows the customer the option to reflex the individual assays of a screening test such as the QUANTA Flash ENA7.

2.3. Quanta Lite assays

Quanta Lite ENA6 was used as a comparator method. This ELISA contains the same number of antigens as the QUANTA Flash ENA7. Historically, Ro52 and Ro60 were called SS-A [4] and therefore counted as one antigen when the Quanta Lite ENA6 was developed. The antigen composition and sources are shown in Table 1. The assay was performed according to the direction of use.

2.4. Precision and linearity studies

Precision and linearity of the assays were verified by performing the required testing according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. For the precision study, the within run, between day, between run, and total precision were determined by running 2 aliquots of the precision samples twice a day in random order, with a minimum of 2 h between each run. The samples were run on the same instrument for each assay and repeated for at least 20 days, according to CLSI guideline EP5-A2. Linearity testing was performed according to CLSI guideline EP6-A (Vol. 23, No. 16), which involved diluting several high titer sera in a serial dilution scheme to span the analytical measuring range (AMR) for each assay.

2.5. Generation of mono-specific samples (Jo-1)

Seven samples with high titers of Jo-1 antibodies were diluted in normal serum to yield negative or low positive results for all other autoantibodies to antigens contained in the ENA7 Screen. Those samples were then used to analyze the sensitivity of QUANTA Flash ENA7 for Jo-1.

2.6. Statistical analyses

The data were statistically evaluated using the Analyse-it Software (ver 1.62; Analyse-it Software, Ltd., Leeds, UK). Linearity results were analyzed by linear regression analysis, and slope, intercept and R^2 values were calculated. Chi-square, Spearman's correlation and Cohen's *kappa* agreement tests were carried out to analyze the agreement between portions and $p < 0.05$ was considered significant. Receiver-operating characteristics (ROC) analysis was used to analyze the discriminatory ability of different immunoassays.

3. Results

3.1. Precision and linearity

Precision study results for all QUANTA Flash CIAs are shown in Table 2. The total precision for all assays varied between 3.8 and 13.8%. The linearity study results are tabulated in Table 3 for all QUANTA Flash CIA assays. All assays demonstrated linearity over the AMR.

Table 1
Antigen composition of QUANTA Flash ENA7 and QUANTA Lite ENA6.

Antigen	QUANTA Flash ENA7	QUANTA Lite ENA6
RNP	Native purified	Native purified
Sm	Native purified	Native purified
SS-A Ro60	Recombinant, insect cells	Native purified
SS-B La	Recombinant, insect cells	Native purified
Ro52	Recombinant, insect cells	Recombinant, insect cells
Scl-70	Recombinant, insect cells	Native purified
Jo-1	Recombinant, insect cells	Native purified

Table 2
Precision data of the QUANTA Flash CIA assays.

	No. of samples	Within run	Between-day	Between-run	Total
ENA7	8	1.8–4.3%	2.6–6.3%	0.0–2.6%	4.0–7.1%
RNP	7	3.4–4.8%	3.0–9.3%	0.0–4.5%	4.8–10.8%
Sm	5	5.3–9.7%	2.7–5.8%	0.5–7.1%	8.3–11.5%
Ro60	9	2.9–5.8%	0.0–2.6%	2.9–9.6%	3.8–10.5%
Ro52	6	4.1–6.9%	6.2–11.6%	0.0–4.1%	9.8–12.5%
SS-B	7	3.7–9.3%	7.2–9.9%	1.8–6.8%	9.7–13.8%
Scl-70	7	2.2–4.5%	0.0–2.6%	1.9–4.2%	4.3–5.0%
Jo-1	9	2.9–5.0%	4.7–6.8%	0.0–3.0%	6.2–8.0%

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