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SPECIAL ARTICLE

# International multi-center evaluation of a novel chemiluminescence assay for the detection of anti-dsDNA antibodies

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**Objective**: Anti-double stranded desoxyribonucleic acid (anti-dsDNA) antibodies are considered fairly specific for systemic lupus erythematosus (SLE) and their quantification is useful for the clinical management of SLE patients. We assessed the diagnostic performance of the QUANTA Flash dsDNA chemiluminescent immunoassay (CIA) in comparison to an ELISA, using patients from five participating countries. The main focus was to evaluate the correlation between anti-dsDNA antibody results from the CIA and global SLE disease activity, as measured by the SLE Disease Activity Index 2000 (SLEDAI-2K). **Patients and methods**: A total of 1431 samples (SLE, $n = 843$; disease controls, $n = 588$) from five countries (Canada, USA, Portugal, Sweden and Spain) were tested with QUANTA Flash dsDNA (Inova Diagnostics, San Diego, CA, USA). Data obtained with the QUANTA Lite dsDNA SC ELISA (Inova Diagnostics) were available for samples from three sites (Canada, USA and Sweden, $n = 566$). The SLEDAI-2K scores were available for 805 SLE patients and a cut-off of $> 4$ was used to define active disease. **Results**: QUANTA Flash dsDNA had a sensitivity of 54.3% for the diagnosis of SLE, combined with 89.8% specificity. Anti-dsDNA antibody levels were significantly higher ($p < 0.0001$) in active SLE (SLEDAI-2K $> 4$; $n = 232$; median value 83.0 IU/mL) versus the inactive patients ($n = 573$; median value 22.3 IU/mL), and the SLEDAI-2K scoring correlated with their dsDNA antibody levels (Spearman's rho $= 0.44$, $p < 0.0001$). Similar but less pronounced findings were also found for the ELISA, in relation to disease activity. **Conclusions**: The QUANTA Flash dsDNA assay showed good clinical performance in a large international multi-center study. Additionally, the strong correlation between anti-dsDNA antibody results and SLEDAI-2K scores supported the potential utility of QUANTA Flash dsDNA for monitoring disease activity. *Lupus* (2016) **25,** 864–872.

**Key words:** anti-dsDNA antibodies; autoantibodies; autoimmune disease; comparative study; diagnostic test; immunoassay; lupus; multicenter study; systemic lupus erythematosus

## Introduction

The detection of anti-dsDNA autoantibodies remains an important diagnostic tool in the diagnosis of systemic lupus erythematosus (SLE)[1] and these antibodies constitute a classification criterion for SLE.[2,3] Anti-dsDNA antibodies are considered fairly specific for SLE and their levels also correlate with disease activity and lupus nephritis, which proves useful for the clinical management of SLE patients.[4–7] Although the standardization of measuring anti-dsDNA antibodies is still poor and different methods yield different results, new technologies were developed in recent years that were shown to be viable alternatives to the traditional methods, such as: Farr radioimmunoassay, ELISA and *Crithidia luciliae* indirect immunofluorescence test (CLIFT).[8–15] Such technologies include multiplex systems, fluoro-enzyme immunoassays (FEIA), and chemiluminescent immunoassays (CIA), which were shown to be comparable to other traditional methods in previous studies.[16–23] The goal of the present multicenter study was to

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assess the diagnostic performance of the QUANTA Flash dsDNA CIA in comparison with an ELISA, using patients from five participating countries. Secondly, we investigated the utility of this assay for the clinical management of SLE patients by analyzing the correlation of results to the SLE disease activity index (SLEDAI-2K) in these patients.

## Patients and methods

### *Patient population and serum samples*

The study included 1431 patient samples from five countries (Canada, USA, Portugal, Sweden and Spain), including 843 samples from SLE patients (median age 44.0 years, 95% CI 43.0–46.0 and 88.7% of female gender) and 588 disease controls (median age 48.0 years, 95% CI 46.0–50.0 and 72.0% female gender), namely other patients with rheumatoid arthritis (RA, $n=109$), ankylosing spondylitis (AS, $n=96$), autoimmune hepatitis (AIH, $n=35$), celiac disease (CD, $n=7$), dermatomyositis (DM, $n=3$), hepatitis C (HCV, $n=40$), juvenile idiopathic arthritis (JIA, $n=33$), primary biliary cirrhosis (PBC, $n=43$), psoriatic arthritis (PsA, $n=23$), systemic sclerosis (SSc, $n=25$), primary sclerosing cholangitis (PSC, $n=4$), Sjögren's syndrome (SjS, $n=57$) and samples from population-based healthy individuals (HI, $n=113$). SLEDAI-2K scores were available for 805 SLE patients and a cut-off of $>4$ was used to define active disease.[24] The diagnoses were established as described in a previous paper[25] or when applicable, according to the standard disease criteria.[2,3] All samples were tested for anti-dsDNA IgG by QUANTA Flash dsDNA CIA (Inova Diagnostics, San Diego, CA, USA).

For comparison, data obtained with the QUANTA Lite dsDNA SC ELISA (Inova Diagnostics) were available for SLE patient samples from three sites (Canada, USA and Sweden, $n=566$).

This study met and was in compliance with all ethical standards in medicine, according to the Declaration of Helsinki.

### *Anti-dsDNA antibody assays*

The QUANTA Flash dsDNA (Inova Diagnostics) assay is a novel CIA that is used on the BIOFLASH instrument (Biokit, 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 BIOFLASH system and QUANTA Flash dsDNA assay was recently described.[17–19,26] The QUANTA Flash assay for this study was developed using synthetic dsDNA coupled to the surface of paramagnetic beads. The cut-off was established during the development of the assay, using a reference population of apparently healthy blood donors, in accordance to Clinical and Laboratory Standards Institute (CLSI) C28-A3c in *Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory: Approved Guidelines*, 3rd Ed.

The upper 95th percentile reference interval limit was calculated to be 26.9 IU/mL. Additionally, the 99th percentile reference interval limit was calculated to be 35.8 IU/mL. Therefore, 27 IU/mL was set as the cut-off, and 27–35 IU/mL was set as equivocal/indeterminate range. Samples with $>35$ IU/mL were considered positive. More detailed assay characteristics can be found in Table 1. QUANTA Lite dsDNA SC (Inova Diagnostics) is an ELISA for the quantitative detection of IgG specific for dsDNA in human serum, as an aid in the diagnosis of SLE in conjunction with other laboratory and clinical findings. Both assays were performed at Inova Diagnostics, according to the direction insert. The characteristics of the assays are summarized in Table 1.

### *Statistical evaluation*

The data were statistically evaluated using Analyse-it software, version 3.90.1 (Leeds, UK). We used Wilcoxon-Mann-Whitney to analyze the titer difference between different groups, the Fisher Exact test to analyze antibody prevalence in different categories and $p$ values $<0.05$ were considered significant for all statistical methods.

**Table 1** Characteristics of the anti-dsDNA antibody assays used in the SLE study

| *Characteristic* | *QUANTA Flash dsDNA* | *QUANTA Lite dsDNA SC* |
|---|---|---|
| Manufacturer | Inova Diagnostics | Inova Diagnostics |
| Technology | CIA | ELISA |
| Assay time | 30 min | 90 min |
| Detection | Quantitative | Quantitative |
| Analytical range | 9.8–666.9 IU/mL | 12.3–1000 IU/mL |
| Cut-off value (ranges) | 9.8–27 = Negative<br>27–35 = Equivocal<br>>35 = Positive | 12.3–30 = Negative<br>30–75 = Equivocal<br>>75 = Positive |
| Cut-off value applied | ≥27 IU/mL | ≥30 IU/mL |
| Antigen source | Synthetic dsDNA | Native calf thymus |

CIA: chemiluminescent assay, dsDNA: double-stranded deoxynucleic acid, ELISA: enzyme-linked immunosorbent assay, SC: standard curve; SLE: systemic lupus erythematosus

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. We used receiver operating characteristics (ROC) analysis to analyze the discriminatory ability of different immunoassays. For the assays having an equivocal range, all data was analyzed using the applied cut-offs listed in Table 1, where the equivocal results were considered positive.

## Results

### *Qualitative and quantitative agreement between anti-dsDNA methods*

Overall in the 566 SLE patients tested by both methods, the ELISA and the CIA showed moderate qualitative agreement ($kappa = 0.59$; 95% CI 0.52–0.65) and good quantitative agreement (Spearman's $rho = 0.78$; 95% CI 0.74–0.81; $p < 0.0001$; data not shown). A total of 230 samples were positive and 219 negative by both methods, 51 were CIA positive but ELISA negative, and 66 were ELISA positive but CIA negative.

### *Clinical performance*

QUANTA Flash dsDNA demonstrated a clinical sensitivity of 54.3% for the diagnosis of SLE ($n = 843$; Table 2) and 89.8% specificity in the control population ($n = 588$; Figure 1(a)). The anti-dsDNA antibody levels showed moderate variations among countries (ANOVA $p = 0.0183$; Figure 1(b)). The highest median titer was found in the cohort from the USA, followed by Spain, Portugal, Canada and Sweden. The ROC curve analysis among SLE patients and disease controls demonstrated an area under the curve (AUC) value of 0.79 (Figure 2(a)). Sensitivity and specificity, as well as positive and negative likelihoods ratios (LRs) and AUC values for QUANTA Flash dsDNA are shown in Figure 2(a).When clinical performance was analyzed in the active SLE patients only ($n = 232$) compared to disease controls, the AUC (0.89), the sensitivity (77.2%) and the OR (29.7) of the CIA increased (Figure 2(b)).

**Table 2** Diagnostic Sensitivity for SLE in patients from five countries

| *Site* | *QUANTA Flash dsDNA CIA Sensitivity (95% CI)* | *QUANTA Lite dsDNA SC ELISA Sensitivity (95% CI)* |
|---|---|---|
| Canada | 50.9% (44.5–57.2%) | 62.8% (56.5–68.8%) |
| USA | 64.8% (53.2–74.9%) | 69.0% (57.5–78.6%) |
| Sweden | 44.4% (38.5–50.5%) | 38.3% (32.6–44.3%) |
| **Canada, USA, Sweden** | **49.6% (45.5–53.8%)** | **52.3% (48.2–56.4%)** |
| Portugal | 56.8% (48.0–65.2%) | N/A |
| Spain | 69.7% (62.0–76.5%) | N/A |
| **All five countries** | **54.3% (51.0–57.7%)** | **N/A** |

CIA: chemiluminescent immunoassay; dsDNA: double-stranded deoxyribonucleic acid; ELISA: enzyme-linked immunosorbent assay; N/A: not applicable; CIA: chemiluminescence immunoassay; SC: standard curve; SLE: systemic lupus erythematosus.

### *Correlation to disease activity (SLEDAI-2K)*

When comparing the novel CIA with the ELISA for the measurement of disease activity, we observed that the titer difference between active and inactive SLE patients was more pronounced using the novel CIA. Using the CIA, the anti-dsDNA antibody levels were significantly higher ($p < 0.0001$; Figure 3(a)) in active SLE ($n = 232$; median value = 83.0 IU/mL) versus in the inactive patients ($n = 573$; median value = 22.3 IU/mL), and the SLEDAI-2K scoring correlated with the dsDNA antibody levels (Spearman's $rho = 0.44$; $p < 0.0001$; Figure 3(b)).

Additionally, the SLEDAI-2K scores differed significantly between the anti-dsDNA positive ($n = 432$, median = 4.0 and mean = 5.4) and negative ($n = 373$, median = 2.0 and mean = 2.4) groups, for the CIA ($p < 0.0001$, data not shown). The positivity rate of the CIA and ELISA in active and inactive SLE patients can be found in Table 3. When analyzing the cohorts from the different sites individually, we observed a significantly higher sensitivity in active SLE (Table 3). The most pronounced difference was found in the cohort from Portugal, where the sensitivity in the active SLE patients was 89.3% compared to 47.9% in the inactive group.

## Discussion

The detection of anti-dsDNA autoantibodies remains an important tool in the diagnosis of SLE[1] and is part of the American College of Rheumatology (ACR) and The Systemic Lupus Collaborating Clinics (SLICC) classification criteria.[2,3] In addition, anti-dsDNA antibodies correlate with disease activity and certain disease phenotypes (such as nephritis).[4–7,15] This large multicenter study demonstrated good diagnostic performance of the QUANTA Flash dsDNA CIA and its utility for the detection of active SLE. In

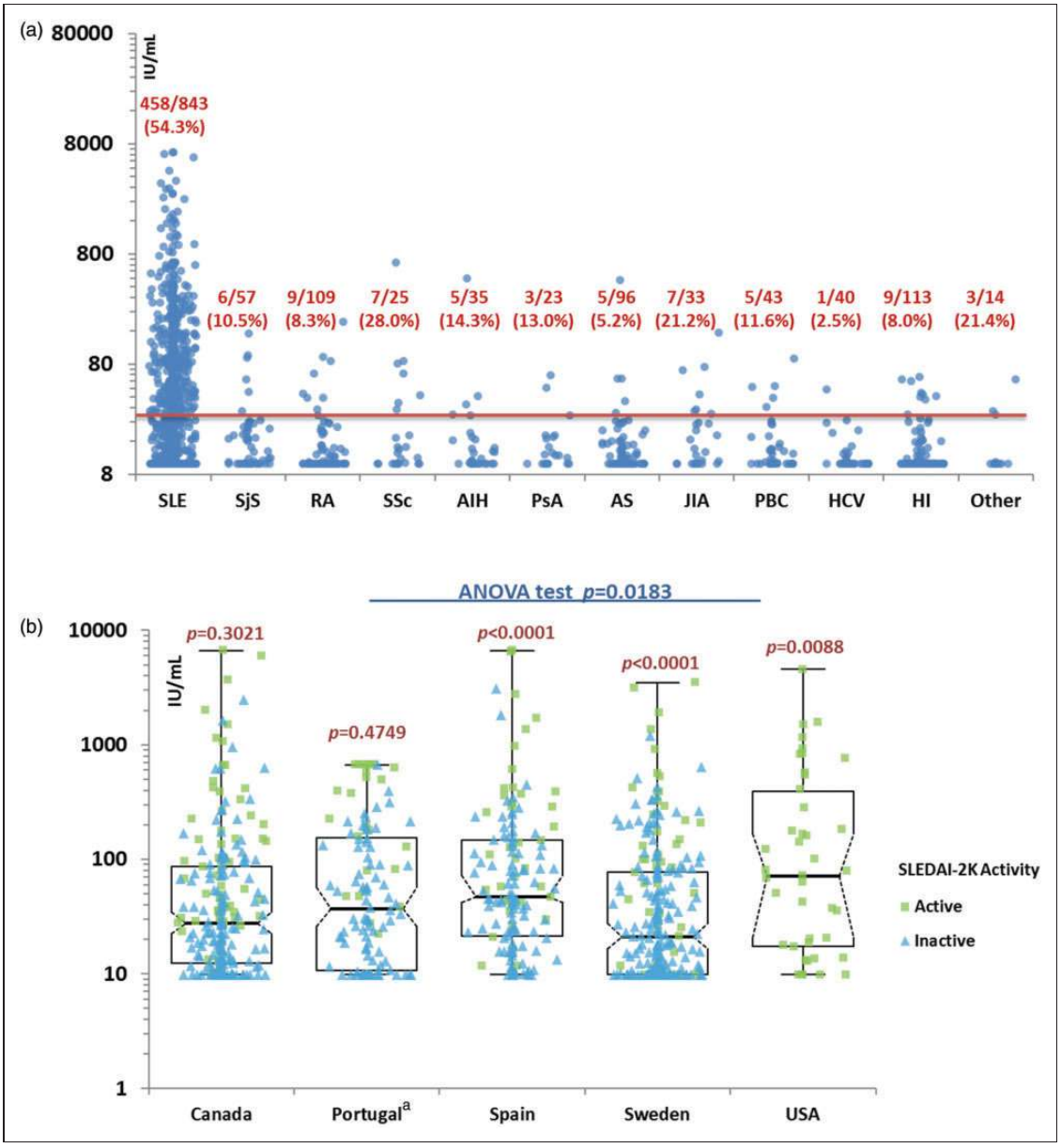


**Figure 1** Comparative descriptive analysis showing the titers of anti-dsDNA antibodies using QUANTA Flash dsDNA CIA in IU/mL. (a) SLE and disease controls. (b) Five countries with separation between active and inactive patients. Red *p*-values indicate the difference between each country and the rest of the sites (Wilcoxon-Mann-Whitney test).
[a]Portugal was the only site that did not use the auto-rerun function for the assay; and therefore, results above 666.9 IU/mL were not obtained.
AIH: autoimmune hepatitis; AS: ankylosing spondylitis; CIA: chemiluminescent immunoassay; dsDNA: double-stranded DNA; HCV: hepatitis C virus; HI: healthy individuals; IU: international units; JIA: juvenile idiopathic arthritis; mL: microliter; other: other diseases category including celiac disease, primary sclerosing cholangitis, and dermatomyositis, PBC: primary biliary cirrhosis; PsA: psoriatic arthritis; RA: rheumatoid arthritis; SjS: Sjögren's syndrome; SLE: systemic lupus erythematosus; SLEDAI-2K: SLE Disease Activity Index 2000; SSc: systemic sclerosis

terms of clinical performance, the sensitivity in SLE (54.3%) and specificity (89.8%) in the disease controls is similar to previous studies on the CIA and other anti-dsDNA solid phase assays; however, it is important to note that in general there is a high degree of variability reported among the studies for both sensitivity and specificity of the anti-dsDNA methods evaluated.[8–10,13,18] In particular, Infantino et al.[18] reported a 96.0% specificity (95% CI 90.1–98.9%) and 39.3% sensitivity (95% CI 27.2–52.7%) for the CIA in an Italian cohort, while this study demonstrated lower

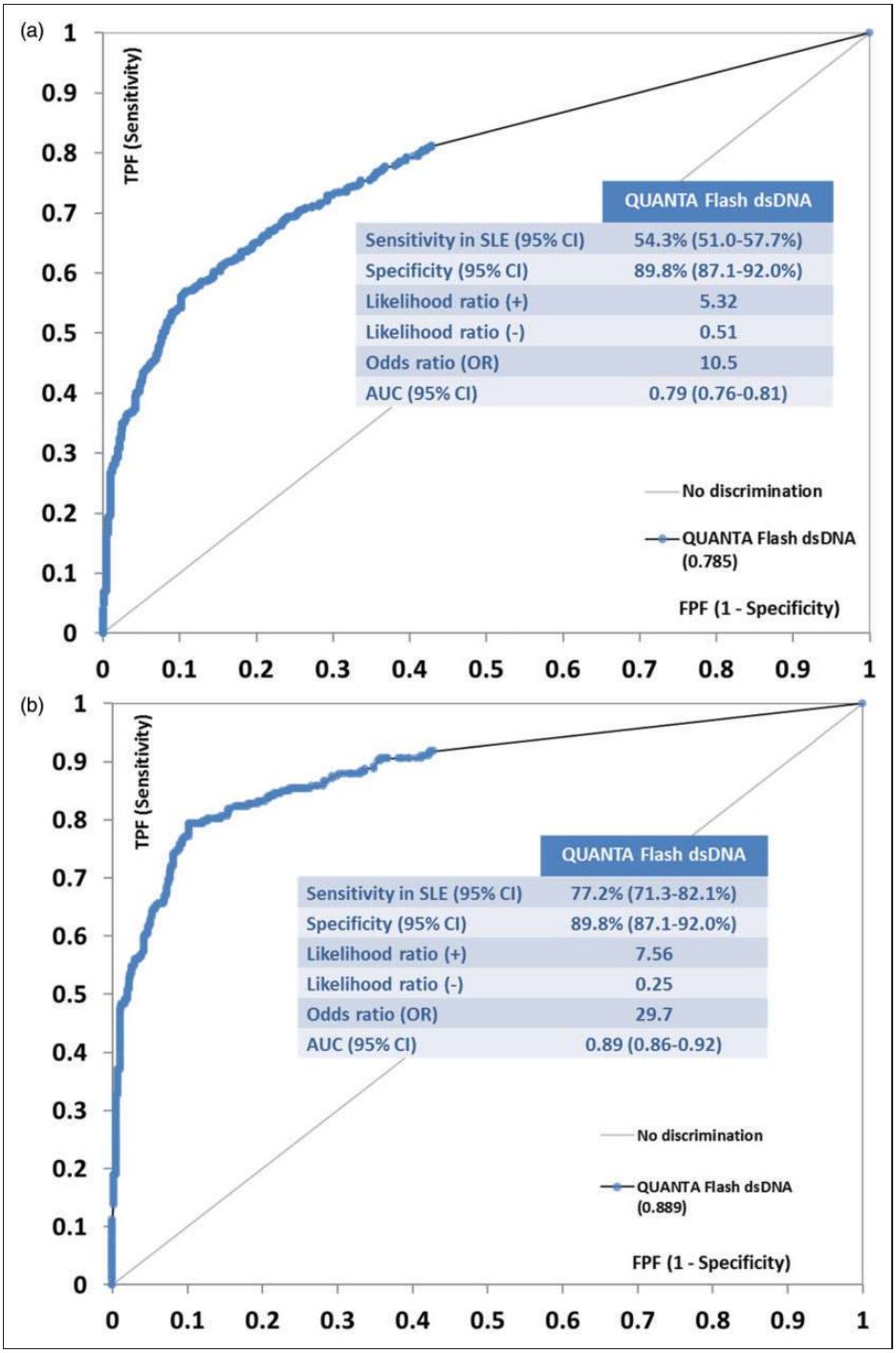


**Figure 2** Clinical performance of QUANTA Flash dsDNA for the detection of anti-dsDNA antibodies. (a) ROC analysis shows the discrimination between SLE patients ($n = 843$) and controls ($n = 588$). (b) The ROC analysis shows the discrimination between active SLE patients ($n = 232$) and controls ($n = 588$).
AUC: area under the curve; FPF: false positive findings; OR: odds ratio; ROC: receiver operating characteristics; TPF: true positive findings; SLE: systemic lupus erythematosus

specificity. The reasons most likely contributing to these observed differences in sensitivity and specificity are the number and type of disease controls included (the Italian study[18] was a much smaller population of patients), and the clinical phenotype of the SLE patients (i.e. the number of ACR criteria fulfilled) involved in the study. Especially for the SLE patients, the prevalence of anti-dsDNA

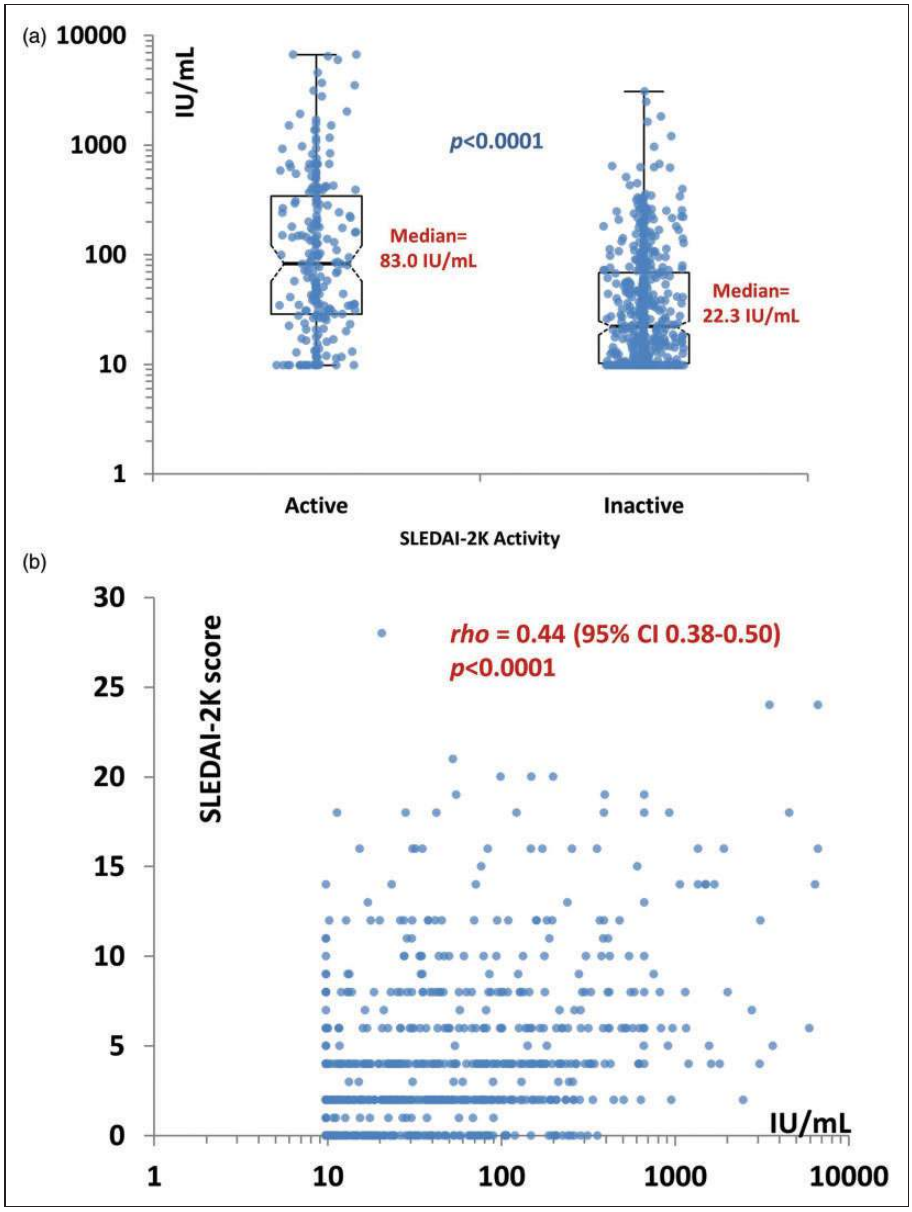


**Figure 3** Correlation of QUANTA Flash dsDNA results to disease activity in SLE patients ($n = 805$). (a) Comparative antibody titer distribution between active SLE and inactive SLE groups. (b) Spearman's correlation of QUANTA Flash dsDNA and SLEDAI-2K score. Axes are shown in logarithmic scale for QUANTA Flash units.
[a]SLEDAI-2K scores include anti-dsDNA as a variable.
IU: international units; SLE: systemic lupus erythematosus; SLEDAI 2K: SLE disease activity index 2000

antibodies in a population can vary based on the following:

- Whether the patients have active or inactive disease;
- If the patients have more severe clinical manifestations (e.g. nephritis);
- The treatment status of the patients; and
- The change in clinical disease activity between study assessment and blood draw.

In this multi-center study, the type of SLE patients included from each country was heterogeneous regarding the four factors listed above. For example, SLE patients from Spain and USA demonstrated the highest prevalence (Table 2) and highest titers (Figure 1(b)) of anti-dsDNA antibodies as measured by the CIA, but this is explained mostly by the fact that these two countries had the highest percentage of active patients (Table 3).

**Table 3** Diagnostic sensitivity SLE in patients with active and inactive disease

| | | | *CIA* | | | *ELISA* | | |
|---|---|---|---|---|---|---|---|---|
| *Site* | *Active/inactive patients* (n) | *Active/ Inactive (%)* | *Active SLE % positive (95% CI)* | *Inactive SLE % positive (95% CI)* | p-*value* | *Active SLE % positive (95% CI)* | *Inactive SLE % positive (95% CI)* | p-*value* |
| Canada | 68/166/0 | 41.0% | 79.4% (68.4–87.3%) | 39.2% (32.1–46.7%) | <0.0001 | 76.5% (65.1–85.0%) | 57.2% (49.6–64.5%) | 0.00071 |
| USA | 43/0/28 | 100.0% | 65.1% (50.2–77.6%) | N/A | N/A | 67.4% (52.5–79.5%) | N/A | N/A |
| Sweden | 50/211/0 | 23.7% | 72.0% (58.3–82.5%) | 37.9% (31.6–44.6%) | <0.0001 | 60.0% (46.2–72.4%) | 33.2% (27.2–39.8%) | 0.0006 |
| **Canada, USA, Sweden** | **161/377/0** | **42.7%** | **73.3% (66.0–79.5%)** | **38.5% (33.7–43.5%)** | **<0.0001** | **68.9% (61.4–75.6%)** | **43.8% (38.8–48.8%)** | **<0.0001** |
| Portugal | 28/94/3 | 29.8% | 89.3% (72.8–96.3%) | 47.9% (38.1–57.9%) | <0.0001 | N/A | N/A | N/A |
| Spain | 43/102/7 | 42.2% | 83.7% (70.0–91.9%) | 61.8% (52.1–70.6%) | 0.0109 | N/A | N/A | N/A |
| **All five countries** | **232/573/38** | **40.5%** | **77.2% (71.3–82.1%)** | **44.2% (40.1–48.2%)** | **<0.0001** | **N/A** | **N/A** | **N/A** |

[a]Disease activity N/A.
CIA: chemiluminescent immunoassay; ELISA: enzyme-linked immunosorbent assay; N/A: not available; SLE: systemic lupus erythematosus

Similarly, SLE patients from Sweden had a lower prevalence and the lowest titers of anti-dsDNA antibodies, but this site also had the lowest proportion of active patients. In comparison to the ELISA, the positivity rate for SLE of the CIA was lower than the ELISA in the cohorts from Canada and USA, but was higher in the cohort from Sweden (Table 2). This illustrates that the performance of an assay might be dependent on the sample population.

The majority of studies comparing different anti-dsDNA antibody assays focused on the ability to discriminate between SLE and controls.[8,10,13,18,27] Only a few studies have analyzed the tests for their performance to measure disease activity in SLE patients.[4–7,15] Our data demonstrated that the novel CIA shows a good correlation to the disease activity measured by the SLEDAI-2K score. This association was found in all cohorts; not only a significantly higher positivity rate (Table 3), but also a significantly higher level of anti-dsDNA antibodies were found in the active *versus* the inactive patients measured by the CIA (Figure 3(a)). The higher level of anti-dsDNA antibodies measured by the CIA in active patients demonstrated the potential utility of the assay to serve as a biomarker of active disease. Although we also found significantly higher positivity rates for the ELISA in the active SLE groups (Table 3), the percent difference between the active and inactive SLE patients was more pronounced for the CIA in all countries and overall.

A potential shortcoming of our study was the approach used to differentiate between active and inactive disease. Analyzing the association between anti-dsDNA antibodies and disease activity in SLE is challenging. We used the SLEDAI-2K score with a cut-off of $> 4$ to differentiate between active and inactive disease, based on the work presented by Yee et al.[24] in 2011, although this cut-off selection is somewhat debatable, because previous studies used a variety of cut-offs (including $> 6, \geq 6, \geq 10$, etc.) to define active disease.[28–33] Additionally, it is important to point out that the cut-off selection presented by Yee et al.[24] underlines the importance of considering the SLEDAI-2K score as a continuous scale, especially in longitudinal studies. Therefore, in this study we also analyzed the relationship of dsDNA positivity by the CIA in relation to the SLEDAI-2K score and found significantly higher scores in the CIA-positive versus the CIA-negative SLE patients ($p < 0.0001$). The score in this study also included anti-dsDNA antibodies as a variable and this could be considered a limitation of the study, because anti-dsDNA antibodies can bias the classification into active and inactive disease; however, the antibodies contributed only two points and different anti-dsDNA assays were used at the five sites to determine the SLEDAI-2K score, which reduced the bias. Longitudinal studies are needed to further analyze the performance of QUANTA Flash dsDNA for monitoring the level of disease activity.

## Conclusions

The QUANTA Flash dsDNA showed good clinical performance in a large international multicenter study. Additionally, the strong correlation between anti-dsDNA antibody results and SLEDAI-2K scores supported the potential utility of QUANTA Flash dsDNA for monitoring disease activity.

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## Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: M Mahler, C Bentow, P Martis, E Wahl and G Lakos are employed at Inova Diagnostics, which sells autoantibody assays. The other authors have no conflict of interest to declare.

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